# Automated Pupillometry using a Prototype Binocular Optical Coherence Tomography System

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## Abbreviations:

BA – Bland-Altman

CI – confidence interval

ICC – Intraclass correlation coefficient

MD – mean deviation

NTG – normal tension glaucoma

RANSAC – random sample consensus

OCT – optical coherence tomography

RAPD – relative afferent pupillary defect

SD – standard deviation

SFM – swinging flashlight method

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This manuscript contains 1 video clip.

# Abstract

**Purpose:** To determine the test-retest reliability and diagnostic accuracy of a binocular optical coherence tomography (OCT) prototype (Envision Diagnostics, USA) for pupillometry.

**Design:** Assessment of diagnostic reliability and accuracy.

**Methods:** Fifty participants with RAPD confirmed using the swinging flashlight method (mean age 49.6 years) and 50 healthy controls (mean age 31.3 years) were examined. Participants twice underwent an automated pupillometry exam using a binocular OCT system that presents a stimulus and simultaneously captures OCT images of the iris-pupil plane of both eyes. Participants underwent a single exam on the RAPDx (Konan Inc, USA), an automated infrared pupillometer. Pupil parameters including maximum and minimum diameter, and anisocoria were measured. The magnitude of RAPD was calculated using the log of the ratio of the constriction amplitude between the eyes. A pathological RAPD was considered to be above ±0.5 log units on both devices.

**Results:** Intraclass correlation coefficient was >0.90 for OCT-derived maximum pupil diameter, minimum pupil diameter, anisocoria. The RAPDx had a sensitivity of 82% and a specificity of 94% for detection of RAPD whereas the binocular OCT had a sensitivity of 74% and specificity of 86%. The diagnostic accuracy of the RAPDx and binocular OCT was 88% (CI: 80-94%) and 80% (CI: 71-87%) respectively.

**Conclusions:** Binocular OCT-derived pupil parameters had excellent test-retest reliability. Diagnostic accuracy of RAPD was inferior to the RAPDx and is likely related to factors such as eye movement during OCT capture. As OCT becomes ubiquitous, OCT-derived measurements may provide an efficient method of objectively quantifying the pupil responses.

# Introduction

Pupillometry is a valuable diagnostic tool to assess the integrity of the visual pathway and evaluate neurological function. The conventional method of assessing the pupil response is by shining a light into each eye while the responses are observed by the examiner's naked eye.

Relative afferent pupillary defects (RAPD) are often observed in asymmetric conditions affecting the retina, optic nerve or anterior aspects of the optic tract, where a lower magnitude of pupil constriction is observed when the light is swung from the affected eye to the unaffected eye. This method of clinical testing is known as the swinging flashlight method (SFM). The SFM requires a trained examiner and is known to be prone to error - subtle defects can be easily missed due to uneven illumination, or if the eye is stimulated off-axis[1]. Likewise, the interpretation of the SFM is difficult in the context of anisocoria, dark irides or poorly reacting pupils[2]. To address such issues, binocular pupillometers have been developed that can objectively quantify parameters of the pupil responses such as anisocoria, maximum and minimum pupil diameter, and dilation and constriction velocity[3]. These single-purpose instruments utilize infrared technology that can capture enface video images of the pupil without stimulating a response. In addition, the flash stimulus and testing environment is controlled, eliminating errors described above. Although these devices can provide objective data for the diagnosis and monitoring of neurological disease, SFM is still the method of choice in busy clinics due to its convenience and low-cost[4].

Optical coherence tomography (OCT) devices are becoming ubiquitous in eye clinics to image ocular structures at micron-resolution. Anterior-segment OCT devices have previously been used to describe pupil parameters and iris dynamics for the study of accommodation[5] and anterior chamber dynamics for insight into primary angle closure[6]. In this report, we describe a new approach of measuring the pupil parameters and responses using an automated binocular optical coherence tomography (OCT) prototype device

(Envision Diagnostics, El Segundo, CA)[7]. This device acquires images of (in this case) the anterior segments, including the iris-pupil planes, of both eyes simultaneously while delivering a controlled light stimulus to either or both eyes on display screens within the device. The pupil parameters can thus be quantified to micrometer resolution precision using OCT technology that most eye care professionals are familiar with. In addition, the instrument's multi-purpose capabilities such as posterior segment imaging, visual acuity and visual field measurement, and ocular motility testing, could further increase the utility of the device in clinical practice[8,9]. We report on the test-retest reliability of the binocular OCT for the measurement of pupil parameters and its ability to detect RAPD in healthy volunteers and patients diagnosed with RAPD, where SFM was used as the reference standard.

# Methods

Approval for prospective data collection and analysis was obtained from a UK National Health Service Research Ethics Committee (London-Central) as part of the PUPIL study (ClinicalTrials.gov Identifier: NCT03081468). Written informed consent was obtained from all participants. The study adhered to the tenets set forth in the Declaration of Helsinki.

Fifty participants with eye disease with a positive RAPD confirmed using SFM by their treating clinician and a study investigator (RC) were recruited from emergency, glaucoma, medical retina, and neuro-ophthalmology clinics at Moorfields Eye Hospital, London. Fifty healthy participants with a self-reported normal ocular examination within the previous year and an absent RAPD on SFM were also recruited. As a ratio of the constriction amplitude is used to calculate RAPD (as described below), the subject's fellow eye serves as a control, and therefore participants were not age or sex matched[10].

Individuals were excluded if they had any significant ocular opacity or ptosis that obscured visibility of the pupil; if they had any ocular, neurological, or systemic disease that might affect the efferent limb of the pupil pathway; or if they were using any systemic or topical medications known to alter pupil size, such as pilocarpine or opiates. Preliminary testing involved visual acuity measurement using the logarithmic visual acuity chart (logMAR) with habitual correction. All visual field tests were undergone on a Humphrey Visual Field Analyzer (Carl-Zeiss Meditec, USA) using the SITA 24-2 algorithm. Habitual refractive error correction was measured using a lensmeter to determine a best spherical equivalent for correction within the binocular OCT device to aid visibility of the fixation target. The mean deviation (MD) results from the participants' visual field test (<6 months) were recorded.

**RAPDx examination**

Participants underwent a single examination using the RAPDx device (Konan Medical USA, Irvine, CA, USA), an automated infrared pupillometer. Each participant was tested in a dimly-lit room (<5 lux) and dark-adapted for 2-3 minutes prior to examination as recommended in the manufacturer's user manual. The participant placed their head against the RAPDx device interface that blocks out the majority of external illumination. The RAPDx presents bright white stimuli monocularly covering a field of view of 30-degrees. The stimulus alternates between the eyes, while the subject continues to view a nominal white background and fixation target as a cyclopean scene. The stimuli were presented for 0.2s duration, with 1.9s between each stimulus. The results from the first pair of stimuli are discarded. The device continues to present visual stimuli until 8 pairs of recordings are obtained. If the device records a blink during the pupil recording, the pair is automatically repeated. The recordings are averaged to output a constriction 'amplitude' and 'latency' RAPD score. The amplitude score was used for this study as a comparable parameter to that produced by the binocular OCT.

Raw data for other pupil parameters was obtained from the manufacturer for analysis. The RAPD measurement was calculated using the mean pupil constriction amplitudes for each eye.

**Binocular OCT examination**

The specifications of the device are described elsewhere[8]. Briefly, this device acquires OCT images of the 'whole-eye' in a single instrument. The display screens within the device can be customized, and for this study displayed a single controlled flash of white light to each eye whilst simultaneously capturing OCT scans of the anterior segments including the iris plane of both eyes.

Each participant was dark-adapted for 2-3 minutes prior to binocular OCT pupillometry examination. The machine is light-proof, blocking out all external light when users place their head within the mask-machine interface. To assess repeatability (test-retest variability), participants underwent two trials on the binocular OCT within the same session, separated by a minimum interval of 15 minutes. The binocular OCT ensures alignment and visualization of the

pupils prior to stimulus presentation as described previously. The OCT captures pupillary images of both eyes every 47ms for 350ms prior to the stimulus, and 4s after stimulus presentation. The left eye is first presented with a fixation target on a black background, and is stimulated with a bright flash of white light of 38x20 degrees with a luminance of 25cd/$m^2$ for 0.25s duration. The fixation target is then presented to the right eye, with realignment of the oculars if necessary. Once aligned, the same controlled stimulus is presented to the right eye. From these images, the pupil margins can be determined, and a circumference measurement can be derived as described below. The binocular OCT generates similar parameters to the RAPDx. These can be used to calculate the constriction amplitude as a ratio between the eyes to generate a RAPD value in log units.

Pupil metrics are derived using 4 horizontal line scans over the central 3mm of the anterior segment. Each line scan is separated by 1mm (Figure 1), generating 8 possible intersections with the pupillary margin. The pupil may occasionally be obstructed in images if it moves off-center - this is especially related to eye movement during capture. Only 2 line scans with the pupil visible, i.e. at least 3 pupil margin points, are required to calculate the pupil diameter by fitting those points to a circle in 3D space, whereas the RAPDx uses an enface 2D image. When more than 3 pupil margin points are available, a random sample consensus (RANSAC) algorithm is used to calculate the diameter by fitting circles to 3 points selected at random. Multiple iterations form a consensus of the best fitting circle.

**Data Analysis and Statistics**

The following pupil parameters were compared for test-retest reliability:

- Maximum/resting pupil diameter (mm)
- Minimum pupil diameter (mm)
- Anisocoria (defined as the difference in pupil diameter between eyes and measured in the 0.5 seconds before stimulus presentation) (mm)
- Constriction amplitude using maximum and minimum pupil diameter (%)

- RAPD (log units) calculated using the following formula, where a positive result indicates a right RAPD and a negative result indicates a left RAPD:

$$RAPD = 10log_{10}\left(\frac{constriction\ amplitude\ left\ eye}{constriction\ amplitude\ right\ eye}\right)$$

Diagnostic accuracy of each device was assessed compared to the SFM, which was used as the ground truth. Receiver operating characteristic curves (ROC) were used for sensitivity and specificity analysis using an absolute value of the RAPD score. Agreement between binocular OCT trials were appraised using the intraclass correlation coefficient for test-retest reliability. Bland-Altman graphs were used for intra- and inter-device comparisons. Proportional bias was assessed using linear regression analysis plotted on the Bland-Altman graphs.

To assess the correlation between RAPD score and visual function, Pearson's correlation coefficient and $R^2$ values from univariable linear regression were used. Inter-eye differences were computed left eye minus right eye values for MD and logMAR visual acuity.

Independent t-tests were used to assess statistically significant differences in demographics and pupil parameters between the healthy and disease groups. Paired t-tests were used to assess intra- and inter-device differences. A P-value of ≤0.05 was considered to be statistically significant.

# Results

The mean age of the cohort with eye disease and a positive RAPD was 49.6 years (interquartile range (IQR): 35-60.5 years), and 52% were female. A summary of the range of eye diseases in this cohort are presented in Table 1. The fifty healthy participants had a mean age of 31.3 years (IQR: 25 to 32 years), and 58% were females. Age, visual acuity in the worse eye, and refractive error were significantly different between the two groups (P≤0.05).

All participants completed both examinations on the binocular OCT, amounting to 100 participants and 200 examinations. One healthy volunteer and one participant with a positive RAPD did not complete the RAPDx examination due to excessive blinking and unreliable detection of the pupil due to a co-existing peripheral iridotomy, respectively. Therefore 49 healthy controls and 49 participants with RAPD were used for comparison of the binocular OCT with the RAPDx.

The binocular OCT examination generates a series of images that can be collated into a video - an example of a participant with a positive right RAPD is presented in Supplementary Video 1 (available online at [URL]). A summary is shown in Figure 2. A quantitative pupillometry report for each participant is generated at the end of the examination, providing a data-rich analysis of the examination (Figure 3).

**Test-retest reliability and intra- and inter-device agreement**

The following pupil parameters were analyzed for test-retest reliability using the intraclass correlation coefficient (ICC): maximum and minimum pupil diameters, anisocoria, constriction amplitude. Pupil diameters from both direct and consensual responses for both healthy and diseased cohorts were aggregated to form a list of 400 test-retests from 100 participants. The intraclass correlation coefficient (ICC) for OCT-derived maximum pupil diameter, minimum pupil diameter, anisocoria, and constriction amplitude was 0.95 (95% confidence interval [CI] 0.94-

0.96), 0.93 (CI: 0.91-0.94), 0.97 (CI: 0.95-0.97), and 0.88 (CI: 0.85-0.90), respectively. For RAPD measurement, the ICC was 0.90 (CI: 0.74-0.87). Minimum pupil diameter, absolute pupil constriction and constriction amplitude were significantly different between the tests for both cohorts, however RAPD measurements were not (Table 2; paired t-test).

Intra- and inter-device agreement is shown in Bland-Altman (BA) plots in Figure 4. The BA plots for both cohorts show a significant proportional bias ($P$<0.001) for test-retest (Figure 4A-B) but with high variability ($R^2$=0.25). This suggests that the RAPD measurements from the first trial were of larger magnitude compared to the second trial. The limits of agreement had a smaller range for the healthy participants (±1.70) than the cohort with eye disease (±2.27). Inter-device agreement was assessed between the second binocular OCT trial and the RAPDx (Figure 4C-D). Limits of agreement were smaller for both cohorts in comparison to test-retest measures, indicating better agreement between the second binocular OCT trial and the RAPDx. The distribution of measured RAPD is illustrated in the violin plots in Figure 5, showing a tighter distribution in the second trial compared to the first trial, with fewer observed outliers. The measured scores on the RAPDx show an even tighter distribution for the healthy cohort in comparison to the binocular OCT.

Paired t-tests showed nearly all pupil parameters were significantly different between the two devices for both groups, as expected due to the different light conditions. RAPD measurements were not statistically different between the devices and groups (Table 2).

**Diagnostic accuracy for RAPD detection**

Results for the second examination on the binocular OCT were used for comparison with the RAPDx. For healthy participants, the mean (SD) RAPD score was +0.01 (±0.21) log units (95% CI: -0.05 to 0.06) using the RAPDx. In comparison, the mean (SD) RAPD score for this cohort using binocular OCT was 0.04 (±0.54) log units (95% CI: -0.19 to 0.12). For participants with eye disease, the mean (SD) RAPD score was -0.0002 (±2.13) log units (95% CI: -0.61 to 0.61) using the RAPD, and +0.07 (±1.97) log units (95% CI: -0.49 to 0.63) using binocular OCT. The RAPDx and binocular OCT agreed on which eye was affected for all participants in the diseased

cohort. In the healthy cohort, the RAPDx and binocular OCT agreed on the affected side in 29 participants (59%).

Absolute values of the RAPD scores were used to measure diagnostic accuracy, with the caveat that this does not take into account whether the instrument correctly identifies the RAPD on the affected eye. The area under the ROC curve (AUC) for the RAPDx device was 0.963, indicating excellent diagnostic ability when using SFM as the reference standard (Figure 6). The AUC for binocular OCT was 0.832, indicating good diagnostic ability but inferior to the RAPDx. The threshold to detect disease whilst optimizing for both sensitivity and specificity was between 0.4 and 0.5 log units for both the RAPDx and binocular OCT. At a threshold of 0.5 log units, the RAPDx had a sensitivity of 82% and a specificity of 94% for detection of RAPD, whereas the binocular OCT had a sensitivity of 74% and specificity of 86%. The diagnostic accuracy of the RAPDx and binocular OCT was 88% (CI: 80-94%) and 80% (CI: 71-87%) respectively. At the 0.3 log units threshold, the RAPDx and binocular OCT sensitivity/specificity was 92/89% and 86/66%, respectively.

**Correlation of RAPD with visual function parameters in cohort with pathological RAPD**

A larger inter-eye difference in mean deviation was associated with a larger RAPD score measured on the binocular OCT (correlation coefficient R=0.58, P<0.001, $R^2$=0.33), and even more strongly associated with RAPD score measured on the RAPDx (R=0.76, P<0.001, $R^2$=0.57). The relationship between visual acuity and RAPD score was similar for both binocular OCT (R=0.74, P<0.001, $R^2$=0.54), and RAPDx (R=0.77, P<0.001, $R^2$=0.59). This suggests a moderately positive relationship between increasing difference of visual acuity and increasing RAPD score.

# Discussion

In this study, pupillometry was performed using two devices with two different technologies: RAPDx, utilizing infrared cameras to image the pupil circumference enface; and a prototype binocular OCT instrument, employing swept-source lasers to capture high-resolution cross-sectional images through the iris and pupil planes. To our knowledge, this is the first time that the binocular pupil responses have been assessed using OCT imaging.

Binocular OCT pupillometry was found to have excellent test-retest reliability for pupil parameters such as maximum and minimum pupil diameter, and anisocoria. Although constriction amplitude performed well on ICC testing, agreement was slightly lower than the other measured parameters. As constriction amplitude is calculated using pupil diameter values, small errors in maximum and minimum pupil diameters can propagate error into constriction amplitude measurement, and consequently RAPD calculation. In addition, the distribution of RAPD was found to be wider for the first trial compared to the second trial although the distributions were not statistically significant. This suggests there may be a learning effect as the user becomes familiar with the device, the fixation target, and how bright the flash will be, reducing measurement error on subsequent trials. This effect is demonstrated in other devices such as perimetry, where fixation and thus test-retest variability improves upon repeat testing[11–13]. Further testing eliminating potential learning effect errors should be carried out in future work to understand the extent to which this and other artefacts contributes to test-retest variability, which may limit the application of such a RAPD measure in clinical practice.

Although the diagnostic accuracy for RAPD detection was inferior to the RAPDx, OCT-derived measurements show promise in detecting these, often subtle, abnormalities. Literature evidence suggests that a RAPD score of within ±0.3 log units is physiological, whereas a threshold of ±0.5 log units is suggested as a cut-off to detect disease[10,14,15], in agreement with our findings - the optimum threshold for disease detection using the binocular OCT was 0.5 log units. However, the specificity of the binocular OCT was worse than the RAPDx at a threshold

of 0.3 log units, recommended as the threshold for presence of a physiological RAPD. We hypothesize that diagnostic accuracy is affected by small eye movements during OCT capture that may cause the pupil to become occluded in one or more B-scans, thus reducing the number of possible iterations of the RANSAC algorithm used to calculate the ratio of constriction amplitude. As a result of fewer iterations, the accuracy of the pupil diameter measurement reduces. The maximum pupil diameter captured pre-stimulus had slightly better test-retest reliability than minimum pupil diameter - generally measured 1-2 seconds later, when the eyes have had the opportunity to move. One advantage of measuring the pupils using OCT is that RANSAC utilizes 3-dimensional space, whereas the RAPDx and other infrared pupillometers use a 2-dimensional enface measurement only. This might be helpful in eyes with strabismus[9].

For this study, we used SFM as the reference standard for detecting the presence or absence of RAPD. Despite its flaws, the SFM is still the most commonly used pupillometry assessment in clinical practice. As a result, false positives and false negatives are likely to occur. In 4 participants with RAPD confirmed by SFM, both the RAPDx and binocular OCT measured a RAPD within ±0.5 log units, and therefore below the threshold for disease detection. Although participants were confirmed with RAPD using the SFM by a trained observer, it is possible that some of these cases were false positives. On follow-up of case notes for these patients, two participants had a diagnosis of optic neuritis 6 months prior to testing and had made a recovery from all other symptoms; and one participant had asymmetric primary open angle glaucoma with a difference in MD of 6dB between the eyes which may not elicit an RAPD on quantitative testing[16]. Another participant had a healthy optic disc in the right eye, and normal tension glaucoma (NTG) in the left eye, with a difference of 0.18 in visual acuity and -16.7 in MD, but only measured a left RAPD of 0.20 and 0.26 log units on the binocular OCT and RAPDx, respectively. This case is likely to be a false negative, however recent literature has shown that those with NTG have a lesser RAPD for a given inter-eye difference in MD compared to those with open angle glaucoma[17]. These cases further support the need for objective methods of performing pupillometry.

In line with previous literature, we found a positive relationship between inter-eye difference in MD and RAPD score measured by both devices[17–20]. Interestingly, we found that there was also an association between visual acuity and RAPD score. Although contradictory results have been reported in the other studies[21–23], it is not surprising that RAPD was found in the eye with the worse visual acuity.

The binocular OCT pupillometry exam has several limitations at present. The RAPDx averages 8 pairs of measurements to minimize noise and the effect of anomalies, whereas the binocular OCT only uses a single measurement. This difference in methodology means the test duration is shorter for the binocular OCT device, which may be worthwhile in busy clinic. However, the signal/noise ratio is poorer, resulting in greater measurement scatter and may increase the impact of random error[22]. In addition, the difference in stimulus characteristics between the devices such as stimulus luminance are likely to elicit a different magnitude of pupil response. These are likely to be a major factors in explaining the underperformance in diagnostic accuracy of the binocular OCT when compared with the RAPDx. Future iterations of the device should allow rapid repetitive stimulation of the pupils, and should follow stimulus characteristics as recommended by Kelbsch et al.[24]. In this work we focus on amplitude measurements to calculate RAPD, however latency measures can also be used. Velocity and amplitude of the pupil light reflex are linearly related[25], so there is no a priori reason to expect any additional information from latency measures as an outcome measure. However, the sampling frequency of the OCT should be improved upon in future iterations for accurate velocity and latency measures that are often informative for other pupil abnormalities in addition to RAPD.

The binocular optical coherence tomography system has shown promise for automated OCT imaging[8], and for novel applications that exploit the binocularity aspect of the device, such as evaluation of strabismus[9]. In the future, pupillometry - an essential aspect of the eye examination, could be performed in an automated manner using OCT. Unfortunately, automated pupilometers such as the Konan RAPDx system are not widely used in clinical practice. This is largely because they are expensive devices, limited to a single purpose. In the real-world busy

clinic, the swinging flashlight method is still the test of choice - often performed by technicians who may not be trained observers. . Thus, an automated system that is capable of performing a comprehensive quantitative examination may be more sensitive for disease detection, and may perhaps provide new insights into the pathophysiology of disease.

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# References


1. Wilhelm H. Neuro-ophthalmology of pupillary function--practical guidelines. *J Neurol*. 1998;245(9):573-583.

2. Meneguette NS, de Carvalho JER, Petzold A. A 30 s test for quantitative assessment of a relative afferent pupillary defect (RAPD): the infrared pupillary asymmetry (IPA). *J Neurol*. 2019;266(4):969-974.

3. Martínez-Ricarte F, Castro A, Poca MA, et al. Infrared pupillometry. Basic principles and their application in the non-invasive monitoring of neurocritical patients. *Neurología (English Edition)*. 2013;28(1):41-51.

4. AMERICAN ACADEMY OF OPHTHALMOLOGY. *2019-2020 BASIC AND CLINICAL SCIENCE COURSE, SECTION 05: Neuro-Ophthalmology*. American Academy of Ophthalmology; 2019.

5. Baikoff G, Lutun E, Ferraz C, Wei J. Static and dynamic analysis of the anterior segment with optical coherence tomography. *J Cataract Refract Surg*. 2004;30(9):1843-1850.

6. Cheung CY-L, Liu S, Weinreb RN, et al. Dynamic analysis of iris configuration with anterior segment optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2010;51(8):4040-4046.

7. Walsh AC. Binocular optical coherence tomography. *Ophthalmic Surg Lasers Imaging*. 2011;42 Suppl:S95-S105.

8. Chopra R, Mulholland PJ, Dubis AM, Anderson RS, Keane PA. Human Factor and Usability Testing of a Binocular Optical Coherence Tomography System. *Transl Vis Sci Technol*. 2017;6(4):16.

9. Chopra R, Mulholland PJ, Tailor VK, Anderson RS, Keane PA. Use of a Binocular Optical Coherence Tomography System to Evaluate Strabismus in Primary Position. *JAMA Ophthalmol*. 2018;136(7):811-817.

10. Satou T, Goseki T, Asakawa K, Ishikawa H, Shimizu K. Effects of Age and Sex on Values Obtained by RAPDx® Pupillometer, and Determined the Standard Values for Detecting Relative Afferent Pupillary Defect. *Transl Vis Sci Technol*. 2016;5(2):18.

11. Heijl A, Lindgren G, Olsson J. The effect of perimetric experience in normal subjects. *Arch Ophthalmol*. 1989;107(1):81-86.

12. Heijl A, Bengtsson B. The effect of perimetric experience in patients with glaucoma. *Arch Ophthalmol*. 1996;114(1):19-22.

13. Wild JM, Dengler-Harles M, Searle AE, O'Neill EC, Crews SJ. The influence of the learning effect on automated perimetry in patients with suspected glaucoma. *Acta Ophthalmol* . 1989;67(5):537-545.

14. Wilhelm H, Peters T, Lüdtke H, Wilhelm B. The prevalence of relative afferent pupillary defects in normal subjects. *J Neuroophthalmol*. 2007;27(4):263-267.

15. Kawasaki A, Moore P, Kardon RH. Variability of the relative afferent pupillary defect. *Am J Ophthalmol*. 1995;120(5):622-633.

16. Tatham AJ, Meira-Freitas D, Weinreb RN, Zangwill LM, Medeiros FA. Detecting glaucoma using automated pupillography. *Ophthalmology*. 2014;121(6):1185-1193.

17. Lawlor M, Quartilho A, Bunce C, et al. Patients With Normal Tension Glaucoma Have Relative Sparing of the Relative Afferent Pupillary Defect Compared to Those With Open Angle Glaucoma and Elevated Intraocular Pressure. *Invest Ophthalmol Vis Sci*. 2017;58(12):5237-5241.

18. Chang DS, Boland MV, Arora KS, Supakontanasan W, Chen BB, Friedman DS. Symmetry of the pupillary light reflex and its relationship to retinal nerve fiber layer thickness and visual field defect. *Invest Ophthalmol Vis Sci*. 2013;54(8):5596-5601.

19. Sarezky D, Volpe NJ, Park MS, Tanna AP. Correlation Between Inter-Eye Difference in Average Retinal Nerve Fiber Layer Thickness and Afferent Pupillary Response as Measured by an Automated Pupillometer in Glaucoma. *J Glaucoma*. 2016;25(3):312-316.

20. Sarezky D, Krupin T, Cohen A, Stewart CW, Volpe NJ, Tanna AP. Correlation between intereye difference in visual field mean deviation values and relative afferent pupillary response as measured by an automated pupillometer in subjects with glaucoma. *J Glaucoma*. 2014;23(7):419-423.

21. Fison PN, Garlick DJ, Smith SE. Assessment of unilateral afferent pupillary defects by pupillography. *Br J Ophthalmol*. 1979;63(3):195-199.

22. Bobak SP, Goodwin JA, Guevara RA, Arya A, Grover S. Predictors of visual acuity and the relative afferent pupillary defect in optic neuropathy. *Doc Ophthalmol*. 1998;97(1):81-95.

23. Thompson HS, Montague P, Cox TA, Corbett JJ. The relationship between visual acuity, pupillary defect, and visual field loss. *Am J Ophthalmol*. 1982;93(6):681-688.

24. Kelbsch C, Strasser T, Chen Y, et al. Standards in Pupillography. *Front Neurol*. 2019;10:129.

25. Bremner FD. Pupillometric evaluation of the dynamics of the pupillary response to a brief light stimulus in healthy subjects. *Invest Ophthalmol Vis Sci*. 2012;53(11):7343-7347.

## Figure Legends

**Figure 1**. Position of horizontal line scans acquired through the iris-pupil planes during pupillometry examination are 1mm apart. The pupil margin in each of these scans (shown by a red dot) is used to calculate the pupil diameter using the random sample consensus (RANSAC) method.

**Figure 2.** (A) Resting diameters pre-stimulus; (B) Flash presented to the left eye, constriction of both pupils observed; (C) Both pupils dilate to their resting diameter; (D) Flash presented to the right eye. Constriction amplitude of both eyes is less than that observed when the flash was presented to the left eye.

**Figure 3.** The binocular OCT pupillometry report displays a graphical output of the pupil diameter versus time, and quantitative measurements such as diameters and velocities.

**Figure 4.** (A) and (B): Intra-device agreement. Bland-Altman graphs for healthy participants (left) and participants with disease (right) to assess the agreement between the two binocular OCT trials. (C) and (D): Inter-device agreement between the binocular OCT and RAPDx. Limits of agreement (±1.96 standard deviation) and the mean is shown as dashed lines with shaded confidence intervals. Regression lines are plotted to highlight proportional bias.

**Figure 5.** Violin plots illustrating the distribution of measured RAPD scores by cohort and device.

**Figure 6.** Receiver operating characteristic (ROC) curves showing the ability to discriminate between positive and negative RAPD using the RAPDx (blue) and binocular OCT (red). The area under the curve (AUC) is greater for RAPDx (AUC 0.963) than binocular OCT (AUC 0.832). Operating points at 0.3, 0.4, and 0.5 log units are indicated by green markers. The

optimal threshold for disease detection for both RAPDx and binocular OCT appears to be between 0.4 to 0.5 log units.

**Clip 1.** Pupillometry using the binocular coherence tomography system

**Table_2**

| | **Healthy (n = 50) vs Disease (n = 50)**<br>**Independent t-test P-value** | | | | | |
|---|---|---|---|---|---|---|
| **Age (years) - Mean (SD)** | 0.001 | | | | | |
| **Sex (% female)** | 0.356 | | | | | |
| **Visual acuity worse eye (logMAR) - Mean (SD)** | <0.001 | | | | | |
| **Visual acuity better eye (logMAR) - Mean (SD)** | 0.761 | | | | | |
| **Refractive error, mean spherical equivalent (dioptres) - Mean (SD)** | 0.032 | | | | | |
| | **Healthy vs Disease**<br>**Independent t-test P-value** | | **Binocular OCT test-retest**<br>**Paired t-test P-value** | | **Binocular OCT vs RAPDx**<br>**Paired t-test P-value** | |
| | **Binocular OCT**<br>**n = 50** | **RAPDx**<br>**n = 49** | **Healthy**<br>**n = 50** | **Disease**<br>**n = 50** | **Healthy**<br>**n = 49** | **Disease**<br>**n = 49** |
| **Maximum pupil diameter (mm) - Mean (SD)** | 0.202 | 0.710 | 0.824 | **All eyes**<br>0.789<br>**Affected eyes**<br>0.870<br>**Unaffected eyes**<br>0.659 | <0.001 | **All eyes**<br><0.001<br>**Affected eyes**<br><0.001<br>**Unaffected eyes**<br><0.001 |
| **Minimum pupil diameter (mm) - Mean (SD)** | 0.458 | 0.592 | <0.001 | **All eyes**<br>0.009<br>**Affected eyes**<br>0.054<br>**Unaffected eyes**<br>0.066 | <0.001 | **All eyes**<br><0.001<br>**Affected eyes**<br><0.001<br>**Unaffected eyes**<br><0.001 |
| **Anisocoria (mm) - Mean (SD)** | 0.043 | Not reported | 0.673 | 0.173 | - | - |
| **Absolute pupil constriction (mm) - Mean (SD)** | 0.003 | <0.001 | <0.001 | **All eyes**<br><0.001<br>**Affected eyes**<br>0.003<br>**Unaffected eyes**<br>0.011 | <0.001 | **All eyes**<br><0.001<br>**Affected eyes**<br><0.001<br>**Unaffected eyes**<br><0.001 |
| **Constriction amplitude (%) - Mean (SD)** | 0.001 | <0.001 | <0.001 | **All eyes**<br><0.001<br>**Affected eyes**<br>0.002<br>**Unaffected eyes**<br>0.001 | 0.112 | **All eyes**<br><0.001<br>**Affected eyes**<br><0.001<br>**Unaffected eyes**<br>0.008 |
| **RAPD (log units) - Mean (SD)** | <0.001 | <0.001 | 0.487 | 0.308 | 0.629 | 0.661 |
| **Absolute RAPD (log units) - Mean (SD)** | <0.001 | <0.001 | 0.160 | 0.240 | 0.063 | 0.130 |

**Table 2.** Statistical significance tests. Independent t-tests were used to assess significant differences between the healthy and disease group. Paired t-tests were used to assess significant differences for intra- and inter-device comparisons. The second test on the binocular OCT was used for inter-device comparisons. Significance is assumed at ≤0.05.

**Table_1**

| | **Healthy (n = 50)** | | **Disease (n = 50)** | |
|---|---|---|---|---|
| **Age (years) - Mean (SD)** | 31.3 (10.1) | | 49.6 (15.9) | |
| **Sex (% female)** | 58 | | 52 | |
| **Eye diseases** | No eye disease present | | Branch retinal artery occlusion<br>Glaucoma<br>Idiopathic optic neuropathy<br>Ischaemic central retinal vein occlusion<br>Non-arteritic ischaemic optic neuropathy<br>Idiopathic optic atrophy<br>Optic nerve compression<br>Optic neuritis<br>Traumatic optic neuropathy | n=1<br>n=22<br>n=2<br>n=1<br>n=1<br>n=2<br>n=2<br>n=17<br>n=2 |
| **Visual acuity worse eye (logMAR) - Mean (SD)** | -0.05 (0.10) | | 0.55 (0.73)* | |
| **Visual acuity better eye (logMAR) - Mean (SD)** | -0.08 (0.08) | | -0.03 (0.12) | |
| **Mean deviation (dB) worse eye - Mean (SD)** | Not performed | | -14.59 (6.58)** | |
| **Mean deviation (dB) better eye - Mean (SD)** | Not performed | | -2.65 (0.45)** | |
| **Refractive error, mean spherical equivalent (dioptres) - Mean (SD)** | -1.43±2.17 | | -0.51 (2.21) | |
| | **Binocular OCT n = 50** | **RAPDx n = 49** | **Binocular OCT n = 50** | **RAPDx n = 49** |
| **Maximum pupil diameter (mm) - Mean (SD)** | 6.78 (1.18) | 5.87 (0.99) | **All eyes**<br>5.80 (1.19)<br>**Affected eyes**<br>5.84 (1.18)<br>**Unaffected eyes**<br>5.77 (1.20) | **All eyes**<br>5.35 (1.00)<br>**Affected eyes**<br>5.33 (0.99)<br>**Unaffected eyes**<br>5.38 (1.04) |
| **Minimum pupil diameter (mm) - Mean (SD)** | 4.94 (1.10) | 4.21 (0.82) | **All eyes**<br>4.32 (1.08)<br>**Affected eyes**<br>4.51 (1.12)<br>**Unaffected eyes**<br>4.12 (1.00) | **All eyes**<br>4.06 (0.87)<br>**Affected eyes**<br>4.25 (0.90)<br>**Unaffected eyes**<br>4.07 (0.93) |
| **Anisocoria (mm) - Mean (SD)** | 0.23 (0.19) | Not reported | 0.34 (0.28) | Not reported |
| **Absolute pupil constriction (mm) - Mean (SD)** | 1.84 (0.43) | 1.66 (0.34) | **All eyes**<br>1.49 (0.47)<br>**Affected eyes**<br>1.33 (0.45)<br>**Unaffected eyes**<br>1.65 (0.45) | **All eyes**<br>1.30 (0.44)<br>**Affected eyes**<br>1.09 (0.38)<br>**Unaffected eyes**<br>1.30 (0.46) |
| **Constriction amplitude (%) - Mean (SD)** | 27.59 (6.33) | 28.38 (4.31) | **All eyes**<br>25.90 (7.38)<br>**Affected eyes**<br>23.07 (7.31)<br>**Unaffected eyes**<br>29.74 (6.28) | **All eyes**<br>24.22 (7.03)<br>**Affected eyes**<br>20.42 (6.52)<br>**Unaffected eyes**<br>24.42 (7.16) |

| | | | | |
|---|---|---|---|---|
| **RAPD (log units) - Mean (SD)** | **Trial 1**<br>0.142 (0.87)<br>**Trial 2**<br>0.036 (0.54) | 0.006 (0.21) | **Trial 1**<br>0.238 (2.54)<br>**Trial 2**<br>0.069 (1.97) | 0.0002 (2.13) |
| **Absolute RAPD (log units) - Mean (SD)** | **Trial 1**<br>0.560 (0.68)<br>**Trial 2**<br>0.36 (0.40) | 0.140 (0.15) | **Trial 1**<br>1.432 (2.10)<br>**Trial 2**<br>1.266 (1.50) | 1.565 (1.71) |

**Table 1.** Participant characteristics. *Visual acuity for 48 participants - 1 participant had perception of light vision, 1 participant had no perception of light vision. (Counting fingers and hand movements converted to 2.0 and 3.0 logMAR respectively). **Visual fields for 45 participants - test not performed in 5 eyes with vision of counting fingers, hand movements, perception of light, and no perception of light.

**Figure 1**

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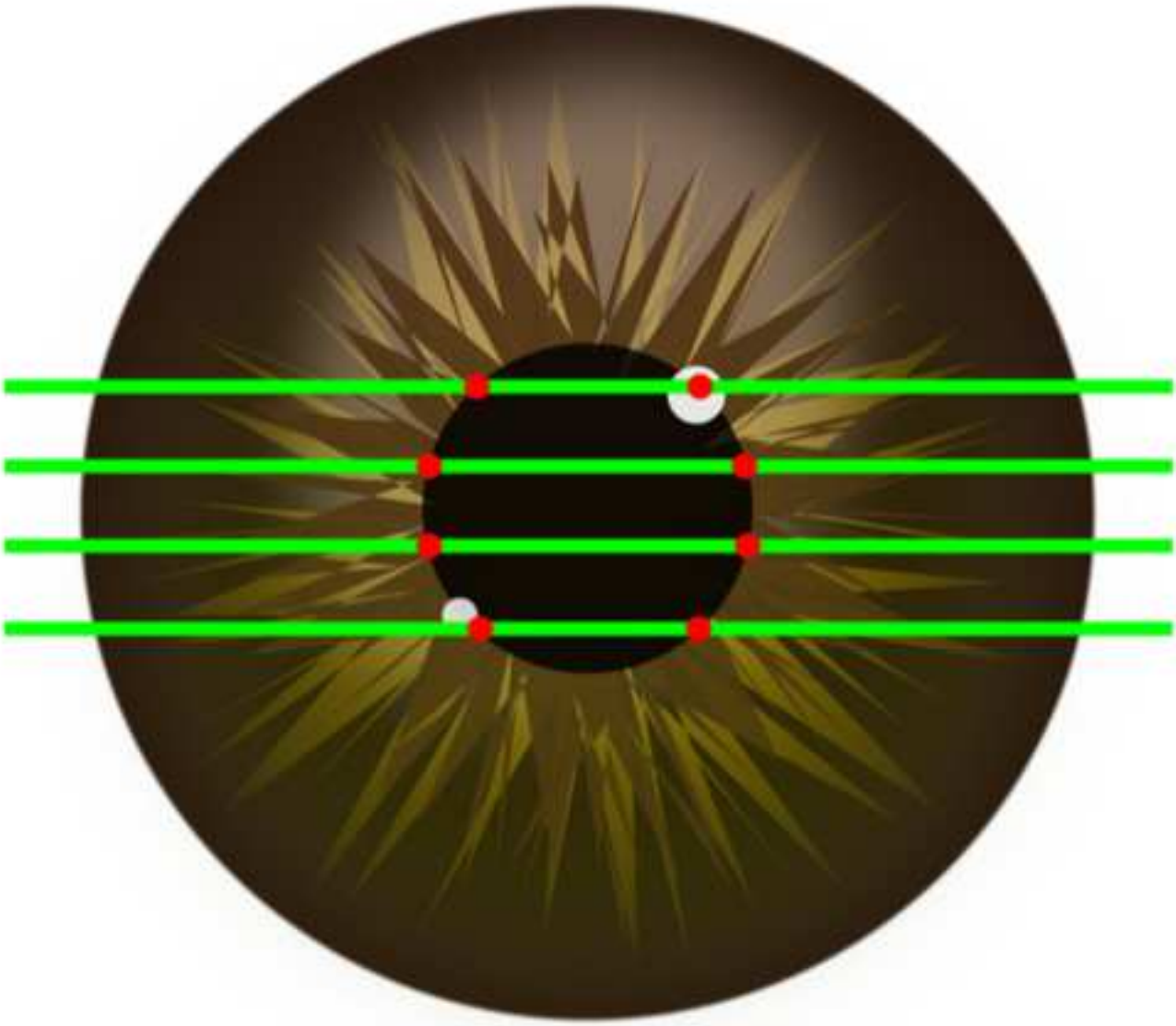

**Figure 2**

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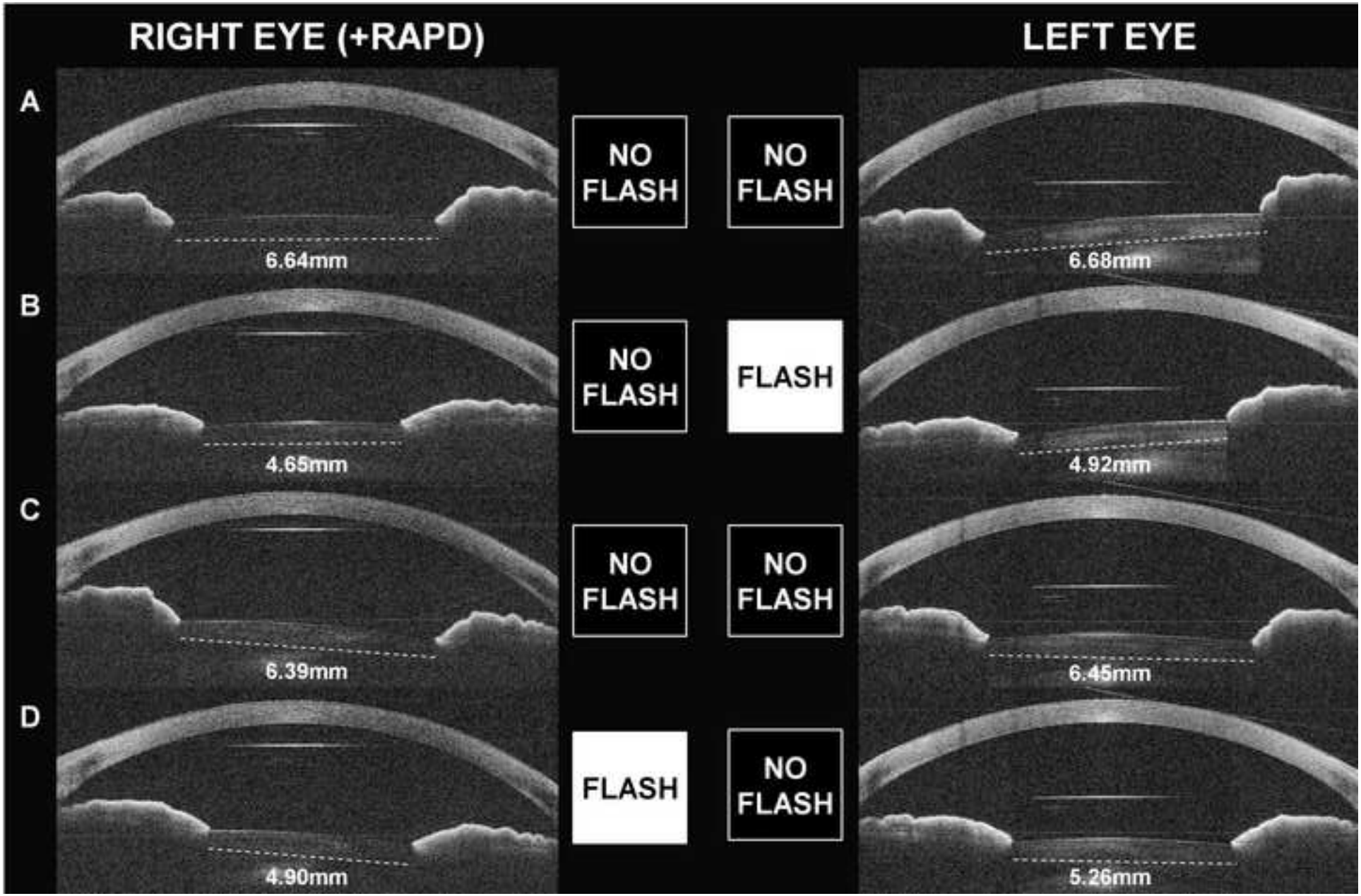

**Figure 3**

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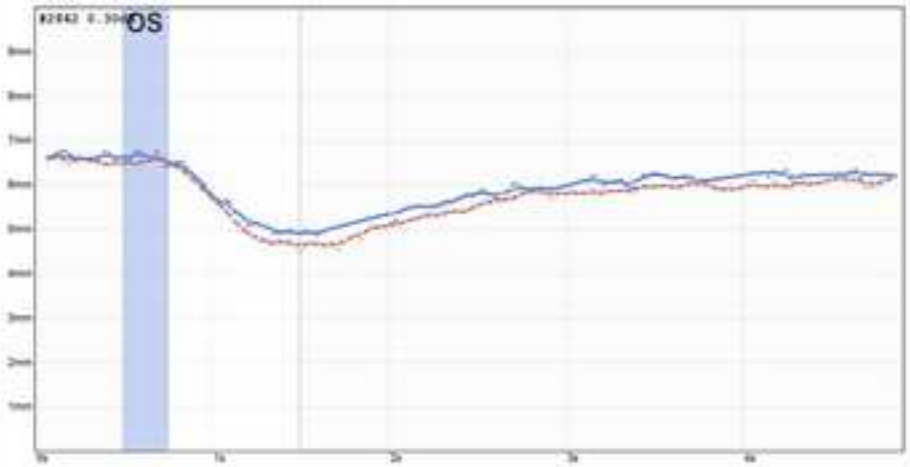


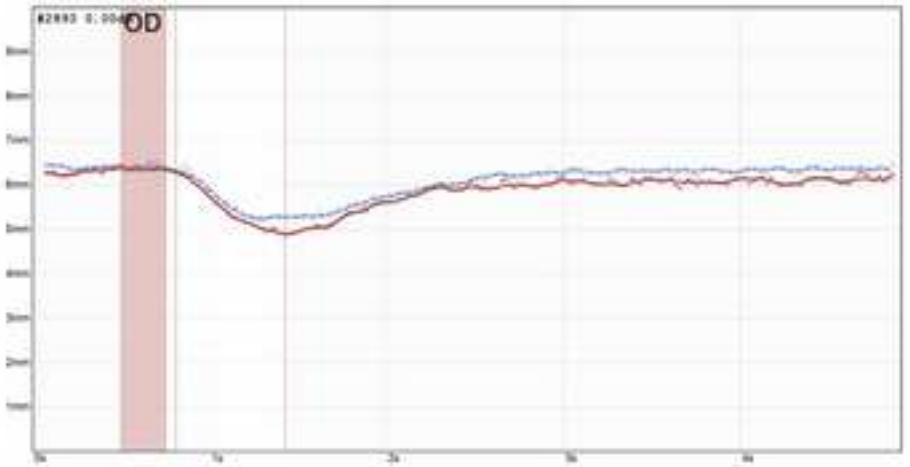


| OS flash | | | OD flash | |
|---|---|---|---|---|
| 0.19 mm (OS) | | Anisocoria | -0.19 mm (OD) | |
| -1.48dB (OS) | | RAPD Abs corrected | 1.48dB (OD) | |
| **Direct (OS)** | **Consensual (OD)** | | **Consensual (OS)** | **Direct (OD)** |
| 6.68 mm | 6.64 mm | **Diameter Maximum** | 6.45 mm | 6.39 mm |
| 4.92 mm | 4.65 mm | **Diameter Minimum** | 5.26 mm | 4.90 mm |
| 1.76 mm | 1.99 mm | **Constriction Amplitude** | 1.18 mm | 1.48 mm |
| 26 % | 30 % | **Constriction Amplitude %** | 18 % | 23 % |
| 0.25 s | 0.26 s | **Constriction Latency** | 0.26 s | 0.31 s |
| 0.75 s | 0.88 s | **Constriction Duration** | 0.52 s | 0.61 s |
| 4.56 mm/s | 4.49 mm/s | **Max Constriction Velocity** | 3.75 mm/s | 3.87 mm/s |
| 2.24 mm/s | 2.25 mm/s | **Avg Constriction Velocity** | 2.30 mm/s | 2.42 mm/s |
| 1.81 mm/s | 1.97 mm/s | **Max Dilation Velocity** | 1.77 mm/s | 2.50 mm/s |
| 0.39 mm/s | 0.46 mm/s | **Avg Dilation Velocity** | 0.31 mm/s | 0.36 mm/s |

Figure 4
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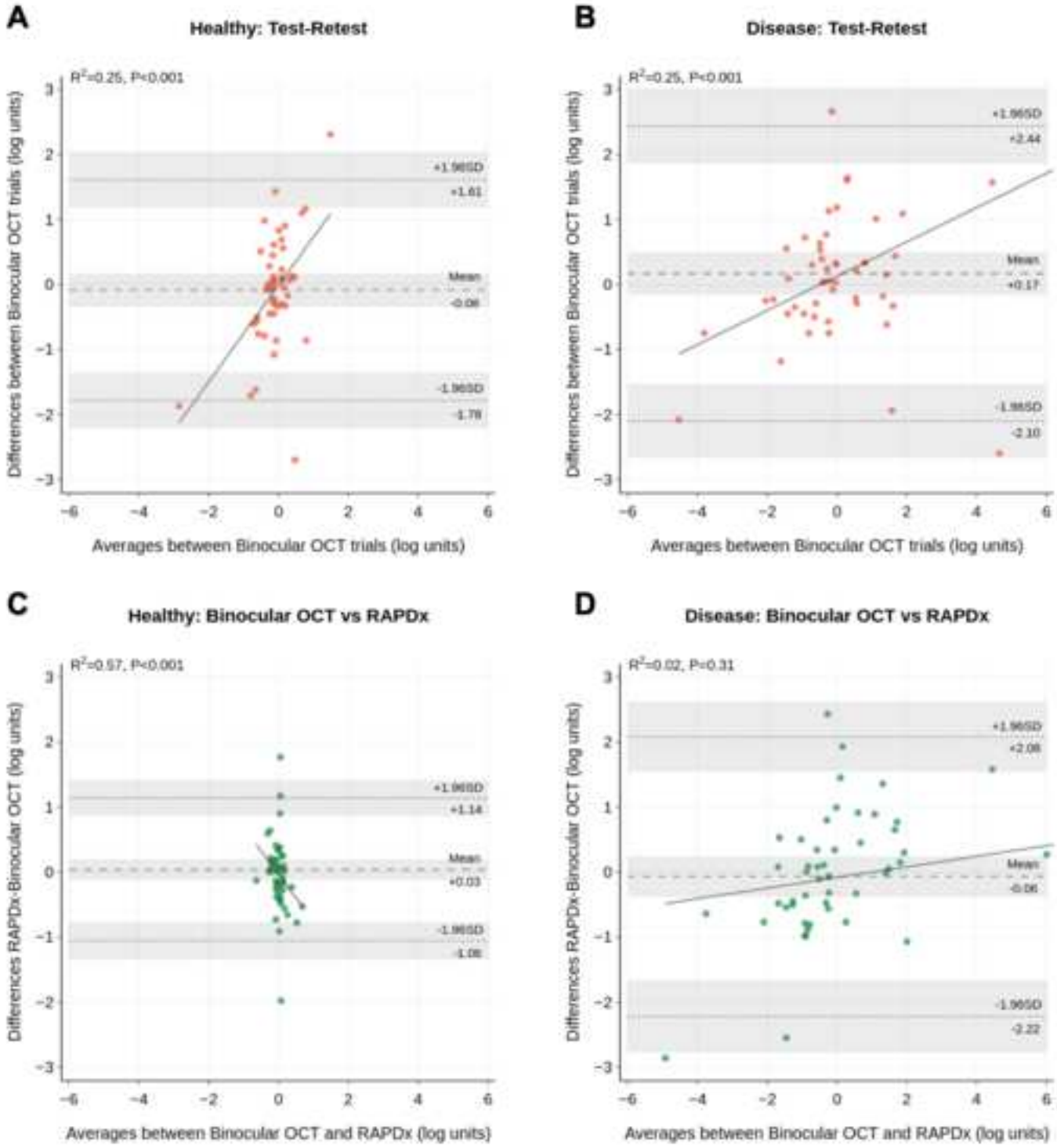

**Figure 5**

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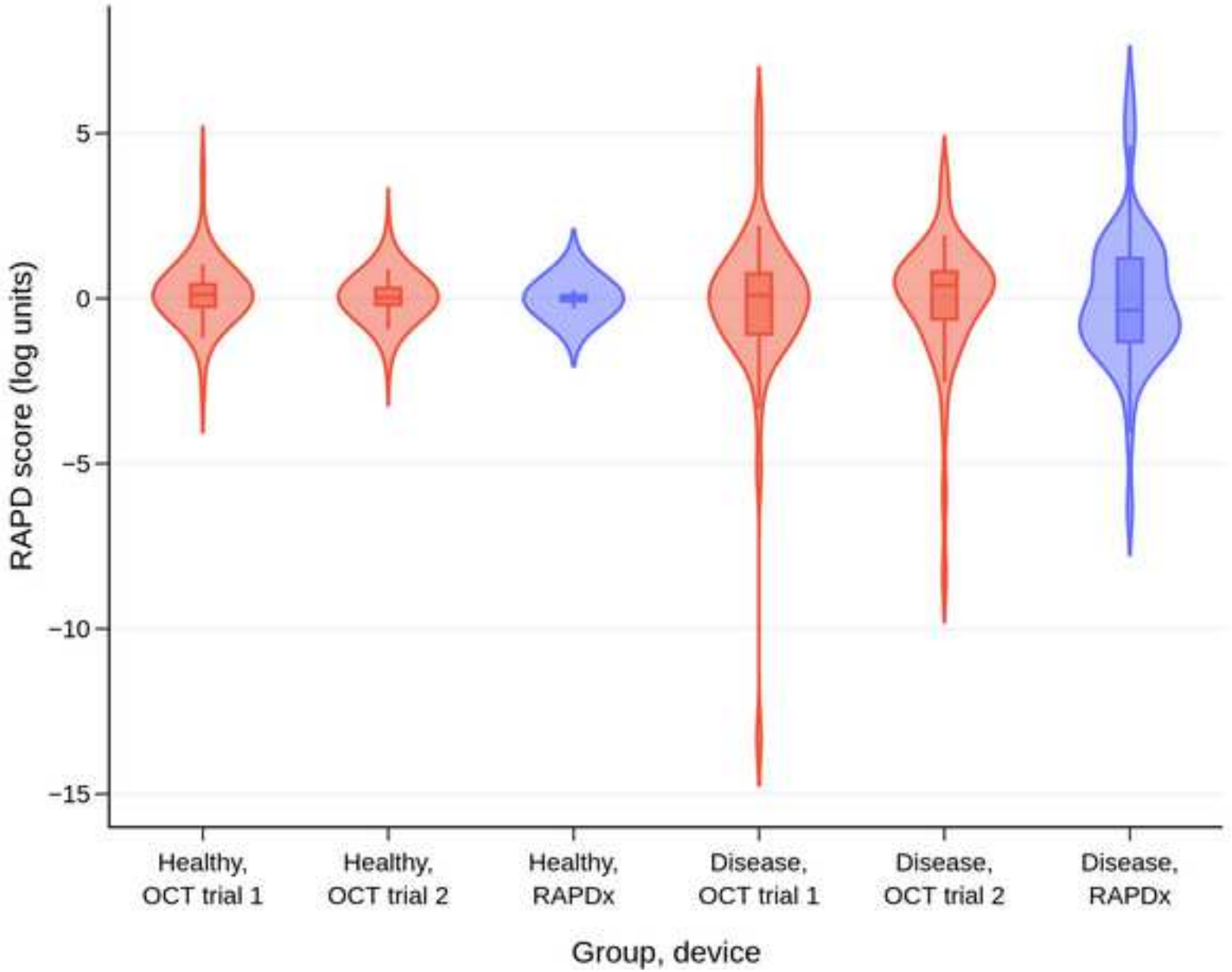

Figure 6

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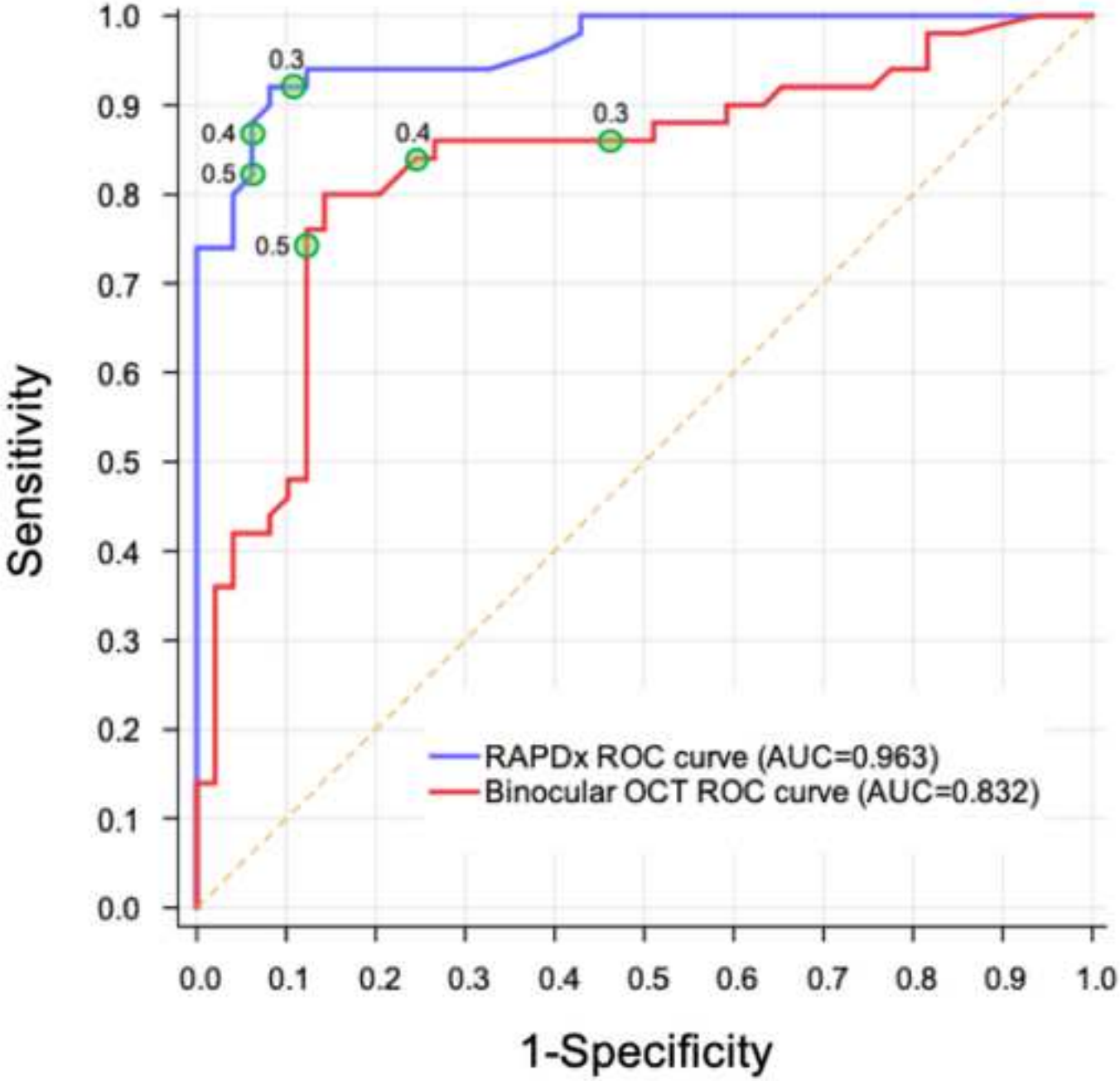

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