Supercontinuum source-based multi-contrast optical coherence tomography for rat retina imaging

JIA-PU SYU,1 WARADEE BUDDHAKOSAI,2 SHIH-JEN CHEN,3,4 CHANG-CHIH KE,5 SHIH-HWA CHIOU,2,3,5,6,7 AND WEN-CHUAN KUO1,6,8

1Institute of Biophotonics, National Yang-Ming University, Taipei 112, Taiwan
2Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan
3Department of Ophthalmology, Taipei Veterans General Hospital, Taiwan
4School of Medicine, National Yang-Ming University, Taiwan
Department and Institute of Pharmacology, National Yang-Ming University, Taipei 112, Taiwan
6Center For Intelligent Drug Systems and Smart Bio-devices (IDS²B), National Chiao Tung University, Hsinchu, Taiwan
7shchiou@vghtpe.gov.tw
8wckuo@ym.edu.tw

Abstract: This study proposed an ultrahigh-resolution multi-contrast optical coherence tomography system integrated with fundus photography for in vivo retinal imaging of rodents. A supercontinuum light source was used in the system, providing an axial resolution of less than 3 µm within 1.8 mm (in the tissue). Three types of tissue contrast based on backscattered intensity, phase retardation, and microvasculature at a capillary level can be simultaneously obtained using the proposed system. Pigmented Long-Evans, non-pigmented (albino) Sprague Dawley, and Royal College of Surgeons rats were imaged and compared. In vivo imaging results were validated with histology.

© 2018 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

1. Introduction

Several retinal diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD), were defined by the degeneration of retina [1,2], and these diseases are not completely understood. Animal eye models [3,4], particularly for mice and rats, are widely used in the preclinical and basic research of ophthalmology because of their short life cycles, structures similar to those of human eye, and various available disease models. Because of noninvasive, in vivo, and high-resolution tomographic imaging capabilities, optical coherence tomography (OCT) has become successful in clinical ophthalmology [5]. However, high axial resolution is required for rodent imaging to distinguish all retinal layers. Therefore, OCT for small animals is still relatively less prevalent in preclinical research.

The new development of broad bandwidth light sources generated ultrahigh-resolution (UHR) OCT [6–9], which could be favorable for assessing pathological changes in animal eyes. Advances in commercially available supercontinuum light sources [6,7] provide several features, including substantial optical bandwidth that allows UHR imaging, excellent spatial coherence, and high illumination power density, thereby providing higher sensitivity than conventional sources can provide. All of these attributes are essential parameters for UHR OCT for small animals.

Because of label-free and depth-resolved features, OCT angiography, which is a functional extension of OCT, has been widely applied in clinical research. Retinal degeneration is associated with retinal blood circulation because of the high metabolic demand of the neuroretina. Therefore, several clinical researches have investigated retinal diseases, such as RP, AMD, and diabetic retinopathy (DM) [10–12], by using OCT angiography. A multitude of functional-extension applications using a supercontinuum source
in small animals has increased rapidly (e.g., visualizing microvasculature in mouse ears and monitoring acute stroke in a mouse model using OCT angiography [13,14] and measuring retinal oxygen metabolic rate noninvasively in rat eyes by spectroscopic OCT) [15,16].

Polarization-sensitive OCT (PS-OCT) is a functional extension of OCT, which uses polarization interferometry with broadband light sources to obtain structural and birefringence tomograms or other polarization properties of biological tissues. The phenomenon of birefringence is observed in media containing ordered arrays of anisotropic structures, such as in the retinal nerve fiber layer (RNFL) and sclera or depolarizing phenomenon caused by retinal pigment epithelium (RPE) and choroid. PS-OCT has been used to assess peripapillary sclera and RNFL in vivo in nonhuman primates [17], healthy albino rats [18], and pigmented and nonpigmented rats [19] with axial resolutions of 12, 7.6, and 5.1 µm, respectively. Changes in polarization states may indicate the presence of tissues in early stages of a pathological process, such as damaged RNFL by glaucoma, RPE-related changes in AMD, and hard exudates in DM [18–21].

The expansion of conventional OCT to multifunctional OCT (i.e., including intensity OCT, OCT angiography, and PS-OCT) enables the simultaneous acquisition of the structure, flow, and phase retardation information of tissues, which provides multiparametric, complementary information on tissue microstructure, blood vessel morphology, and anisotropic structures [22–25]. For example, Wang et al [26] proposed multi-contrast OCT-based tractography for reconstructing micrometer-scale fiber pathways in the brain. Moreover, Jones matrix OCT was demonstrated for the Doppler and polarization-sensitive imaging of the posterior eye [27]. Furthermore, Augustin et al. [28] longitudinally observed spontaneous retinal-choroidal neovascularization in a mouse model with a threefold contrast.

This study aimed to develop a supercontinuum source-based multi-contrast OCT for the imaging of the retina degeneration in rats. Compared with previous threefold contrast setups, an all-fiber circular-state (CS) spectral domain (SD) PS-OCT with ultrahigh resolution was proposed. Bulk quarter wave plates (QWPs) were replaced by fiber optics polarization controllers (PCs) to realize a single-mode (SM) fiber optics PS-OCT. With the use of OCT angiography calculation, such a system enables the simultaneous imaging of three intrinsic contrasts: backscattered intensity, phase retardation (PR), and microvasculature at the capillary level. This study performed UHR multi-contrast OCT for Royal College of Surgeons (RCS−/−) rat retina imaging. The RCS rat is an acceptable photoreceptor degeneration model, which is used to determine the safety and efficacy of drugs or therapies used for treating several retinal diseases, and the genetic defect in RCS rats causes the inability of the RPE to phagocytose shed photoreceptor outer segments [29,30]. Moreover, pigmented Long-Evans (LE) and nonpigmented Sprague Dawley (SD) rats were imaged for comparison with RCS rats. The aforementioned in vivo imaging results were validated through histology and fundus photography.

2. Materials and methods

2.1 Setup of multi-contrast OCT

Figure 1 presents the schematic of the proposed multi-contrast OCT. A supercontinuum laser (NKT Photonics, Denmark) was used as a light source. The emission spectrum of the light was shaped by optical filtering to achieve a central wavelength and spectral bandwidth of 860 nm and 200 nm, respectively, with a theoretical axial resolution of 1.8 µm (in tissue). The light source was linearly polarized using a fiber optics polarizer. A fiber coupler with a coupling ratio of 90/10 placed beside the polarizer was arranged to split the light power into the two fiber arms of the interferometer, such that 90% was for the reference arm and 10% for the sensing arm.

The light reflected from the measured sample and the mirror of the reference arm to PC-1 and PC-2, respectively, was then propagated through the same SM fiber back to an SM
coupler. A fiber-type polarization beam splitter (PBS) then divided the reflected beams onto horizontal and vertical parts. After transmission gratings, two dispersed spectra were formed and recorded with two 4096-element line scan cameras (Wasatch Photonics). Line scan sensors were operated in a line period (77 μs), and A-scan rates of 13 kHz were achieved. Two-dimensional (2-D) OCT imaging was then performed by scanning the sample with a galvo-scanning mirror with an incident power of approximately 0.75 mW. Moreover, fundus photography was integrated into this system by inserting a dichroic mirror in the sample arm.

Before using the proposed multi-contrast OCT to capture the birefringence data, a calibration procedure was required for adjusting the polarization states of the PC to the same conditions in conventional bulk optics PS-OCT. The procedure was as follows: First, terminate the reflected light in the reference arm, and adjust PC-1 to convert the light propagating in the horizontal (H)-axis into the vertical (V)-axis after passing through a QWP with 45° to the horizontal axis and a PBS, at which PC-1 can be assumed as a fiber optics QWP with an incident azimuth angle of 45° to generate circularly polarized light. Second, replace the QWP and PBS with a mirror, and introduce a PC-3 to compensate for the polarization change when light was reflected from the mirror back to spectrometers. Adjust PC-3 to convert the light into the V-axis after reflecting from the mirror. The extinction ratio of the spectrometer\textsuperscript{V} divided by spectrometer\textsuperscript{H} must be set higher than 36 dB. Finally, terminate the reflected light in the sensing arm, and adjust PC-2 to balance the power of the reflective light until it arrives equally at the two spectrometers. This process can produce two polarized, linearly orthogonal modes with equal power, at which PC-2 can be assumed to act as a QWP oriented at 22.5° to the H-axis.

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{schematic.png}
\caption{Schematic of multi-contrast OCT system. CF: color filter, BS: beam splitter, PC: polarization controller, DM: dichroic mirror, and glass: a glass for dispersion compensation. Contact lens: a Plano-Concave lens (Ocular Instruments).}
\end{figure}

### 2.2 Image processing

After data acquisition, post-processing steps (Fig. 2) were performed based on our previously published work [31]. The A-scan signal obtained through inverse Fourier transform of the acquired fringe data can be expressed as follows:

\begin{align}
FT^{-1}\{S^v(k)\} &\rightarrow S^v(z) = \sqrt{R^v(z)} \sin(\delta(z))\exp(i\varnothing_v) \quad (1) \\
FT^{-1}\{S^h(k)\} &\rightarrow S^h(z) = \sqrt{R^h(z)} \cos(\delta(z))\exp(i\varnothing_h) \quad (2)
\end{align}
where \( V \) and \( H \) denote the vertical and horizontal polarization channels, respectively. \( R_s(z) \) is the sample reflectivity, the \( \sin(\delta(z)) \) and \( \cos(\delta(z)) \) terms are retardation moduli modulated by the birefringence of the measured samples. The amplitude \( \sqrt{R_s(z)}(\sin \delta(z)) \), \( \sqrt{R_s(z)}(\cos \delta(z)) \) of interference signals was used to calculate the structure \( (R_s) \) and phase retardation \( (\delta) \) images as prescribed for traditional bulk optics-type CS PS-OCT [32]. Vascular images were then acquired through complex signal calculations in an optical microangiography (OMAG) algorithm [33]. The flow signal can be written as follows:

\[
Flow(x,z) = \frac{1}{N-1} \sum_{i=0}^{N-1} |S_{i,v}^{v}(x,z) - S_{i,v}^{v}(x,z)| + \frac{1}{N-1} \sum_{i=0}^{N-1} |S_{i,h}^{h}(x,z) - S_{i,h}^{h}(x,z)|
\]

(3)

Here, \( S_{i,v}^{v}(x,z) \) is the complex value OCT data of the \( i \)-th frame at a lateral position of \( x \) and depth \( z \) in the vertical and horizontal channels, respectively. \( N = 5 \) is the number of frames to be calculated to one flow frame. Vascular contrast was achieved because flow regions and the bulk tissue provided several different values. To reduce the artifacts generated by laser jitter and eye motion, bulk motion compensation and phase compensation methods [34] were used in this study. After the compensation method, the OMAG algorithm could separate the flow tissue from the static tissue.

Figure 2 presents an image processing flowchart. Interference signals were acquired using two spectrometers. After resampling, dispersion compensation, and Fourier transform, three contrast images were simultaneously obtained. The structure image was obtained using the following formula: \( R_s(x,z) = (S_{i,v}^{v})^2 + (S_{i,h}^{h})^2 \), one structure image was averaged from five frames. Angiography performed using \( S_v^{v} \) and \( S_h^{h} \), which were then combined together. A retardation image was calculated using \( \tan^{-1}(S_{i,v}^{v}/S_{i,h}^{h}) \). To determine the microstructure of the retina, three-dimensional (3D) reconstruction performed using 2000 two-dimensional (2D) OCT images recorded to a 3D data set having a volume of \( 1 \times 1 \times 1 \) mm\(^3\) corresponding to \( 400 \times 400 \times 2048 \) pixels in the \( X \), \( Y \), and \( Z \) directions of the retina. Manual retinal layer segmentation was then performed for the visualization of vascular structure through separate projections from different layers of the retina.

Fig. 2. Image processing flowchart.

2.3 Animal model and histological analysis

In this study, the three strains of rats with different eye conditions were used, namely one 8–10-week old pigmented-eye LE rat, two 8–10-week old nonpigmented-eye (albino) SD rat, and two 12-month old hereditary retinal dystrophy rat RCS. All are male rats.

The RCS rat is a natural animal model of autosomal recessive RP. This rat strain harbors homozygous null mutation in gene encoding receptor tyrosine kinase (MERTK), which functions in the phagocytosis process. In the eye, MERTK is expressed only in the RPE.
Therefore, the RPE of the RCS rat cannot eliminate the shed photoreceptor outer segment, which is accumulated as debris and induces cell loss and retinal degeneration. At approximately 18 days postnatal, the RCS rat rapidly develops photoreceptor cell loss and progressive degenerated retinas [35,36].

In this study, all animals were fed ad libitum and were kept in ventilation cages (2 rats per cage) with 12/12 h light/dark cycle, optimal temperature, and humidity-controlled conditions. All animals were maintained under anesthesia with an intraperitoneal injection of ketamine (100 mg/kg) + dexmeditomidine hydrochloride (dexamitor) (1 mg/kg) throughout the experiment. The study was approved by the Institution of Animal Care and Use Committee at National Yang-Ming University. At the end of observation, the rats were sacrificed and enucleated. The eyes were collected and fixed in 4% paraformaldehyde for 4 h at 4°C. The fixed samples were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin for a histopathological analysis. The histological change was assessed by an experienced pathologist.

3. Results

Figure 3 depicts the depth-dependent decay (logarithmic scale) for the mirror at different depths, where the measured signal to noise ratio (SNR) in dB was calculated to be 20 times the base 10 logarithm of the ratio of the A-scan peak height to the mean noise floor. An SNR of more than 40 dB was obtained for all the peaks less than 1.8 mm. The optimal axial resolution measured was 3.1 μm at a depth of 0.2 mm in air, which corresponds to 2.3 μm in the tissue (n = 1.33).

For the performance verification of the proposed UHR multi-contrast OCT system for retardation measurement, a Berek’s polarization compensator (BPC) placed in front of a mirror was used as a standard sample. For a short-term stability test, the BPC was set at 42° then measurements were continuously obtained for 5 min. Figure 4(a) indicates that the peak-to-peak variation value was only approximately 0.5° within 5 min, which is considerably longer than the time required for acquiring a 3D retinal data set. In this study, the acquisition time for each 3D set was 77 s. For a long-term stability test, measurements were obtained for 1 min at intervals of 20 min for 2 h. Figure 4(b) illustrates a slight increase of approximately 2° within 2 h, indicating acceptable system stability. For the accuracy test, the BPC was set at various tilt angles for a retardation set step, increasing from 0 to 180° at an interval of 15°. The measured retardation (Fig. 4(c)) indicated satisfactory linearity with a deviation of 7.9°.
which was the largest in the diagram. To validate that the retardation is independent of fast-axis orientation, retardation values were set at 0°, 15°, 30°, 45°, 60°, 75°, and 90° at different axis orientations, namely 0°, 22.5°, 45°, 67.5°, and 90°. The experimental results (Fig. 4(d)) indicate that retardation measurements were independent of fast-axis orientations, thereby validating that PC-1 and PC-2 exhibited equal effects from the bulk optics QWPs. A large deviation of retardation was observed at 90° of set retardation. This deviation may be attributed to the wavelength dependence of birefringence in the SM fiber and BPC [37].

Figures 5(a) and 5(b) present OCT images of an SD rat and an RCS rat, respectively. The stratification of a healthy retinal layer (Fig. 5(a)), including the nerve fiber layer/ganglion cell layer (NFL/GCL), inner plexiform (IP), inner nuclear (IN), outer plexiform (OP), outer nuclear (ON), external limiting membrane (ELM), photoreceptor inner segment/outer segment (IS/OS), RPE, and choroid. For retinal thickness measurement, a region of interest (ROI) was selected close to the central areas with flattened profiles in Figs. 5(a) and 5(b), including approximately 10 A-scans. After laterally averaging Rs(z) within the ROI, a reflectivity signal was obtained as a function of depth as illustrated in Figs. 5(c) and 5(d) for SD and RCS rats, respectively. Therefore, retinal thickness was defined as the thickness from the NFL/GCL layer to the RPE, which was calculated using manually selected peak position; the pixel numbers between each layer were counted, where one pixel corresponded to 1.39 μm distance. Finally, the average thickness value in ten ROIs (i.e., from ten OCT images positioned 2 mm away from an optic disc) was 187.4 in the SD rat. The retinal thickness calculated using OCT images was close to that in histology (Fig. 5(e), with an average thickness of near 177.6 μm, measured with manual selection by Matlab software). Figure 5(b) illustrates retinal degeneration in a 55-week-old RCS rat. A significant reduction in retinal thickness was observed (i.e., only 98.0 μm from the surface to RPE, p < 0.05), which was almost half of that in Fig. 5(a). The OP, ON, and photoreceptor layer (IS, OS) were absent. The same result was observed in histology (Fig. 5(f), 84.2 μm), but a smaller thickness in histology occurred due to the shrinkage during specimen preprocessing.
Fig. 5. OCT images of a SD rat (a) and an RCS rat (b). A lateral average from ten A-scans of the reflectivity profile along the depth defining the different retinal layers in SD (c), and RCS (d) rats. (e) & (f): histology of SD and RCS rat. 3D reconstruction video of (a) and (b) are illustrated in supplemental information. NFL/GCL: nerve fiber layer/ganglion cell layer, IP: inner plexiform, IN: inner nuclear, OP: outer plexiform, ON: outer nuclear, ELM: external limiting membrane, IS/OS: photoreceptor inner segment/outer segment, RPE: retinal pigment epithelium (Visualization 1 and Visualization 2).

Comparison of the fundus photography of an SD rat (Fig. 6(a)) with that of an RCS rat (Fig. 6(f)) indicates the vascular pattern in the RCS rat is more tortuous than that in the SD rat; however, other features were similar. Thus, a 3D volume of OCT angiography was projected onto the 2D forward (en face) view by maximum intensity projection (MIP) to visualize the details of the vascular change. The OCT scanning region was indicated using a black rectangle in fundus images (Fig. 6(a) and 6(f)). Before performing MIP, manual segmentation was used for avoiding the surface noise and eliminating upper vasculature...
covering the lower small blood capillaries. The rodent retina can be separated into three layers, namely retinal arteries and veins within the NFL and GCL, the intermediate capillary networks within the IP layer that supply nutrients to the inner retina, and the deep capillary networks within the OP layer that supply nutrients to the outer retina. Figures 6(b)-6(d) and 6(g)-6(i) demonstrate the retinal vascularity within these three layers of the retina in the SD and RCS rats, respectively. The vessels within NFL and GCL in the RCS rat (Fig. 6(g)) were discontinuous because of weak back-reflected intensity. Although the IP layer and OP layer of the RCS rat exist, we were not able to find any vessel distribution within IP layer and OP layer in OCT angiography (Figs. 6(h) and 6(i)). Figures 6(e) and 6(j) demonstrate the depth-color coded microvascular en-face projection of the healthy SD rat and RCS rat, respectively.

![Fig. 6. Fundus photography (a, f) and three layers of OCT angiography of a healthy SD rat (b: NFL-GCL; c: IP layer; d: OP layer) and RCS rat (g: NFL-GCL; h: IP layer; i: OP layer) respectively. (e & j) depth-color coded microvascular en-face projection of the healthy SD rat and RCS rat (blue: NFL-GCL layer, green: IP layer, red: OP layer).](image)

Figures 7(a) and 7(b) are OCT structure images of an SD rat and an RCS rat, respectively. Figures 7(c) and 7(d) present the extension of the conventional structure OCT to functional OCT by polarization contrast enhancement, which is beneficial for providing additional intrinsic contrast (birefringence properties). In general, the pseudo color represented the retardation angle should start from the sclera, which is the same as that in a healthy SD rat (Fig. 7(c)), whereas the boundary of sclera was blurry in the RCS rat (Fig. 7(d)). After validation through histology (Fig. 7(g) and 7(h)), we speculated that it was due to the degeneration of choroid and sclera, and the dysregulation of elasticity or connective tissue formed in the RCS rat due to old age. To quantitatively evaluate the retardation change, a distance from RPE to the retardation value reaches 45° was measured. Figures 7(e) and 7(f) demonstrate a representative retardation signal as a function of depth in the SD and RCS rats, respectively.
Figure 8 summarized the finding of UHR multi-contrast OCT for small animal retina imaging, which retrieves additional information beyond that of the conventional OCT reflectivity-only map. The representative structure, retardation, and angiography map in an SD rat are depicted in Figs. 8(b), 8(e), and 8(h), respectively. The results for an RCS rat are illustrated in Figs. 8(c), 8(f), and 8(i). The results for an LE rat are presented in Figs. 8(d), 8(g), and 8(j). Compared with the healthy albino rat (SD) (Fig. 8(b)) and abnormal RCS rat (Fig. 8(c)), light penetration depth into the sclera in a healthy LE rat (Fig. 8(d)) was considerably shallow because of the melanin content in the retina of a normal LE rat. Moreover, because of the light depolarization property of melanin, the retardation angle after the RPE layer diminished rapidly in the LE rat (Fig. 8(d)) compared with that in the SD rat (Fig. 8(e)) or RCS rat (Fig. 8(f)). The mean distances from RPE to the position of $\delta = 45^\circ$ were 38.5 $\mu$m (LE rat), 62.9 $\mu$m (SD rat), and 78.3 $\mu$m (RCS rat), respectively. However,
measurements from NFL/GCL to the RPE layer demonstrate the thickness were similar in the SD (198.4 μm in Fig. 8(b)) and LE rats (204.8 μm in Fig. 8(d)). Furthermore, depth-color-coded angiography demonstrates similar and normal microvascular distribution in both healthy the SD (Fig. 8(h)) and healthy LE rats (Fig. 8(j)).

Relative to the healthy SD or LE rats, the photoreceptors of the RCS rat were degenerated and caused the significant collapse of the retinal layer. Figure 7(c) demonstrates that the retina in the RCS rat degenerates with apparently reduced stratification and thinner thickness the aforementioned RPE layer (90.5 μm). Retardation imaging in Fig. 8(f) indicates degeneration in choroid and sclera. The standard deviations of the distance from RPE to the first δ = 45° within different lateral positions were 3.7μm (SD), 5.3 μm (LE), and 17.9 μm (RCS), respectively. Depth-color-coded angiography (Fig. 8(i)) confirmed the blood vessels in the retina were degraded, which hindered the visibility of intermediate capillary networks within the IP layer, and deep capillary networks within the OP layer. The vessel area density in Fig. 8(i) was only 20.7%, which was less than that in the SD (36.9%) or LE (38.3%) rats. This indicates a problem of nutrient supply and metabolism in the retinal layer of the late-stage RCS rat.

![Multi-contrast OCT images](image)

Fig. 8. Multi-contrast OCT images (a) schematic plot, (b-d): structural image, (e-g) retardation image, (h-j) depth-color coded microvascular enface projection (blue: NFL-GCL layer; green: IPL layer, red: OPL layer). The SD rat (b, c, and h); RCS rat (c, f, and i); and LE rat (d, g, and j).
4. Discussion and conclusion

The expansion of conventional OCT to multi-contrast OCT enables the multiparametric analysis of pathological processes, such as in wet AMD, choroidal neovascularization can lead to macular edema and retinal hemorrhage, and the development of fibrosis is very common and associated with neovascularization [38]. In DM, retinal and choroidal vascular circulations both are altered, such abnormal retinal capillaries leak extracellular lipids; this phenomenon then leads to leakage of hard exudates that depolarize the light. In this study, an in vivo RCS−/− rat with the rapid loss of photoreceptor cells applied is used as a model for RP. Compared with the previous threefold contrast setups, this study proposed to use a supercontinuum source-based CS SD PS-OCT with a resolution of less than 3 µm in the tissue. All SM fiber optic setup ensures minimally stringent requirements on the lens alignment in this scheme, and therefore easily perform a compact and portable system. Moreover, three intrinsic contrasts—backscattered intensity, phase retardation, and microvasculature at the capillary level—can be simultaneously obtained. High resolution for rodent imaging enables the system to distinguish each layer of the retina clearly and to delineate the IS/OS of the photoreceptor layer (such as in Fig. 5(a)), whose degeneration is primarily responsible for reduction in total retinal thickness. The retardation contrast can provide the information of the changes in choroid and sclera, and depolarization properties from melanin. In the RCS rat, uneven retardation change indicates degeneration in choroid and sclera. Fundus photography can rapidly perform 2D en-face inspections, presenting shallow vascularity and vessel branches: however, the underlying microvessels are not visible. Blood vessels in various depth layers of the retina in the SD and LE rats can be observed by depth-color-coded OCT angiography where vascular contrast was achieved from the flow signal in vascular regions. In the RCS rat, the vessel within NFL and GCL was still observed; however, no vascularity was found within IP layer and OP layer. The previous report using fluorescein angiography has demonstrated the patches of hypofluorescence in the RCS rat retina. The severe capillary of the nonperfusion area was identified in the aged RCS rat (older than 6 months) and correlated to devoid capillaries observed through NADPH-d histology [39]. Capillary nonperfusion is caused by insufficient tissue perfusion, which the crucial blood delivery process in the retina. Several attempts have been made to illustrate the role of photoreceptor cells in the retinal vascular degeneration, particularly in diabetic retinopathy and genetic-mediated photoreceptor degeneration [40–44]. A mechanism by which photoreceptor degeneration could mediate retinal vasculature degeneration was explained in animals with opsin-deficiency-induced photoreceptor degeneration [40,42]. These animals have significantly reduced vascular density. Particular opsin and rhodopsin expressions are specific to photoreceptor cells. Therefore, opsin deficiency from the photoreceptor loss may be the probable cause of retinal vascular degeneration in the RCS rats. In conclusion, UHR multi-contrast OCT system enables the simultaneous acquisition of the microstructure, blood vessel morphology, and anisotropic properties of tissues, which may provide multi-parametric, complementary information in a lesion.

Funding
Taiwan Ministry of Science and Technology, R.O.C Grant (MOST) (105-2112-M-010-002-MY3), and the “Center for Intelligent Drug Systems and Smart Bio-devices (IDS’B)” from the Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

Disclosures
The authors declare that no conflicts of interest related to this article.
References

1. S. P. Daiger, S. J. Bowne, and L. S. Sullivan, “Perspective on genes and mutations causing retinitis pigmentosa,” Arch. Ophthalmol. 125(2), 151–158 (2007).

2. S. Nusinowitz, Y. Wang, P. Kim, S. Habib, R. Baron, Y. Conley, and M. Gorin, “Retinal Structure in Pre-Clinical Age-Related Macular Degeneration,” Curr. Eye Res. 43(3), 376–382 (2018).

3. E. Vecino, “[Animal models in the study of the glaucoma: past, present and future],” Arch. Soc. Esp. Oftalmol. 83(9), 517–519 (2008).

4. E. L. Fletcher, A. I. Jobling, U. Greferath, S. A. Mills, M. Waugh, T. Ho, R. U. de Jongh, J. A. Phipps, and K. A. Vessey, “Studying age-related macular degeneration using animal models,” Optom. Vis. Sci. 91(8), 878–886 (2014).

5. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and et, “Optical coherence tomography,” Science 254(5035), 1178–1181 (1991).

6. W. Yuan, J. Mavadia-Shukla, J. Xi, W. Liang, X. Yu, S. Yu, and X. Li, “Optimal operational conditions for supercontinuum-based ultrahigh-resolution endoscopic OCT imaging,” Opt. Lett. 41(2), 250–253 (2016).

7. N. Nishizawa, H. Kawagoe, M. Yamanaika, M. Matsushita, K. Mori, and T. Kawabe, “Wavelength Dependence of Ultrahigh-Resolution Optical Coherence Tomography Using Supercontinuum for Biomedical Imaging,” IEEE J. Sel. Top. Quantum Electron. 25(1), 1–15 (2019).

8. J. Barrick, A. Doblas, M. Gardner, P. Sears, L. Ostrowski, and A. L. Oldenburg, “Supercontinuum Parallel Line-field Optical Coherence Tomography for High Sensitivity, Kilohertz Frame Rate Imaging,” in Optics in the Life Sciences Congress (Optical Society of America, San Diego, California, 2017), p. BoM4A.1.

9. U. Schmidt-Erfurth, R. A. Leitgeb, S. Michels, B. Povazay, S. Sacu, B. Hermann, C. Ahlers, H. Sattmann, C. Scholka, A. F. Fischer, and W. Drexler, “Three-dimensional ultrahigh-resolution optical coherence tomography of macular diseases,” Invest. Ophthalmol. Vis. Sci. 46(9), 3393–3402 (2005).

10. W. Choi, N. K. Waheed, E. M. Moul, M. Adhi, B. Lee, T. De Carlo, V. Jayaraman, C. R. Baumal, J. S. Duker, and J. G. Fujimoto, “Ultrahigh Speed Swept Source Optical Coherence Tomography Angiography of Retinal and Choriopticillaris Alterations in Diabetic Patients with and without Retinopathy,” Retina 37(1), 11–21 (2017).

11. M. Battaglia Parodi, M. V. Cicinelli, A. Rabiolo, L. Pierro, M. Gagliardi, G. Bolognesi, and F. Bandello, “Vessel density analysis in patients with retinitis pigmentosa by means of optical coherence tomography angiography,” Br. J. Ophthalmol. 101(4), 428–432 (2017).

12. M. Pilioto, L. Frizziiero, A. R. Daniele, E. Convento, E. Longhin, F. Guidolin, R. Parazzoni, F. Cavarzeran, and E. Miden, “Early OCT angiography changes of type 1 CNV in exudative AMD treated with anti-VEGF,” Br. J. Ophthalmol. 2018, 311752 (2018).

13. J. Yi, S. Chen, V. Backman, and H. F. Zhang, “In vivo functional microangiography by visible-light optical coherence tomography,” Biomed. Opt. Express 5(10), 3603–3612 (2014).

14. Q. Liu, S. Chen, B. Soetikno, W. Liu, S. Tong, and H. Zhang, “Monitoring acute stroke in mouse model using laser speckle imaging-guided visible-light optical coherence tomography,” IEEE Trans. Biomed. Eng. 65, 2136–2149 (2018).

15. S. P. Chong, C. W. Merkle, C. Leahy, R. Radhakrishnan, and V. J. Sriniavsan, “Quantitative microvascular hemoglobin mapping using visible light spectroscopic Optical Coherence Tomography,” Biomed. Opt. Express 6(4), 1429–1450 (2015).

16. J. Yi, W. Liu, S. Chen, V. Backman, N. Sheibani, C. M. Sorenson, A. A. Fawzi, R. A. Linsenmeier, and H. F. Zhang, “Visible light optical coherence tomography measures retinal oxygen metabolic response to systemic oxygenation,” Light Sci. Appl. 4(9), e334 (2015).

17. J. Dwelle, S. Liu, B. Wang, A. McElroy, D. Ho, M. K. Markey, T. Milner, and H. G. Rylander 3rd, “Thickness, phase retardation, birefringence, and reflectance of the retinal nerve fiber layer in normal and glaucomatous non-human primates,” Invest. Ophthalmol. Vis. Sci. 53(8), 4380–4395 (2012).

18. M. Pircher, C. K. Hitzenberger, and U. Schmidt-Erfurth, “Polarization sensitive optical coherence tomography in the human eye,” Prog. Retin. Eye Res. 30(6), 431–451 (2011).

19. C. Schütze, M. Wedel, B. Baumann, M. Pircher, C. K. Hitzenberger, and U. Schmidt-Erfurth, “Progression of retinal pigment epithelial atrophy in antiangiogenic therapy of neovascular age-related macular degeneration,” Am. J. Ophthalmol. 159(6), 1100–1114 (2015).

20. S. Fialová, M. Augustin, M. Glösmann, T. Himmel, S. Rauscher, M. Gröger, M. Pircher, C. K. Hitzenberger, and B. Baumann, “Polarization properties of single layers in the posterior eyes of mice and rats investigated using high resolution polarization sensitive optical coherence tomography,” Biomed. Opt. Express 7(4), 1479–1495 (2016).

21. J. Lammer, M. Bolz, B. Baumann, M. Pircher, B. Gerendas, F. Schlani, C. K. Hitzenberger, and U. Schmidt-Erfurth, “Detection and analysis of hard exudates by polarization-sensitive optical coherence tomography in patients with diabetic maculopathy,” Invest. Ophthalmol. Vis. Sci. 55(3), 1564–1571 (2014).

22. B. Park, M. C. Pierce, B. Cense, S. H. Yun, M. Mujat, G. Tearney, B. Bouma, and J. de Boer, “Real-time fiber-based multi-functional spectral-domain optical coherence tomography at 1.3 microm,” Opt. Express 13(11), 3931–3944 (2005).

23. B. Park, M. Pierce, B. Cense, and J. de Boer, “Real-time multi-functional optical coherence tomography,” Opt. Express 11(7), 782–793 (2003).
24. H. Ren, Z. Ding, Y. Zhao, J. Miao, J. S. Nelson, and Z. Chen, “Phase-resolved functional optical coherence tomography: simultaneous imaging of in situ tissue structure, blood flow velocity, standard deviation, birefringence, and Stokes vectors in human skin,” Opt. Lett. 27(19), 1702–1704 (2002).

25. M. C. Pierce, B. Hyle Park, B. Cense, and J. F. de Boer, “Simultaneous intensity, birefringence, and flow measurements with high-speed fiber-based optical coherence tomography,” Opt. Lett. 27(17), 1534–1536 (2002).

26. H. Wang, A. J. Black, J. Zhu, T. W. Stigen, M. K. Al-Qaisi, T. I. Netoff, A. Abosch, and T. Akkin, “Reconstructing micrometer-scale fiber pathways in the brain: multi-contrast optical coherence tomography based tractography,” Neuroimage 58(4), 984–992 (2011).

27. M. J. Ji, Y. J. Hong, S. Makita, Y. Lin, K. Kurokawa, L. Duan, M. Miura, S. Tang, and Y. Yasuno, “Advanced multi-contrast Jones matrix optical coherence tomography for Doppler and polarization sensitive imaging,” Opt. Express 21(16), 19412–19436 (2013).

28. M. Augustin, S. Fialová, T. Himmel, M. Glösmann, T. Lenghe imer, D. J. Harper, R. Plasenzotti, M. Pircher, C. K. Hitzenberger, and B. Baumann, “Multi-Functional OCT Enables Longitudinal Study of Retinal Changes in a VLDLR Knockout Mouse Model,” PLoS One 11(10), e0164419 (2016).

29. S. Rösch, C. Aretzweiler, F. Müller, and P. Walter, “Evaluation of Retinal Function and Morphology of the Pink-Eyed Royal College of Surgeons (RCS) Rat: A Comparative Study of in Vivo and in Vitro Methods,” Curr. Eye Res. 42(2), 273–281 (2017).

30. R. C. Ryals, M. D. Andrews, S. Datta, A. S. Coyner, C. M. Fischer, Y. Wen, M. E. Pennesi, and T. J. McGill, “Long-term Characterization of Retinal Degeneration in Royal College of Surgeons Rats Using Spectral-Domain Optical Coherence Tomography,” Invest. Ophthalmol. Vis. Sci. 58(3), 1378–1386 (2017).

31. W. C. Kuo, C. M. Lai, Y. S. Huang, C. Y. Chang, and Y. M. Kuo, “Balanced detection for spectral domain optical coherence tomography,” Opt. Express 21(16), 19280–19291 (2013).

32. W. C. Kuo, N. K. Chou, C. Chou, C. M. Lai, H. J. Huang, S. S. Wang, and J. J. Shyu, “Polarization-sensitive optical coherence tomography for imaging human atherosclerosis,” Appl. Opt. 46(13), 2520–2527 (2007).

33. C. L. Chen and R. K. Wang, “Optical coherence tomography based angiography [Invited],” Biomed. Opt. Express 8(2), 1056–1082 (2017).

34. J. Lee, V. Srinivasan, H. Radhakrishnan, and D. A. Boas, “Motion correction for phase-resolved dynamic optical coherence tomography imaging of rodent cerebral cortex,” Opt. Express 19(22), 21258–21270 (2011).

35. E. Nandrot, E. M. Dufour, A. C. Provost, M. O. Péquignot, S. Bonnel, K. Gogat, D. Marchant, C. Rouillac, B. Sépulcre de Condé, M. T. Biloreau, C. Shaver, J. L. Dufier, C. Marsac, M. Lathrop, Menasche, and M. M. Abitbol, “Homozygous deletion in the coding sequence of the c-mer gene in RCS rats unravels general mechanisms of physiological cell adhesion and apoptosis,” Neurobiol. Dis. 7(6 Pt B), 586–599 (2000).

36. J. E. Dowling and R. L. Sidman, “Inherited retinal dystrophy in the rat,” J. Cell Biol. 14(1), 73–109 (1962).

37. W. Eickhoff, Y. Yen, and R. Ulrich, “Wavelength dependence of birefringence in single-mode fiber,” Opt. Lett. 20(19), 3428–3435 (1981).

38. M. Friedlander, “Fibrosis and diseases of the eye,” J. Clin. Invest. 117(3), 576–586 (2007).

39. H. J. Zambarakji, D. J. Keegan, T. M. Holmes, A. S. Halfyard, M. P. Villegas-Perez, D. G. Charteris, F. W. Fitzke, and D. O. Lund, “High resolution imaging of fluorescein patterns in RCS rat retinae and their direct correlation with histology,” Exp. Eye Res. 82(1), 164–171 (2006).

40. H. Liu, J. Tang, Y. Du, A. Saadane, D. Tonade, I. Samuels, A. Veenstra, K. Palczewski, and T. S. Kern, “Photoreceptor Cells Influence Retinal Vascular Degeneration in Mouse Models of Retinal Degeneration and Diabetes,” Invest. Ophthalmol. Vis. Sci. 57(10), 4272–4281 (2016).

41. Y. Feng, Y. Wang, Z. Yang, L. Wu, S. Hoffmann, T. Wieland, N. Gretz, and H. P. Hammes, “Chronic hyperglycemia inhibits vasoregression in a transgenic model of retinal degeneration,” Acta Diabetol. 51(2), 211–218 (2014).

42. T. E. de Gooyer, K. A. Stevenson, P. Humphries, D. A. Simpson, T. A. Gardiner, and A. W. Stitt, “Retinopathy is reduced during experimental diabetes in a mouse model of outer retinal degeneration,” Invest. Ophthalmol. Vis. Sci. 47(12), 5561–5568 (2006).

43. J. S. Penn, S. Li, and M. I. Naash, “Ambient hypoxia reverses retinal vascular attenuation in a transgenic mouse model of autosomal dominant retinitis pigmentosa,” Invest. Ophthalmol. Vis. Sci. 41(12), 4007–4013 (2000).

44. A. H. Milam, Z. Y. Li, and N. R. Fariss, “Histopathology of the human retina in retinitis pigmentosa,” Prog. Retin. Eye Res. 17(2), 175–205 (1998).