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Flexible wide-field optical micro-angiography based on Fourier-domain multiplexed dual-beam swept source optical coherence tomography

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Abstract

Wide-field optical coherence tomography angiography (OCTA) is gaining interest in clinical imaging applications. In this pursuit, it is challenging to maintain the imaging resolution and sensitivity throughout the wide field of view (FoV). Here, we propose a novel method/system of dual-beam arrangement and Fourier domain multiplexing to achieve wide field OCTA when imaging the uneven surface samples. The proposed system provides two separate FoVs, with flexibility that the imaging area, focus of the imaging beam and imaging depth range can be individually adjusted for each FoV, leading to either 1) increased system imaging FoV or 2) capability of targeting two regions of interests that locate at depths with large difference between each other. We demonstrate this novel method by employing 100 kHz laser source in a swept source OCTA to achieve an effective 200 kHz sweeping rate, covering a 22×22 mm FoV. The results are verified by a SS-OCTA system employing a 200 kHz laser source, together with the experimental demonstrations when imaging whole brain vasculature in rodent models and skin blood perfusion in human fingers, showing the capability of proposed system to image live large samples with complex surface topography.

Graphical Abstract

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A novel system of dual-beam arrangement and Fourier domain multiplexing can achieve wide field OCT and OCT angiography when imaging uneven surface samples. The proposed system provides two separate field-of-views (FoV), with flexibility that the imaging area, focus of the imaging beam and imaging depth range can be individually adjusted for each FoV. The abstract image shows a wide-field angiography result from dual-beam setup, where each hemisphere of mouse brain is scanned by individual OCT beams, demonstrating the extension of image area without extra time or data collection cost.

Keywords

Optical coherence tomography; Medical and biological imaging; Imaging systems; Three-dimensional Image Acquisition; Multiplexing

1. Introduction

Optical coherence tomography (OCT) is a three-dimensional (3D) imaging technique that provides non-invasive, high-resolution cross-sectional images in real time¹. Over the past

decade, OCT has been widely used in many clinical applications, e.g. ophthalmology^{2,3}. It has also witnessed rapid growth in medical research and various industrial applications⁴. As a functional extension of the OCT technique, OCT-based angiography (OCTA) has recently made its way to clinical translation, largely due to its ability of providing rapid, safe and non-invasive images of depth-resolved microcirculation networks, which facilitates the visualization and interpretation of vascular involvements with pathological conditions. Ultra-high sensitive optical micro-angiography (OMAG) is introduced in 2010⁵, which brings superior performance in terms of imaging capillary level microcirculations. Ever since, a number of new developments were accomplished to improve the imaging speed⁶, system stability², field-of-view³ and sensitivity⁷. OMAG technique was successfully translated to clinical ophthalmology to aid in the diagnosis of ocular diseases, such as diabetic retinopathy, glaucoma, and age-related macular degenerations⁸⁻¹⁰.

To date, majority of efforts have been devoted to the technical developments of functional OCT systems, towards the following directions: 1) High-speed imaging. Imaging speed not only plays critical role in the practicality of the applications, but also benefits various functional OCT modalities, such as OCTA, tissue dynamics¹¹, and elastography^{12,13}. 2) Wide-field, long-range imaging. Extended imaging range can be highly beneficial for many clinical applications, especially for imaging samples with a complex surface topography, for example when it is required to image whole eye¹⁴ and whole brain¹⁵. Surgical microscope¹⁶ would also benefit from an OCT system with extended imaging range. Today, there is an increasing interest in the field of dermatology to visualize the cutaneous microcirculation as well as the tissue structure with wide field-of-view¹⁷. 3) High resolution. Maintaining high spatial resolution throughout the OCT depth range could significantly improve the amount of information one can obtain through OCT imaging.

Improvements in imaging speed have largely relied on the advancement of high-speed swept lasers. On the other hand, parallel detection may also be used to improve image speed, albeit with a concern whether the system is cost effective and clinical translatable¹⁸. Various types of high-speed swept-source OCT (SS-OCT) have been introduced, allowing significantly higher speed performance than spectral domain OCT (SD-OCT). High-speed OCT with A-scan rate from 200 kHz to multi-megahertz were demonstrated by vertical-cavity surface-emitting laser (VCSEL)^{14,19}, Fourier domain mode-locked (FDML) laser^{20,21}, and aperiodic swept laser²²⁻²⁴. The sensitivity roll-off performance, as well as robustness to motion artifacts of these SS-OCT systems are proven superior to SD-OCT. Moreover, ultra-high imaging speed also facilitates the extensions of field-of-view under the constraint of imaging time required to make patients feel comfortable. The drawback of high sweeping rate is the inevitable reductions of photons that can be received by the detector, leading to reduced sensitivity. In addition, it also requires a higher detection bandwidth to maintain the axial ranging distance²⁵.

The extension of field-of-view can be relatively easy to achieve, by simply employing a long focal length object lens and higher A-scan rate^{21,26}. However, an inherent challenge that comes with extended field-of-view is the limitation of system depth-of-focus (DOF) imposed by the Gaussian optics. For a conventional OCT imaging system with Gaussian detection beam, the image quality is only considered acceptable within the DOF, as defined

by twice the Rayleigh length, expressed as²⁷ $\text{DOF} = 4\lambda/\pi\text{NA}^2$, where NA stands for the numerical aperture of the objective lens. Given that the OCT lateral resolution is inversely proportional to the NA, the DOF can be considered proportional to the square of lateral resolution. This explains the tradeoff between lateral resolution and the DOF for most OCT systems. Although this limitation may not be a concern for retinal imaging, it would severely affect the lateral resolution for imaging large volume objects for wide-field OCT applications. Hence, various approaches have been proposed to mitigate the issue of limited DOF, examples including dynamic focusing²⁸, Bessel beam illumination^{29, 30}, synthetic aperture³¹⁻³³, etc. However, the benefits from these focus-extension methods usually come with cost of computation time, sensitivity loss, or increased image acquisition time.

In addition to the above-mentioned endeavors, multiplexing configurations have been explored in a few studies, which usually demonstrates composited benefits. Technologies of space-division multiplexing³⁴ and interleaved OCT^{35, 36} have brought multifold imaging speed and FoV extension. Along the similar tactic, here we propose a novel method to achieve a flexible wide filed imaging for OCT and OCTA, in which a specially designed dual-beam sample arm and Fourier domain multiplexing are implemented by leveraging the benefit of multi-centimeter coherence length that the swept laser brings. Compared to a conventional setup, dual-beam system can achieve extended and flexible FoV, with extended depth-of-focus, at no extra cost of data collection or computation resources.

2. Experimental implementation

2.1 OCT imaging system design

Figure 1 illustrates the experimental setup of the proposed dual-beam SS-OCT system. We have evaluated the setup with two OCT laser sources in this study, both were MEMS-VCSEL swept sources (SL1310V1-10048, and SL1310V1-20048, Thorlabs, Inc.), operated at 100 kHz and 200 kHz swept rate, respectively. Both lasers had a 1305 nm central wavelength and 100 nm spectrum bandwidth. They had built-in A-line trigger signal, i.e. wavelength sweep trigger. K-linear sampling clock were provided by a Mach-Zehnder interferometer (MZI) clock module integrated within the laser sources. OCT systems were implemented with fiber-based Michelson interferometer by launching the light into a wideband 50:50 coupler. A pair of XY galvanometric mirrors (GVS002, Thorlabs, Inc.) were synchronized with the swept source and controlled by a 12-bit high-speed analog output board (National Instruments, PCI-6713). A wide-field OCT-optimized scanning microscopy lens (Thorlabs, LSM05) was used in the sample arm to deliver the light onto the sample, with an effective focal length of 110 mm, providing a spot size ($1/e^2$) of $\sim 31 \mu\text{m}$. The depth of focus was estimated as 0.63 mm, defined by twice the Rayleigh length. An adjustable free-space reference arm was employed to match the optical delay with that in the sample arm. The backscattered light from the two arms were combined by the 50:50 coupler, and then delivered to a high-speed dual-balanced photodetector with 1.6GHz bandwidth (PDB480C-AC, Thorlabs, Inc.). A high-speed digitizer (ATS9373, AlazarTech, Inc.) was used to detect the OCT interference signal. The raw OCT data was transmitted to a host computer (Intel Xeon E5-2630 v3) through 8-lane PCI-Express Gen3 interface. OCT system control was implemented on LabVIEWTM platform to automatically control all the

operations including system calibration, galvo-scanning, system synchronization, data acquisition and streaming, and real-time imaging preview of the OCT results.

During imaging, the optical power delivered on the sample from each beam was 5 mW, giving a maximum measured sensitivity of ~102 dB and 97 dB, respectively, for the two beams (see below).

Dual-beam sample arm—Before delivering OCT illumination beam onto sample, it was split into two beams through a 50/50 fiber coupler (Figure 1). For brevity, these two beams are named as beam 1 and 2, respectively. A 635nm visible laser was also coupled into the two beams for visual aiming purpose. Both beams were guided to free-space with collimator C1 and C2, while the positioning of the collimators were adjustable along the optical axis, so that the optical delay of each beam can be adjusted individually. Both beam paths shared the same x-y galvo-scanners and objective lens. Beam 2 was directed by a mirror M1, onto the scanning mirrors. Mirror M1 controlled the angle between the two collimated beams, which eventually determined the spatial separation between two scan spots on sample. The typical configuration of beam separation distance was 10 mm, corresponding to an angle of 4.45° between two collimated beams. Due to the loss in the extra 50/50 fiber coupler, the over-all OCT sensitivity had approximately 3 dB loss, compared to a conventional single-beam setup, with the same level of illumination energy per beam.

Adjustable focus—Imaging flexibility is a major purpose of the dual-beam setup. An independently adjustable focal depth design was implemented to extend the depth of focus of the whole system. The focus of beam 1 was adjustable and beam 2 was fixed at ~10 mm optical delay. On the optical path of beam 1, a 4-f system was inserted before the scanning mechanism. The 4-f system featured a fixed lens and an adjustable lens, such that beam divergence could be controlled by varying the distance between the pair of lenses. The 4-f system, together with objective lens, functioned as a variable focal lens group, allowing the two beams to position their focus at different depths, so that large volume sample with uneven surface could be imaged. To bring the focus closer to image probe, the adjustable lens should be moved closer to the fixed lens in the 4-f system, so that the beam is slightly diverging before entering the objective lens. Conversely, moving the adjustable lens away from fixed lens would shift the focus farther away from the nominal focal plane. The effective focal length of the lens group can be calculated by the equation derived from 3-element combined lens:

$$EFL = \frac{f_0 \left(d_0 - \frac{f(d-f)}{d-2f} \right)}{d_0 - \left(f_0 + \frac{f(d-f)}{d-2f} \right)} \quad (1)$$

Here f_0 is the focal length of the objective lens, d_0 is the distance between the objective lens and the fixed element of 4-f system, d is the distance between the 4-f lens pair, and f is the focal length of both elements in 4-f system.

Fourier-domain multiplexing—For a multi-beam scanning system, a most straightforward way to acquire data is to use parallel channels to collect the data from each beam; however, the cost-efficiency of this approach can be easily surpassed by Fourier-domain multiplexing which also simplifies data collection and mitigates data streaming burden. Utilizing the property of excellent coherence length that the swept laser source gives, the signals from different beams can be arranged into different coherence length bands, which can be achieved by adjusting the optical delays of sample beams. To facilitate this configuration with extended depth range, we programmed the digitizer to acquire data in “dual-edge sampling” mode, i.e., the digitizer was taking two ADC samples for every clock cycle, one on the rising edge, and another on the falling edge. In this way, the effective sampling rate was doubled, compared to the default configurations of MZI k-clock provided by the laser source. For each A-line sweep, 5120 sampling points are acquired to cover the resulting spectral interferogram. The sampling rate of 100 kHz and 200 kHz lasers are ~900 MHz and ~1,800 MHz, respectively, determined by $[2 \times (\text{central frequency of k-clock})]$. The imaging range of the two VCSEL lasers were both elevated to 24 mm, compared to a default 12 mm range. In our experiments, the first 8 mm band was assigned to beam 1, and 8~16 mm band was assigned to beam 2.

2.2 Dual-beam imaging protocol

For each dual-beam scanning, the scan protocol adopts a B-M-C scan pattern: on each cross-sectional position, B-scan imaging was repeated 4 times, and the slow scan axis was traversed through 1000 lateral positions to complete a C-scan. B-scan repetition rate was 180 Hz, allowing a time for fast-axis scanner fly-back. The total time was ~22.2 seconds for collecting a 3D data volume to represent 3D structural and vascular information of the sample.

100 kHz laser source—500 A-lines were acquired for each B-scan, and 1000 cross-section positions are scanned. The resulting *en-face* image from each beam was rectangular shape, with 1:2 aspect ratio, covering $11 \times 22 \text{ mm}^2$ area. Combined *en-face* image from two beams was 1000×1000 pixels, giving a field of view of $22 \times 22 \text{ mm}^2$.

200 kHz laser source—1000 A-lines were acquired for each B-scan, with 1000 cross-section positions. Total B-scan number is 4000. FoV for each beam is $12 \times 12 \text{ mm}^2$. Single-beam 200 kHz configuration is also employed to verify the imaging performance of dual-beam 100 kHz configuration, with the same $22 \times 22 \text{ mm}^2$ FoV. Imaging frame rate and time consumption was the same for single-beam 200 kHz and dual-beam 100 kHz. The imaging protocol parameters are listed in Table 1.

Imaging procedure—Dual-beam system brings some degrees of complexity in system operations compared to conventional point-and-shot OCT systems. The imaging procedure is as follows: (1) Place sample under the probe and position it into the FoV of beam 1. (2) Adjust mirror M1 to aim beam 2 onto the second field-of-interest. (2) Adjust sample height position so that the image from beam 2 is located at ~10 mm optical delay. (3) Move collimator C1 to place the image of beam 1 at ~2 mm optical delay. (4) Adjust 4-f lens to focus on sample of beam 1. (5) Start acquisition.

2.3 OCT structural and angiography image processing

As a pre-processing step to extract complex data, OCT reference spectrum was subtracted from interference signal to remove DC bias³⁷. DFFT was performed with point number of 5120, to extract depth-resolved OCT signal. Negative frequencies in Fourier domain was truncated as redundancy. Complex image was split into two datasets, where 0~8 mm and 8~16 mm optical delay ranges in the Fourier domain correspond to beam 1 and 2, respectively. OCT structural image was reconstructed using the modulus of the complex data, and visualized in dB scale. For angiography images at each cross-sectional position, four repeated B-scan complex data was firstly processed by the numerical phase-stabilization technique proposed in ³⁸, then eigendecomposition-based clutter filtering technique ^{7, 39} was used for optical micro-angiography.

3. Results

3.1 Assessment of dual-beam OCT system performance

To assess the system sensitivity performance over an extended ranging distance with the dual-beam hardware setup described in the last section, the following procedure was taken for measuring the roll-off performance: a mirror was installed in the sample arm, and one beam on the sample arm was blocked. The system point spread functions (PSF) at a series of optical delays were measured by varying the optical path length in the reference arm. The results are shown in Figure 2, the PSFs were measured in total of 36 times, with 0.5 mm optical delay intervals. The first 8 mm of OCT ranging distance was assigned to beam 1, while the range of 8 to 16 mm was assigned to beam 2, as shown with two series of blue and red PSF plots. Benefiting from the excellent instantaneous line width of VCSEL source, the dual-beam OCT system is proven to possess low sensitivity roll-off. The -3 dB OCT signal roll-off distance was measured to be 9 mm in air. Due to the optical mismatches between the K-clock MZI and the OCT interferometer, there were inevitable errors in the k-space linearity. Therefore, the PSF performance shows slight roll-off and side-lobe with the increase of the optical delay. In our setup, the imaging range beyond 16 mm was intentionally omitted, as shown with gray plots in Figure 2.

Figure 2 also gives the measured full width half maximum(FWHM) of the PSFs against the ranging distance, as the estimation of axial resolutions. Within the imaging range of 0~15 mm, the mean value of axial resolution was 15.2 μm , which is close to the theoretical axial resolution of $\sim 13 \mu\text{m}$ for a spectral bandwidth of 100 nm at the central wavelength of 1310 nm. The overall sensitivity roll-off for 16 mm range was ~ 2 dB, while the axial resolution maintains at approximately 15 μm across whole range. Consequently the sensitivity is slightly inconsistent between two beams, which is difficult to eliminate completely, due to imperfections in k-clock. The image of beam 2 was approximately 3dB lower than beam 1, which also leads to slightly lower sensitivity in OCTA images of this beam.

3.2 Double imaging speed achieved by Dual-beam OCT

The dual-beam SS-OCT imaging was firstly conducted on an intact-skull mouse brain, with the purpose to demonstrate high-speed wide-field functional OCT imaging. With dual-beam setup proposed in Section 2.1, the imaging speed of 100 kHz sweep rate light source can

achieve an equivalent A-line rate of 200 kHz. As validation, the same mouse brain was imaged using 100 kHz VCSEL laser source with dual-beam configuration, then immediately followed by an imaging session with 200 kHz VCSEL source configured in single beam set-up, under the same imaging conditions.

The mouse we used in the study was a C57BL/6 mouse (Charles River Laboratories), aged 10 weeks, and body weight 24g. It was anesthetized by isoflurane at 1.5% concentration, inhaled with 0.2 L/min O₂ and 0.8 L/min air supply. The body of the mouse was warmed by a thermostat pad to maintain the temperature. A simple surgery was conducted to expose the entire skull: the hair on scalp was shaved, and the scalp was cut across the median line, and displaced to expose skull. After adding several drops of saline, the entire open skull was covered with a plastic film to prevent de-hydration on the skull. To suppress motion artifacts from animal breathing/heart-beating, the head of the mouse was immobilized with ear-bars that applied a mild pressure on the skull in OCT imaging sessions. All experimental animal procedures prepared for this pilot study of imaging were in accordance with a protocol reviewed and approved by the Institute of Animal Care and Use Committee (IACUC) of University of Washington (Protocol # 4262-01).

Imaging results from both 100 kHz and 200 kHz laser sources are shown in Figure 3. Here, the 200 kHz setup was operated under conventional wide-field OCTA protocol²⁴ with a single sample beam configuration. A representative OCT structural B-scan image is displayed in Figure 3(a), and the corresponding OMAG image is given in the form of intensity-coded transparent overlay, as shown in Figure 3(b). The arteries and veins in the pial network, as well as some penetrating vessels are clearly visualized in the OMAG image. The OMAG sensitivity maintains at satisfying level across all depth range, thanks to the outstanding roll-off performance of VCSEL-based SS-OCT. In order to better present the rich information of the multi-dimension data, every voxel in the 3-D OMAG volume was color-coded by vessel depth, and transparency was assigned according to OMAG intensity, afterwards the volume was collapsed as an *en-face* projection image, as given in Figure 3(c). The wide-field SS-OCTA system is capable of delivering high-resolution visualization of the blood vessels, including the associated networks of middle cerebral arteries (MCA), which is close to the edge of skull, and located relatively deep relative to the apex of the brain surface. Here the vessel depth is defined by the distance from the vessel to the surface of skull, which is given by the automatic segmentation⁴⁰. Note that due to the resolution limit of the relatively low NA object lens in this setup, it is difficult to identify individual subsurface microvasculature. The OMAG image (Figure 3b) also suffers from projection artifacts that is commonly seen in OCTA results; however, these artefacts are negligible in *en-face* projection images.

Figure 3(d-f) presents the images captured by the dual-beam setup, utilizing the significantly slower speed VCSEL laser source of 100 kHz. As shown in Figure 3(d), the top and bottom half of the OCT image are assigned to beam 1 and 2 separately. Although the A-scan count is only 500 over one B-scan, which is half of the number of A-scans compared to Figure 3(a), these two images present the same resolution, field-of-view, and almost identical image information. Only the image sensitivity appears lower for the bottom part (beam 2) of dual-beam image. However, the sensitivity difference can be eliminated if swept lasers with better

roll-off performances are employed in future image systems (e.g. akinetic SS-OCT^{22, 23}). OMAG cross-sectional image and en-face projection images are displayed in the same scheme as Figure 3(b,c). Figure 3(f1) and (f2) are the results from applying separately segmentations of beam 1 and beam 2 imaging volumes. The image quality and level of details from the dual-beam system are reasonably close to that of higher A-line rates. The time consumption to acquire the same $22 \times 22 \text{ mm}^2$ would have cost 44.4 seconds using 100 kHz source on a conventional setup, while dual-beam configuration has reduced the time cost to 1/2.

3.3 Extended DOF and field-of-view with Dual-beam OCT

As introduced by our previous work on ultra-wide field OCTA systems, multiple applications can benefit from extended field-of-view²³. The design of the flexible dual-beam setup was aimed to facilitate wide-field OCTA, by overcoming the DOF limit, and deliver optimal lateral resolution in different designated depth ranges. To demonstrate the extended DOF, we performed *in vivo* OCTA imaging on two human fingers simultaneously, using the dual-beam setup employing 200 kHz swept laser. As the imaging targets, the left ring finger was arranged in perpendicular overlapping position with the right finger, which induced about 10 mm height difference between the nail-fold areas of two fingers. The height deviation could represent a typical wide-field example with complex surface topography. To enhance the optical penetration, we dropped some glycerol on the ring finger for refractive-index matching⁴¹. Figure 4(a) shows a photo of the targeted sample. One laser scan line is seen on each finger, indicating the cross-sectional scan position. Beam 1 was aimed at the top finger, while beam 2 aimed at the bottom finger. There was $\sim 1 \text{ mm}$ separation between two scan lines. A typical, multiplexed B-scan image is shown in Figure 3(b), where the optical delays of two beams were adjusted to position the region of interests of each finger in their designated ranging distance of the system, i.e. beam 1 was assigned to 1-8 mm and beam 2 assigned to 8-16 mm ranging distance. The foci of two beams were tuned accordingly to provide optimal lateral resolution on each finger, where the focus position for beam 1 was approximately 3mm away from the zero-delay line, while it was about 11 mm for beam 2.

Figure 4(c1, c2) shows the *en-face* projection OMAG image of left and right ring finger from beam 1 and beam 2, respectively. The FOV is as wide as $12 \times 12 \text{ mm}^2$ for each beam, which covered the entire nail fold region and part of peripheral area including the distal edge, nail plate, lateral nail folds, lunular, cuticle, and eponychium. Using structural information, surface of skin was segmented. This topological information was used for calculating vessel depth, which was then color-coded to display Figure 4(c1, c2). The vascular networks in these regions are revealed in excellent details, including most of capillary vessels. A variety of different patterns and diameters in the vessels can be identified. In Figure 4(c1), some vessels in the nail plate region appear distinct in red color, since those vessels are located deep under the nail plate of $\sim 1 \text{ mm}$ thickness. OCT structural image from each beam are displayed in Figure 4(d1, d2), with corresponding OMAG overlay images displayed in Figure 4(e1, e2). On both set of images, high-resolution structural and angiography information are presented with details. Epidermis, dermis and

subcutaneous tissue are revealed and layered structures can be clearly observed, as well as the blood vessels in these layers.

Note that the lateral resolution is preserved well on the images from both beams. This is not possible with conventional, single-beam setup. The “depth of view” for our object lens is specified as 0.63 mm at ~1300 nm wavelength, while the dual-beam setup can extend this limit for the applications where sample presents large height variations.

4. Discussion and conclusion

There are multiple benefits from the dual-beam configuration demonstrated in this study, and the flexibility is the key innovation. The capability to individually adjust two OCT detection beams brings excellent flexibility for image planning, optical alignment, and parallel monitoring of dynamic biological processes. As an example, dual-beam OMAG imaging of the entire mouse brain was achieved, with no extra cost of imaging time. The complete map of the cerebrovascular networks in brain could serve as a powerful inspective tool for a variety of neurological studies such as stroke, Alzheimer, and vision research. The flexible placement of two field-of-views also allows the imaging system to target at two anatomical symmetric sites simultaneously, with one site as the control and the other as the test for physiological monitoring, for example in the case of neuronal stimulation, localized neural injury, etc.

In conventional ultra-long ranging distance OCT imaging²³, most of the image range would appear out-of-focus due to the depth-of-focus limits. Here with dual-beam and flexible re-focusing capability, such limitations could be mitigated. Further improvements can be achieved using an automatic focus lens, which may relax the requirements for optical alignment and patient fixation in clinical practice.

Another advantage that comes with dual-beam or multi-beam configurations is the potential to use lower illumination energy on each individual beam, while maintaining the same sensitivity performance by averaging. This is effectively distributing the high-energy detection beam into a number of low energy beams, which is in favor of applications of higher laser safety concerns, such as retinal imaging.

With rapid growth in SS-OCT ranging-distance, the elevated burden of data sampling, streaming and processing is pushing the requirements for high-speed electronics, memory and computing power to their limits. Fourier domain multiplexing proposed in this study can somehow relax the requirements by multiple folds. All the imaging results presented in this paper were obtained without extra requirements for sampling speed, memory or computing time, thus the multiplexing approach could be a preferred solution for cost-effective imaging systems.

There can be some degrees of trade-off in the application of dual-beam techniques: firstly, splitting and re-combining the beam will inevitably introduce some sensitivity loss, due to the laser energy loss in the extra optical components. The dual-beam setup also brings extra system complexity. If the coherence range performance of the laser source is not optimal, the sensitivity decay can also bring non-uniform image quality between different beams. In this

case, the Fourier domain multiplexing process should be carefully designed considering the balance between image sensitivity and cross-channel aliasing. Image range assigned to each beam should be as small as possible, without inducing any aliasing on the image of two beams. There are also some limitations in extending the DOF using dual-beam setup, especially in ultra-wide field imaging, such as human-brain imaging in clinical operation room. While using two foci for imaging two separated FoVs is natural and straight-forward, using multiple foci to produce single FoV will lead to non-uniform image quality across the FoV. This will require advanced image stitching, or utilizing real-time adaptive focusing during the scanning procedure.

Good axial sensitivity roll-off performance is one prerequisite for Fourier domain multiplexing. Due to the excellent performances of instantaneous linewidth provided VCSEL laser source, the dual-beam system has brought outstanding imaging sensitivity and signal roll-off performances for both beams. The roll-off performances in this study is considerably lower than that reported in ultra-long range^{42, 43} VCSEL SS-OCT setups, due to the differences in MZI-optical clock delay, detector bandwidth, and RF phase calibration⁴⁴. It should be noted that much higher multiplexing factors could be achieved, with increased number of detection beams, given the capability of more than 10 cm coherence length of the swept laser sources.

In summary, a flexible OCT/OCTA imaging system based on dual-beam, and Fourier domain multiplexing has been demonstrated for wide-field high-resolution imaging with extended depth-of-focus. Not only OCT structural images, but also highly sensitive optical micro-angiography imaging was demonstrated using this setup, showing the capability to extend this configuration to wide-field functional imaging of the samples with uneven surface topology. Using 100 kHz laser source, the dual-beam OCT system has demonstrated an effective imaging speed of 200 kHz, covering a 22×22 mm field-of-view. With the proposed dual-beam system employing a high-speed 200 kHz laser source, dual field images of 12×12 mm have been demonstrated for a living subject with complex surface topography. For both field-of-views, the richness of blood vessels was visualized with sufficient details, ranging from superficial capillaries to deep dermis vessel networks. The presented results indicate the great potential of dual-beam/multi-beam system in the medical research fields such as for ophthalmic applications, neurophotonics imaging, and dermatological angiography. It is expected that a variety of clinical applications related to large-volume imaging could benefit from the proposed methods.

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Figure 1. Schematic of the Dual-beam SS-OCT system based on VCSEL swept source

Figure 2. Sensitivity roll-off and resolution measurements of the dual-beam SS-OCT system, measured from 100 kHz VCSEL laser source and ~900 MHz sampling clock.

Figure 3.

Imaging results on mouse brain with intact skull. (a) A representative structural B-scan image on mouse brain, acquired with single beam, 200 kHz swept laser. (b) OMAG image overlay on structural image at same position of (a). (c) *En-face* projection OMAG image of whole mouse brain, with color-coded depth and transparency-coded intensity. (d) A representative structural B-scan image on mouse brain, acquired with the dual-beam, 100 kHz swept laser. (e) OMAG image overlay on structural image at same position of (d). (f1, f2) *En-face* projection OMAG image visualized with same scheme as (c). (f1) Left brain from beam 1 and (f2) Right brain from beam 2.

Figure 4.

Imaging results from Dual-beam SS-OCT setup. (a) Photograph of imaging sample. (b) One representative OCT B-scan, at the center of imaging field. (c1, c2) En-face angiography result, presented as maximum intensity projection with color-coded depth, from Beam 1 and Beam 2 respectively. (d1, d2) OCT structural B-scan images at selected positions. (e1, e2) corresponding OMAG B-scan images overlay onto structural images.

Table 1
Scan protocol parameters in dual-beam OCTA experiments

Laser source	100kHz VCSEL	200 kHz VCSEL	
Beam configuration	Dual beam	Dual beam	Single beam
Lines per B-scan	500	1000	1000
Slow scan steps	1000	1000	1000
Repeated B-scan	4	4	4
Image area per beam	$11 \times 11 \text{ mm}^2$	$12 \times 12 \text{ mm}^2$	$12 \times 12 \text{ mm}^2$
Image area combined	$11 \times 22 \text{ mm}^2$	Two separate $12 \times 12 \text{ mm}^2$	$12 \times 12 \text{ mm}^2$