Original Article

# Reproducibility of Perfusion Parameters of Optic Disc and Macula in Rhesus Monkeys by Optical Coherence Tomography Angiography

**Jing Li[1,2], Yi-Quan Yang[1,2], Di-Ya Yang[1], Xiang-Xiang Liu[1,2], Yun-Xiao Sun[1,2], Shi-Fei Wei[1,2], Ning-Li Wang[1,2]**

[1]Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology and Visual Sciences Key Laboratory, Beijing 100730, China

[2]Beijing Institute of Ophthalmology, Beijing Tongren Hospital, Capital Medical University, Beijing 100005, China

## Abstract

**Background:** Optical coherence tomography (OCT) angiography is a novel technique by which we can detect the local perfusion of fundus directly. The aim of this study was to evaluate the reproducibility of optic disc and macular flow perfusion parameters in rhesus monkeys using OCT angiography.

**Methods:** Eighteen healthy monkeys (18 eyes) were subjected to optic disc and macula flow index measurements via a high-speed and high-resolution spectral-domain OCT XR Avanti with a split-spectrum amplitude de-correlation angiography algorithm. Right eye was imaged 3 times during the first examination and once during each of the two following examinations. The intra-visit and inter-visit intraclass correlation coefficients (ICCs) were both determined.

**Results:** The average flow indices of the four optic disc area layers were 0.171 ± 0.009 (optic nerve head), 0.015 ± 0.004 (vitreous), 0.052 ± 0.009 (radial peripapillary capillary), and 0.167 ± 0.011 (choroid). Average flow indices of the four macula area layers were 0.044 ± 0.011 (superficial retina), 0.036 ± 0.011 (deep retina), 0.016 ± 0.009 (outer retina), and 0.155 ± 0.013 (choroid). Intra-visit (ICC value: 0.821–0.954) and inter-visit (ICC value: 0.844–0.899) repeatability were both high.

**Conclusions:** The study is about the reproducibility of optic disc and macular perfusion parameters as measured by OCT angiography in healthy rhesus monkeys. Flow index measurement reproducibility is high for both the optic disc and macula of normal monkey eyes. OCT angiography might be a useful technique to assess changes when examining monkeys with experimental ocular diseases.

**Key words:** Flow Index; Macula; Optic Disc; Optical Coherence Tomography Angiography

## Introduction

As a vital part of visual pathway, retina plays an important role in the generation and maintenance of vision. Normal perfusion of retinal vessels is the essential requirement for retina to maintain its normal function.[1] As we all know, a variety of instruments and techniques have been used to measure retinal perfusion, such as laser speckle imaging, laser Doppler flowmetry, and Doppler optic coherence tomography.[2] However, these are limited in terms of clinical applications and research as they are nonquantitative and/or invasive. Recently, a method for measuring local circulation has been developed using high-speed optical coherence tomography (OCT) to perform quantitative angiography.[3] Using a split-spectrum amplitude de-correlation angiography (SSADA) algorithm, flow in the optic disc and macula can be quantified.[3-17]

Although the reproducibility of optic disc and macular flow perfusion parameters in normal people has been

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**Address for correspondence:** Prof. Ning-Li Wang, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University; Beijing Ophthalmology and Visual Sciences Key Laboratory, Beijing 100730, China
E-Mail: wningli@vip.163.com



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previously reported,[16,17] the reproducibility of flow perfusion parameters in the optic discs and maculae of ocularly normal monkeys is unclear. The reproducibility of a technique is of primary importance when addressing its suitability for use in detecting disease. Therefore, the current study was conducted to evaluate the reproducibility of optic disc and macular flow perfusion parameters in normal rhesus monkeys as measured by OCT.

## Methods

A total of 18 normal adult male rhesus monkeys (*Macaca mulatta*) were studied. The animal production license number is SCXK (Beijing) 2010-0007, with a mean age of 6.5 years (range 5.5–7.5 years) and a mean weight of 7.7 kg (range 7.0–8.7 kg).

### Ethics statement

This study was also evaluated and approved by the Institutional Animal Care and Use Committee of Capital Medical University. The rhesus monkeys involved in this study were all purchased from Beijing Institute of Xieerxin Biology Resource, which was the largest nonhuman primate center in the North part of China and specialized in breeding laboratory nonhuman primate. Then, the rhesus monkeys were housed and monitored at the large animal room of Capital Medical University according to the National Standard of China, which was named as laboratory animal institutions' general requirements for quality and competence (GB/T 27416-2014) during the 3 years after the point of experimental implantation.

Monkeys were anesthetized with intramuscular (i.m.) injection of ketamine HCL (20 mg/kg) + midazolam (0.2 mg/kg). Anesthesia was maintained during examinations and operations with supplemental doses of i.m. ketamine (10 mg/kg), as needed.

### Image acquisition

Monkeys had not undergone any interventions and had not been involved in any previous studies. Right eyes of each monkey were examined. The animals were anesthetized, as above, for all examinations. Tropicamide phenylephrine eye drops (0.5%, Santen-China, Beijing, China) were administered topically to both eyes to dilate the pupils. A rigid Plano contact lens was placed onto the corneal surface during the *in vivo* imaging. Artificial tears (Refresh Plus, Allergan, Irvine, CA, USA) were used to maintain corneal moisture.

Both eyes of each monkey were scanned using the RTVue XR with AngioVue (software version 2014.0.1.68; Optovue, Inc., Fremont, CA, USA), with a light source centered at 840 nm, a bandwidth of 45 nm, and an A-scan-rate of 70,000 scans/s. Scans of both the optic disc and macular areas were acquired. The scan sizes of optic disc and macular area were 4.5 mm × 4.5 mm and 3.0 mm × 3.0 mm, respectively. In detail, optic disc area was divided into four layers, which were optic nerve head (ONH) (all above retinal pigment epithelium layer), vitreous, radial peripapillary capillary (RPC) (between the nerve fibers), and choroid layers. Macular area was also divided into four layers, which were superficial retina (upper line: internal limiting membrane with offset of 3 microns; lower line: inner plexiform layer with offset of 15 microns), deep retina (upper line: inner plexiform layer with offset of 15 microns; lower line: inner plexiform layer with offset of 70 microns), outer retina (upper line: inner plexiform layer with offset of 70 microns; lower line: retinal pigment epithelium reference layer with offset of 30 microns), and choroid layers (upper line: retinal pigment epithelium reference layer with offset of 30 microns; lower line: retinal pigment epithelium reference layer with offset of 60 microns). All images were taken by the same trained examiner (Jing Li) and all measurements were repeated 3 times during the first examination and once at each of the two following examinations. Flow index, which is the key parameter of perfusion, was calculated automatically using the above software and was defined as the average de-correlation value as previously described.[10,14]

### Statistical analysis

Right eye from each monkey was chosen randomly for final analysis. All statistical analyses were performed by using SPSS statistics version 19.0 (SPSS, Inc., Chicago, IL, USA). Flow index measurements were analyzed as continuous variables. The measurements are presented as mean ± standard deviation (SD). The intra-session repeatability was calculated with three continuous results obtained during the first visit. Intra-session intraclass correlation coefficient (ICC) was also calculated. The inter-session reproducibility was determined using three results obtained during each visit. The inter-session ICC was then calculated. ICC values of 0.8–1.00 indicate almost perfect agreement between measurements and values <0.50 indicate poor to fair agreement. We also performed an analysis of variance and calculated the *P* value to evaluate the difference between the measurements during three visits.

## Results

A total of 18 eyes of 18 adult male rhesus monkeys were included in the study. Both eyes of each monkey were examined. The average flow indexes of four optic disc layers were 0.171 ± 0.009 for the ONH layer, 0.015 ± 0.004 for the vitreous layer, 0.052 ± 0.009 for the RPC layer, and 0.167 ± 0.011 for the choroid layer. The average flow indices of the four macula layers were 0.044 ± 0.011 for the superficial retinal layer, 0.036 ± 0.011 for deep retinal layer, 0.016 ± 0.009 for outer retinal layer, and 0.155 ± 0.013 for choroid layer. Flow indices of the four optic disc layers and four macula layers at three visits had no statistical difference (each $P > 0.05$) [Table 1 and Figures 1, 2].

Intra-visit (ICC values: 0.821–0.954) and inter-visit (ICC values: 0.844–0.899) repeatability were both high. The highest intra-visit ICC value in optic disc area was in the RPC layer (0.931) and the highest intra-visit ICC value in macular area was in the choroid capillary layer (0.954). Correspondingly, the highest inter-visit ICC value in optic disc area was in the vitreous layer (0.892) and the highest

inter-visit ICC value in macular area was in the outer retinal layer, which was 0.899 [Tables 2 and 3].

## Discussion

This study establishes a normative database for perfusion of the optic disc and macular areas in healthy nonprimates. In our study, average flow index of four layers of optic disc area were 0.171 for ONH layer, 0.015 for vitreous layer, 0.052 for RPC layer, and 0.167 for choroid layer, respectively. Average flow index of four layers of macula area were 0.044 for superficial retina layer, 0.036 for deep retina layer, 0.016 for outer retina layer, and 0.155 for choroid layer.

OCT angiography, which was first described in 2012, has now made it possible to study and quantify perfusion of both the optic disc and the macula.[10-17] There are a growing number of researches that focus on the new technique to study the changes of optic and macular perfusion in various ocular diseases, such as glaucoma,[8] age-related macular degeneration (AMD),[6,18] diabetic retinopathy,[19] retinal vein occlusion,[20] and so on.

**Table 1: Normal values of flow index in normal monkey eyes**

| Flow index | Values (mean ± SD) | Values (first quartile) | Values (third quartile) | *F* | *P* |
|---|---|---|---|---|---|
| Optic disc | | | | | |
| ONH | 0.171 ± 0.009 | 0.166 | 0.177 | 0.009 | 0.933 |
| Vitreous | 0.015 ± 0.004 | 0.013 | 0.017 | 0.054 | 0.994 |
| RPC | 0.052 ± 0.009 | 0.046 | 0.058 | 0.013 | 0.668 |
| Choroid | 0.167 ± 0.011 | 0.159 | 0.177 | 0.035 | 0.272 |
| Macula | | | | | |
| Superficial retina | 0.044 ± 0.011 | 0.035 | 0.053 | 0.013 | 0.903 |
| Deep retina | 0.036 ± 0.011 | 0.026 | 0.040 | 0.029 | 0.85 |
| Outer retina | 0.016 ± 0.009 | 0.010 | 0.021 | 0.144 | 0.904 |
| Choroid capillary | 0.155 ± 0.013 | 0.148 | 0.163 | 0.009 | 0.694 |

ONH: Optic nerve head; RPC: Radial peripapillary capillary; SD: Standard deviation.

Results of this study found that both intra-visit (ICC values: 0.821–0.954) and inter-visit (ICC values: 0.844–0.899) reproducibility of flow index measurements were high. Wei *et al.* found that this technique could be used to measure macular perfusion in normal people and had high repeatability (1.3% intra-visit and 2.1% inter-visit coefficients of variation).[16] Yu *et al.* investigated macular perfusion in healthy Chinese individuals using the spectral-domain system RTVue-XR Avanti and found that the mean ICC between two measurements from 15 human eyes was 0.910 for vessel area density and 0.925 for the flow index, which were all higher than results in the current study.[17] One reason might be that the monkeys were anesthetized with ketamine HCL and midazolam, and thus lacked of ability to fixate on a target, as the humans did. In addition, there was some slight eye movement in the anesthetized monkeys, which was difficult to avoid.

This study had several potential limitations. First, anesthesia might cause a decrease in ocular perfusion. In other words, the actual flow index of the optic disc and macular areas in rhesus monkeys might be higher than indicated in the current study. Second, these results might be valid for rhesus monkeys; however, it is not clear whether the results regarding the reproducibility of the flow index measurements are applicable in other species. Third, the SSADA technique does not directly measure the perfusion of optic disc; however, measures the peri-ONH region, which reflects perfusion of the ONH. Finally, since our results

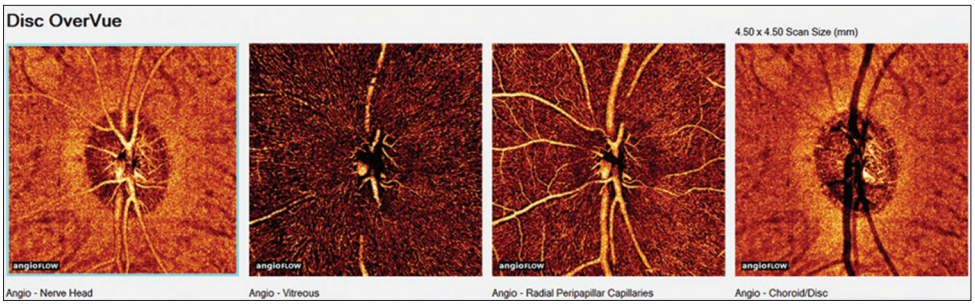


**Figure 1:** Optical coherence tomography angiograms of optic disc area (4.5 mm × 4.5 mm) in normal monkey eyes.

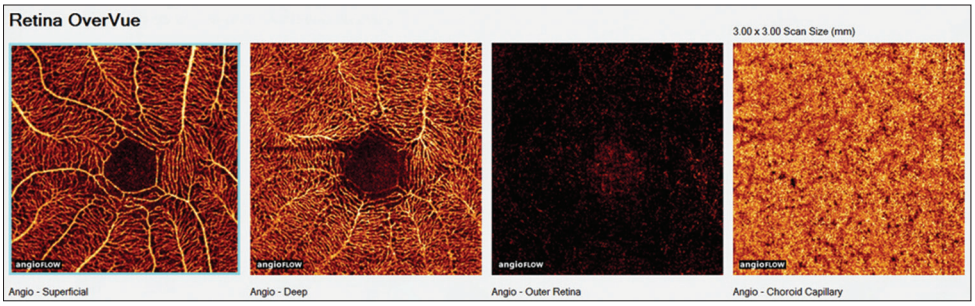


**Figure 2:** Optical coherence tomography angiograms of macular area (3.0 mm × 3.0 mm) in normal monkey eyes.

**Table 2: Intra-visit reproductivity of flow index measurements in normal monkey eyes**

| Flow index | Intra-visit ICC | 95% *CI* (range) |
|---|---|---|
| Optic disc | | |
| ONH | 0.821 | 0.756–0.922 |
| Vitreous | 0.900 | 0.797–0.958 |
| RPC | 0.931 | 0.856–0.971 |
| Choroid | 0.867 | 0.736–0.943 |
| Macula | | |
| Superficial retina | 0.909 | 0.814–0.962 |
| Deep retina | 0.880 | 0.760–0.949 |
| Outer retina | 0.901 | 0.798–0.958 |
| Choroid capillary | 0.954 | 0.902–0.981 |

ONH: Optic nerve head; RPC: Radial peripapillary capillary; ICC: Intraclass correlation coefficient; *CI*: Confidence interval.

**Table 3: Inter-visit reproductivity of flow index measurements in normal monkey eyes**

| Flow index | Inter-visit ICC | 95% *CI* (range) |
|---|---|---|
| Optic disc | | |
| ONH | 0.849 | 0.788–0.902 |
| Vitreous | 0.892 | 0.815–0.919 |
| RPC | 0.859 | 0.781–0.939 |
| Choroid | 0.844 | 0.783–0.885 |
| Macula | | |
| Superficial retina | 0.866 | 0.734–0.943 |
| Deep retina | 0.857 | 0.718–0.939 |
| Outer retina | 0.899 | 0.795–0.958 |
| Choroid capillary | 0.898 | 0.794–0.957 |

ONH: Optic nerve head; RPC: Radial peripapillary capillary; ICC: Intraclass correlation coefficient; *CI*: Confidence interval.

were obtained on normal eyes, caution must be exerted if the results are applied to monkeys with ocular diseases, such as glaucoma or AMD.

In conclusion, this is a rare study of normal values and reproducibility of flow indexes using RTVue XR with AngioVue to perform quantitative angiography in normal monkey eyes. There was a high reproducibility of RTVue XR with AngioVue-assisted measurements of the flow index measurements in normal monkey eyes. These results suggest that the RTVue XR with OCT angiography technique might be suitable and creditable to assess changes during follow-up examinations of experimental ocular diseases, particularly diseases in the optic disc and macula areas.


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## Conflicts of interest

There are no conflicts of interest.

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