Vascular parameters of normal cynomolagus macaques and healthy humans obtained by fundus fluorescence angiography and optical coherence tomography angiography

CURRENT STATUS: ACCEPTED

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DOI:
10.21203/rs.2.85/v2

SUBJECT AREAS
Internal Medicine Specialties

KEYWORDS
Cynomolgus macaques, Healthy humans, Vascular, Fundus fluorescence angiography (FFA), Optical coherence tomography angiography (OCT-A), Vessel density (VD)
Abstract

Background: To provide and compare normal vascular parameters for cynomolgus macaques and healthy humans, compare the advantages and disadvantages of fundus fluorescence angiography (FFA) and optical coherence tomography angiography (OCT-A) in angiography, and establish an eye parameter database for cynomolgus macaques and healthy humans. Methods: 5 normal cynomolgus macaques with a mean age of 4.60±0.55 years were studied for data collection. A Heidelberg Spectralis® HRA+OCT was used to obtain parameters for FFA. The macula of 28 healthy humans (14 males and 14 females), with a mean age of 25.11±6.21 years and the optic discs of 9 healthy humans (4 males and 5 females) with a mean age of 28.56±6.78 years were measured. The vessel density (VD) was measured using an RTVue XR with AngioVue. The scan sizes of the macula and optic discs were 3×3 mm and 4.5×4.5 mm, respectively. Results: FFA of cynomolgus macaques revealed stages similar to those observed in humans. OCT-A was used to image the superficial and deep capillary plexuses and radial peripapillary capillary network. The highest entire en-face mean VD in the macular area was 68.19±0.75% in the choroid capillary layer. In both layers of the optic disc, the VD in the nasal quadrant was lower than the VD in the inferior temporal quadrant. Similarities and significant differences in VD between healthy humans and cynomolgus macaques were obtained using OCT-A. Conclusions: This study provides normal vascular parameters for cynomolgus macaques using FFA and OCT-A to help establish an optical parameter database for cynomolgus macaques and compare VD between healthy humans and cynomolgus macaques to promote choroid-retinopathy research. Trial registration: Current Controlled Trials NCT03692169

Background
The retina plays a vital role in vision. The metabolic activity of the retina is higher than other human tissues\(^1,2\); therefore, the vascular circulation of the retina is complex. A variety of techniques have been used to measure retinal perfusion. Fundus fluorescence angiography (FFA) is routinely used to evaluate retinal vascular retinopathy\(^3\) as it can analyse the choroid-retinal vasculature and show vascular leakage and neovascularization\(^4\). However, this method has limitations, such as the intravenous injection of contrast agents that can lead to some side effects\(^5,6\). Additionally, further details of the deeper capillary plexus are unable to be extracted from FFA images due to limited depth perception. In contrast, optical coherence tomography angiography (OCT-A) is a novel imaging method\(^7-9\) that detects the flow of blood via intrinsic signals without requiring an intravenous agent. OCT-A visualizes and divides the retina into various layers, clearly showing the capillary vessels in each layer. The ability of OCT-A to quantify the blood flow and vascular density of the retinal choroidal circulation is essential to the study of ophthalmology worldwide.

Due to their close similarity to humans in terms of optical structure and function, nonhuman primates such as cynomolgus macaques play a crucial role in ophthalmic disease research\(^10-13\). The FFA and OCT-A vascular parameters for normal humans have been previously reported\(^1,14,15\), and researchers have reported flow perfusion parameters for normal monkeys\(^16\). However, very little literature is available on the vascular density parameters for the macula and optic disc of ocularly normal cynomolgus macaques, and the comparison of VD between healthy humans and cynomolgus macaques using OCT-A has not been conducted. Our study aimed to evaluate macular vascular circulation parameters in normal cynomolgus macaques using OCT-A and FFA and to compare the advantages and disadvantages of the two angiography techniques. Furthermore, for a better use of nonhuman primate models in studies of ophthalmic
diseases, we compared the vascular parameters between healthy humans and cynomolgus macaques.

Methods

Animals

Data were collected from five normal adult male cynomolgus macaques with a mean age of 4.60±0.55 years and a mean weight of 4.44±0.88 kg. The animal production licence number was SCXK(YUE)2014-0027 (GuangZhou Blooming-Spring Biological Technology Development Co., Ltd., Guangzhou, China). Before starting the experiments, all five animals were determined to be ocularly normal and healthy. After these experiments, the animals continued to receive good care and will be used in subsequent studies.

Preparation of animals

Animals were anaesthetized with an intramuscular injection (i.m.) of ZoletilTM 50 (VIRBAC S.A., France), which is a combination of tiletamine-zolazepam (4 mg/kg). Anaesthesia was maintained during examinations by intravenously injecting supplemental doses of ZoletilTM 50 (1/3 of the initial dose) as needed. Body temperature was maintained at 37°C using a water circulating heating pad. Pupils were fully dilated to approximately 9 mm in diameter with tropicamide phenylephrine eye drops (0.5%, Santen pharmaceutical Co.,Ltd, Japan). Sodium hyaluronate eye drops (0.3%, Santen pharmaceutical Co.,Ltd, Japan) were used to maintain corneal moisture. A restraining device was used to maintain stable positioning of the animal’s eyes and head. The eyelids were opened with a lid speculum.

Healthy humans
Healthy humans were recruited prospectively from the Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, Guangdong between December 2017 and December 2018. The macula of 28 healthy humans (14 males and 14 females) with a mean age of 25.11±6.21 years and the optic discs of 9 healthy humans (4 males and 5 females) with a mean age of 28.56±6.78 years were measured. The Ethics Committee of the Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, Guangdong approved the research protocol, which followed the recommendations of the Declaration of Helsinki. Written informed consent was obtained from each patient before any examinations were performed. Each participant’s medical history and records were carefully reviewed for retinal and cardiovascular diseases. Only participants satisfying the inclusion and exclusion criteria were included. Subsequently, participants underwent OCT-A examinations using an RTVue XR instrument with AngioVue (software version 2017.1.0.155; Optovue, Inc., Fremont, CA, USA)

Data acquisition

FFA

Five cynomolgus macaques were used in this experiment. FFA images were obtained using specialized equipment (SpectralisHRA + OCT; Heidelberg Engineering, Germany) in the FA mode. A 24 G intravenous indwelling needle was inserted into the ulnar vein of the monkey. The needle was attached to a 1 ml syringe containing fluorescein sodium (20%, 0.05 ml/kg). The intravenous dye was infused over 3 s. Photographic documentation of the posterior pole and peripheral retina was conducted over 18 min.

OCT-A
Both eyes of the animals and healthy humans were scanned using RTVue XR with AngioVue (software version 2017.1.0.155; Optovue, Inc., Fremont, CA, USA). The scan size was 3×3 mm for the macular disc and 4.5×4.5 mm for the optic disc.

Vessel density (VD) is defined as the percent area occupied by blood vessels. AngioVue automatically divides the scanned area of the macular disc into four sections according to vascularity as follows: the superficial layer (upper line: ILM with a 3 micron offset; lower line: IPL with a 16 micron offset), the deep retinal layer (upper line: IPL with a 16 micron offset; lower line: IPL with a 69 micron offset), the outer retinal layer (upper line: IPL with a 69 micron offset; lower line: REP with a 16 micron offset) and the choroid capillary layer (upper line: REP with a 31 micron offset; lower line: RPE with a 60 micron offset). The scanned area used to determine the VD in the optic disc was divided into the following sections: the nerve head (upper line: ILM with a 0 micron offset; lower line: ILM with a 150 micron offset) and the radial peripapillary capillary (RPC; upper line: ILM with a 0 micron offset; lower line: NFL with a 0 micron offset). A single trained researcher acquired all images.

AngioAnalytics flow density map software automatically evaluates the VD of the scanned area. In the macular area, the inner and outer rings with diameters of 1 and 3 mm, respectively, centred on the fovea were scanned and divided into the following six sections: fovea, parafovea, temporal (T), superior (S), nasal (N) and inferior (I), where the fovea centre was automatically determined from the relevant OCT data. In the optic disc area, a ring with a width of 0.75 mm centred on the disc was scanned and automatically divided into seven sections as follows: inside disc (ID), nasal (N), inferior nasal (IN), inferior temporal (IT), superior temporal (ST), superior nasal (SN) and temporal (T).
Statistical analysis

The test images acquired using FFA and OCT-A were loaded into Photoshop CS6. The FFA images for the macular and optic disc areas were obtained and enlarged to match the details observed in the OCT-A images. IBM SPSS statistics version 19.0 (SPSS, Inc. Chicago, USA) was used to analyse the data. The measurements are presented as means standard deviations (SD). The comparisons of VD in different layers and different regions of each layer were analysed using the Bonferroni analysis. The level of significance adopted in the present study was $P<0.05$.

Results

Stages of FFA

Human FFA is mainly divided into the following stages: the early arterial stage, arterial stage, arteriovenous stage, venous stage and later stage. In our study, FFA images from cynomolgus macaques tended to exhibit similarities to human angiographic stages. As shown in Fig. 1, the mean arm retina circulation time (RCT) was $5''11\pm1''35$ s, and most fluorescence dissipation occurred at $17'18\pm1'21$ min.

VD of the fovea and parafoveal region

The superficial capillary plexus is readily observed in fluorescein angiographic images of the central macular region; however, FFA is unable to image this region at a greater depth. Fig. 2 and Fig. 4 show the four levels of choroid-retinal capillary network images in cynomolgus macaques and healthy humans obtained using OCT-A, including the superficial layer, deep retinal layer, outer retinal layer, and choroid capillary layer. In cynomolgus macaques, a comparison of the complete en-face VD among the four layers revealed that
the choroid-capillary layer had the highest VD. In contrast, the parafoveal region in the outer retinal layer had the lowest VD 39.60±3.85%. The four peripapillary sections of each retinal layer did not show significant differences. Similarities and significant differences in the comparison of the VD of healthy humans and monkeys were observed in areas of the two retinal layers as shown in Table 1 and Fig. 6. In the superficial layer, there are no significant differences between two groups. However, it is observed that VD in the healthy human retina is higher than in the cynomolgus macaque retina in the fovea area of deep layer.

VD of the optic disc and peripapillary region

Compared to OCT-A, the fluorescein angiographic image of the optic disc and surrounding regions showed the RPC network less clearly, as shown in Fig. 3 and 5. In Table 2, a comparison of the measurements of two layers of the cynomolgus macaque retina revealed that the mean VDs of the entire en-face, ID region and peripapillary region were different (P<0.01). The VD of the ID region was higher in the nerve head. In the peripapillary region, the VD was much higher in the RPC layer (P<0.01). Regarding the six peripapillary regions of each retinal layer, a significantly higher VD was in the IT section than in the N section (P<0.05) of the nerve head layer. In the RPC layer, the VD was lower in the N quadrant than in the IT and ST quadrants (P<0.05). Fig. 6 and Table 2 show the VD of two levels of choroid-retinal capillary network in healthy humans obtained using OCT-A, including the nerve head and RPC layer. In the nerve head layer, VDs in the peripapillary, N, SN, T regions were significantly different in two groups. In the RPC layer, VDs in whole area and various partitioned area were higher in healthy humans.

Discussion

FFA is a conventional, qualitative technology used to diagnose retinal vascular diseases,
such as diabetic retinopathy (DR) and central retinal vein occlusion (CRVO)\textsuperscript{17,18}. Using a special intravenous dye, FFA is one of the most valuable methods for evaluating the retinal vasculature. FFA records the filling process of the choroid-retinal vasculature and has a wide field of view. Additionally, FFA analyses the choroid-retinal vasculature and show vascular leakage and neovascularization\textsuperscript{4}. In the present study, FFA provided clear images of retinal vascular activity. Fluorescence angiography showed that cynomolgus macaques have stages similar to humans, including the early arterial stage (before retinal artery filling), arterial stage (whole retinal artery filling), arteriovenous stage (retinal vein laminar flow), venous stage (whole retinal vein filling) and later stage (fluorescence dissipation). The mean arm RCT was $5.11\pm 1.35$ s. In humans, the RCT is usually $7''-15''$ s. This difference may be due to the blood vessel diameter and blood flow velocity.

OCT-A is a novel imaging method\textsuperscript{6,19}. OCT-A was developed as an extension of OCT that clearly shows the vascular circulation in the retina and choroid\textsuperscript{20,21}. Unlike FFA, OCT-A detects blood flow via an intrinsic signal without the use of any intravenous agents, thereby avoiding any serious side effects arising from fluorescent dyes. FFA can be time consuming, as the spread of the fluorescent agent is limited by the rate of blood flow, leading to a long waiting period before any vascular images are obtained. Alternatively, OCT-A saves time with quick scanning speeds. FFA unquestionably provides two-dimensional (2D) images of vascular circulation\textsuperscript{3}, but with a limited depth perception and detailed investigation of vessels\textsuperscript{4}. The development of OCT-A solves these problems. OCT-A visualizes blood flow and divides the retina into various layers, clearly showing capillary vessels in each layer, not just transparent vessels. Therefore, deeper capillary plexuses, which are often mistaken for background choroid fluorescence in FFA, have been observed using OCT-A\textsuperscript{4,22}. Interestingly, the OCT-A image in which the RPC network is readily visible and the
fluorescein angiographic image of the same region in our study showed considerable similarities with research by Richard F in humans. No previous study has shown the RPC network using fluorescein angiography.

Researchers can quantitatively analyse vessel parameters using high-resolution imaging with OCT-A. Therefore, in the present study, we further compared the VD of the macula, optic disc and surrounding regions using OCT-A. Four levels of choroid-retinal capillary networks are present in the macula of cynomolgus macaques, including the superficial layer, the deep retinal layer, the outer retinal layer, and the choroid capillary layer. A comparison of the complete en-face VD among the four layers revealed the highest VD in the choroid capillary layer. These results are consistent with Florence Coscas’ research in humans. One possible explanation for this finding is that the deep layer is formed by a homogenous capillary vortex, whereas the superficial layer is formed by transverse capillaries alone. Additionally, the superficial layer may artificially influence the VD assessment of the DCP1. The lowest VD is observed in the foveal area than in other sections of the retinal layer, probably due to the foveal avascular zone (FAZ).

The VD of the entire en-face and ID section was higher in the nerve head layer. In contrast, the VD of the peripapillary region was much higher in the RPC layer. In both optic disc layers, the VD in the N quadrant was lower than the IT quadrant. Overall, OCT-A provides better images of the RPC network. The blood supply associated with early optic disc lesions in patients with glaucoma is derived from the microcirculation of the posterior ciliary artery; hence, an observation of the RPC layer is beneficial in the early diagnosis of glaucoma.

Despite the novelty of OCT-A, particularly in assessing different diseases, researchers have not clearly determined how OCT-A may be used for disease management, particularly...
in animal models. Currently, nonhuman primates, particularly cynomolgus macaques, play a very important role in the research of ophthalmic diseases due to their similarities to humans in terms of the optical structure and function. Therefore, a better understanding of traditional FFA and novel OCT-A parameters and a comparison of differences between healthy humans and cynomolgus macaques will help establish these techniques, particularly OCT-A, as methods for the diagnosis of diseases in animal models.

We further compared the VD of normal humans and cynomolgus macaques in the area of the nerve head, RPC network and the layers of the superficial and deep retina, and all of the data showed some similarities and differences between two groups. In macula, the VDs of the superficial and deep layer are similar in both cynomolgus macaques and healthy humans. Only in the fovea area of deep layer, VD of healthy human is much higher. Significant differences in the whole RPC layer of the optic disc were observed between the two groups and in the nerve head layer, healthy humans present higher VDs in various sections. Differences between two groups of VD are more obvious in the optic discs than the macula. These meaningful similarities and differences should take into account in the animal researches about human optical diseases, especially vascular diseases in macula and optic discs.

Conclusions

In conclusion, we evaluated macular vascular circulation parameters in normal cynomolgus macaques using OCT-A and FFA and compared the advantages and disadvantages of the two angiography techniques. Furthermore, we analysed the VD parameters of the choroid-retinal vasculature and compared the vascular parameters between healthy humans and cynomolgus macaques. Obviously, the retinal structure of cynomolgus macaques was very similar to healthy humans; thus, we can use this animal model to better study the
development of human optical diseases. However, some differences in the VD were observed between the two groups, indicating that when we use animal models to study optical diseases, we should also consider the functional differences between animals and humans and take these into account in the animal researches about human optical diseases, especially vascular diseases in macula and optic discs. Overall, our research provides the normal vascular parameters of cynomolgus macaques and healthy humans, promoting the establishment of an eye parameter database for nonhuman primates and animal models of ophthalmic diseases. However, our study has certain limitations that must be addressed. For example, we used cynomolgus macaques as the experimental animal and we did not clearly determine whether these results are applicable to other species. Our future goal is to conduct an extensive study across different species, comparing the similarities and differences in FFA and OCT-A parameters between healthy humans and nonhuman species to facilitate the research of ophthalmic diseases.

List Of Abbreviations

Fundus fluorescence angiography (FFA), Optical coherence tomography angiography (OCT-A), Vessel density (VD)

Declarations

Ethics approval and consent to participate:

Cynomolgus macaques: Ethical approval was provided by the Animal Experimental Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-Sen University with the following reference number: SYXK(YUE)2016-114. We conscientiously abided by the ethical principles of animal welfare and accepted the Commission’s supervision and inspection at any time.

Healthy humans: Ethical approval was provided by the Ethics Committee of the Zhongshan
Ophthalmic Center, Sun Yat-Sen University, Guangzhou, Guangdong with the following reference number: 2017KYPJ113. We conscientiously abided by the ethical principles of the Declaration of Helsinki and accepted the Commission’s supervision and inspection at any time.

Consent to publish: Written informed consents were obtained from the patients for publication of any information contained within the manuscript itself. A copy of the written consent is available for review by the Editor of this journal.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding authors upon reasonable request.

Competing interests: All authors are affiliated with the State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, Guangdong 510060, China. The author(s) have no potential competing interests to declare.

Funding: This study was supported by grants from the National Natural Science Foundation of China (81570839) to Prof. Yongxin Zheng. Prof. Yongxin Zheng had full access to all data presented in the study and take responsibility for the integrity of the data and the accuracy of the data analysis and Guided the writing and modification of the article. Leading the team to complete the writing of this article with Prof. Chenjin Jin.

Authors’ contributions:

YZ and CJ had full access to all data presented in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: JP, LZ, YZ, and CJ; Acquisition, analysis, or interpretation of data: JP, LM, and JJ; Drafting of the manuscript: JP and LZ; Critical revision of the manuscript for important intellectual content:
JP, LZ, YZ, LM, JJ, and CJ; Statistical analysis: JP, LZ; Administrative, technical, or material support: JP, LZ, YZ, and CJ; Study supervision: LZ, YZ and CJ. All authors have read and approved the manuscript.

Acknowledgements: The National Natural Science Foundation of China fund support to this research and We thank all the patients who participated in this study.

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Tables

Table 1 VD (X±SD) of various sections in the macula of cynomolagus macaques and healthy humans.

|                         | Whole (%) | Fovea (%) | Para (%) | T (%)      | S (%)      | N (%)      | I (%)      |
|-------------------------|-----------|-----------|----------|------------|------------|------------|------------|
| Cynomolgus macaques (n=5) |           |           |          |            |            |            |            |
| Mean age:               | 4.60±0.55 years | Gender: Male |
| Superficial             | 44.32±1.6%  | 22.53±4.4% | 46.12±1.6% | 46.07±1.52 | 45.87±2.80 | 46.29±2.11 | 46.28±2.42 |
| Deep                    | 50.05±2.4%  | 21.47±4.2% | 53.95±2.6% | 54.07±2.53 | 53.86±2.42 | 53.95±2.36 | 54.08±4.50 |
| Outer retina            | 42.99±3.8%  | 65.59±3.9% | 39.60±3.8% | 38.98±4.08 | 39.84±4.34 | 39.21±4.63 | 40.41±3.34 |
| Choroid cap             | 68.19±0.7%  | 70.42±1.2% | 68.09±0.8% | 68.49±1.81 | 67.78±1.27 | 68.48±1.04 | 67.61±1.05 |
| Healthy humans (n=28)   |           |           |          |            |            |            |            |
| Mean age:               | 25.11±6.21 years | Gender: 14 males and 14 females |
| Superficial             | 42.82±4.7%  | 19.00±7.2% | 45.09±5.0% | 43.92±5.57 | 47.30±4.41 | 44.05±5.49 | 45.04±5.94 |
| Deep                    | 51.38±5.4%  | 29.82±9.2% | 54.35±5.8% | 54.96±5.08 | 53.35±6.58 | 54.50±5.38 | 54.56±6.77 |
Whole en-face (Whole), fovea, parafovea (Para), temporal (T), superior (S), nasal (N), inferior (I).

Table 2 VD (X±SD) of various sections in the optic disc of cynomolgus macaques and healthy humans.

|                | Whole (%) | ID (%) | Peri (%) | N (%) | I (%) | IT (%) | ST (%) | SN (%) | T (%) |
|----------------|-----------|--------|----------|-------|-------|--------|--------|--------|-------|
| Nerve head     |           |        |          |       |       |        |        |        |       |
| Cynomolgus macaques (n=5) | 50.78 ±4.66 | 44.11 ±4.88 | 54.20 ±5.60 | 48.97 ±6.04 | 57.27 ±6.03 | 62.12 ±1.43 | 58.87 ±4.70 | 55.30 ±5.93 | 53.54 ±7.07 |
| Healthy humans (n=9) | 55.50 ±1.89 | 49.02 ±6.72 | 60.63 ±3.00 | 57.37 ±4.83 | 60.80 ±4.28 | 63.87 ±3.21 | 62.30 ±4.90 | 61.75 ±3.35 | 61.75 ±4.60 |
| RPC            |           |        |          |       |       |        |        |        |       |
| Cynomolgus macaques (n=5) | 48.43 ±3.16 | 33.13 ±4.15 | 54.94 ±4.97 | 49.72 ±6.06 | 56.86 ±5.60 | 62.41 ±1.19 | 61.23 ±5.84 | 52.96 ±4.40 | 55.84 ±5.88 |
| Healthy humans (n=9) | 54.83 ±1.27 | 41.46 ±10.22 | 62.51 ±3.37 | 58.54 ±4.93 | 62.31 ±5.62 | 66.78 ±4.60 | 64.94 ±4.59 | 63.17 ±3.87 | 64.32 ±3.07 |

Whole en-face (Whole), peripapillary (Peri), inside disc (ID), nasal (N), inferior nasal (IN), inferior temporal (IT), superior temporal (ST), superior nasal (SN), temporal (T).

Figures
Figure 1

The FFA images of cynomolgus macaques

Description: (A) IR image of normal cynomolgus macaques. (B) Multi-color image. (C) AF image. fluorescence angiography of cynomolgus macaques in (D) arterial stage, (E) arteriovenous stage, (F) venous stage, and (G) later stage.

Figure 2

The FFA images in the macula matched with the OCT-A images of cynomolgus macaques.

Description: Optical coherence tomography angiography of the four capillary plexuses (A) Retinal layer in optical Coherence tomography. (B) Ring centering on macula with foveal ring (1 mm), four quadrants (superior, inferior, temporal, and nasal, 3mm). (C) The En-face images. (D) Normal virtual colored macular vascular density (Dark blue to red areas indicate areas of no flow to high flow density).

Figure 3

The FFA images of capillary plexuses in the optic disc matched with the OCT-A images of cynomolgus macaques.

Description: Optical coherence tomography image in the optic disc and surrounding region of nerve head layer and radial peripapillary capillary network. (A) Retinal layer in optical Coherence tomography. (B) A ring with width of 0.75mm, centering on the disc, automatically divided the scanned area into seven sections. (C) The En-face image of same region. (D) Normal virtual colored optic disc vascular density (Dark blue to red areas indicate areas of no flow to high flow density).
Figure 4

The OCT-A images of healthy humans in the macula

Description: (A) Retinal layer in optical Coherence tomography. (B) Ring centering on macula with foveal ring (1 mm), four quadrants (superior, inferior, temporal, and nasal, 3mm). (C) The En-face images.

Figure 5

The OCT-A images of healthy humans in the optic disc

Description: (A) Retinal layer in optical Coherence tomography. (B) A ring with width of 0.75mm, centering on the disc, automatically divided the scanned area into seven sections. (C) The En-face image of same region. areas indicate areas of no flow to high flow density).

Figure 6

Histogram of vessel density (VD) in macula and optic disc of cynomolgus macaques and healthy humans.

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

NC3Rs ARRIVE Guidelines Checklist (fillable).pdf