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Article

Repeatability of Retinal Sensitivity Measurements Using a Medmont Dark-Adapted Chromatic Perimeter in Healthy and Age-Related Macular Degeneration Cases

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Purpose: To determine the intrasession and intersession test-retest repeatability of retinal sensitivity measurements using a dark-adapted chromatic perimeter (DACP).

Methods: For intrasession testing, retinal sensitivity within the central 24° for the 505-nm stimulus was measured after 20, 30, and 40 minutes of dark adaptation (DA) and for the 625-nm stimulus was measured after the first and second 505-nm tests. For intersession testing, retinal sensitivity for both stimuli was measured after 30 minutes of DA at baseline and 1 month. The point-wise sensitivity (PWS) difference and coefficient of repeatability (CoR) of each stimulus and group were determined.

Results: For intrasession testing, 10 age-related macular degeneration (AMD) and eight control subjects were recruited. The overall CoR for the 505-nm stimulus was 8.4 dB for control subjects and 9.1 dB for AMD cases, and for the 625-nm stimulus was 6.7 dB for control subjects and 9.5 dB for AMD cases. For intersession testing, seven AMD cases and 13 control subjects returned an overall CoR for the 505-nm stimulus of 8.2 dB for the control and 11.7 dB for the AMD group. For the 625-nm stimulus the CoR was 6.2 dB for the control group and 8.4 dB for the AMD group. Approximately 80% of all test points had a PWS difference of ± 5 dB between the two intrasession or intersession measurements for both stimuli.

Conclusions: The CoR for the DACP is larger than that reported for scotopic perimeters; however, the majority of test points had a PWS difference of ± 5 dB between tests.

Translational Relevance: The DACP offers an opportunity to measure static and dynamic rod function at multiple locations with an acceptable reproducibility level.

Introduction

It has been shown that rod function, particularly the dynamic components, becomes abnormal in the early stages of age-related macular degeneration (AMD),¹⁻⁴ and that rod function continues to decline as the severity of the disease increases.^{5,6} These findings have generated interest from many groups in evaluating the utility of rod functional parameters as functional biomarkers of AMD severity and progression.^{5,7,8}

To date, the study of rod function in AMD has been based upon the use of a modified Humphrey

Field Analyzer (HFA; Zeiss Humphrey Systems, Dublin, CA)⁹⁻¹¹ or a dark-adaptation technique, such as the AdaptDx (MacuLogix, Hummelstown, PA),^{12,13} where measurements have been taken at only a single retinal location. However, because progression to late disease starts very locally, it has been postulated that testing rod function at multiple retinal locations will improve the detection of localized changes. Furthermore, studying rod function at multiple retinal locations will provide insight into the time course between functional loss and structural change in the early stages of AMD. Yet, the ability to assess dynamic rod function at multiple

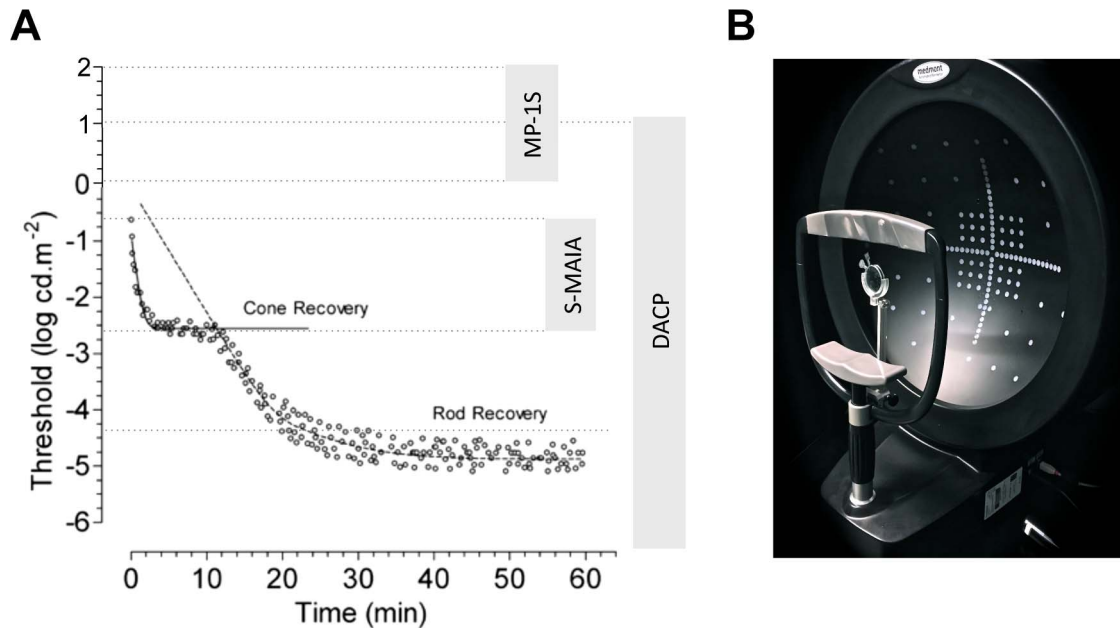


Figure 1. (A) Dark-adaptation curve from a healthy eye and the dynamic ranges of various scotopic perimeters. The DACP (505 nm, cyan stimulus) has the greatest dynamic range compared with the S-MAIA (505 nm, cyan stimulus) and the MP-1S (white stimulus). For the MP-1S, the dynamic range without a neutral density filter is shown. When neutral density filters were applied the minimum stimulus intensity could be further reduced and the dynamic range can be extended. Note that the minimum stimulus intensity of the S-MAIA is around the rod–cone break of the dark-adaptation curve in a healthy eye. (B) The Medmont DACP.

locations within the macula has been limited. Currently, static rod function at multiple locations can be measured using a scotopic microperimeter, such as the Nidek MP-1S (Nidek Technologies, Padova, Italy)^{14–16} or the scotopic Macular Integrity Assessment (S-MAIA; CenterVue, Padova, Italy) microperimeter.^{17,18} However, these systems have a limited dynamic range of test locus luminance, and thus it is difficult to detect subtle changes in rod function in the early stages of AMD, with floor and ceiling effects being problematic.¹⁹ Furthermore, when retinal sensitivity is reduced, it is difficult to determine whether it is a rod- or cone-mediated response with the MP-1S. The S-MAIA has stimuli with two different wavelengths (505 and 627 nm), and in theory has the ability to perform two-color perimetry for assessing rod function.¹⁷ However, the lowest stimulus intensity of the S-MAIA is 0.0025 cd.m^{-2} ($-2.6 \text{ log cd.m}^{-2}$),¹⁸ which is approximately the level of the rod–cone break of the dark-adaptation curve in healthy eyes (Fig. 1A); hence, isolated rod responses can not be guaranteed. Recently, a dark-adapted chromatic perimeter (DACP) was developed (Medmont Pty Ltd International, Victoria, Australia), which has a large dynamic range covering the entire range of dark-adapted rod-mediated function.⁸ A comparison of

the dynamic ranges for DACP, S-MAIA, and MP-1S is shown in Figure 1A.

We have recently reported the utility of the DACP for assessing both the static and dynamic rod function at multiple retinal loci in eyes with intermediate AMD.⁸ Given the clinical potential of the DACP in assessing and monitoring rod function at multiple retinal loci, the aim of this study was to evaluate the intrasession and intersession test–retest repeatability of the DACP in nonneovascular AMD participants, and age-matched controls.

Methods

Subjects with nonneovascular AMD were recruited from existing research cohorts at the Centre for Eye Research Australia (CERA) and private eye clinics. Inclusion criteria for this study were age of at least 50 years and best-corrected visual acuity (BCVA) of 20/60 or better. Participants with AMD had to have drusen of $>125 \mu\text{m}$ with or without any AMD pigmentary changes in both eyes.²⁰ Subjects with evidence of current or past neovascular AMD in either eye were excluded. Control participants were recruited from spouses, friends, and relatives of the AMD participants and staff of CERA or the

University of Melbourne. Participants with no apparent aging changes or normal aging changes (drusen $\leq 63 \mu\text{m}$) were included in the control group. Exclusion criteria for both groups included people with significant ocular media opacity, diabetic retinopathy, glaucoma, severe neck and spinal problems, and having medications that might affect the retinal function. A separate cohort of subjects was recruited for the intrasession and intersession test–retest repeatability. The Human Ethics Committee of the Royal Victorian Eye and Ear Hospital approved the study. Written informed consent conforming to the tenets of the Declaration of Helsinki was acquired from all participants.

Procedures

BCVA and low-luminance visual acuity (LLVA) was assessed using 4-m logMAR chart. Only one eye, the eye with better BCVA, was selected in each subject as the study eye for dark-adapted retinal sensitivity measures. If both eyes had equal vision, the right eye was selected as the study eye. To minimize the variation in the level of background bleaching among the participants the study eye was patched immediately after visual acuities were measured and the eye was dark adapted for 20 minutes. All tests involving exposure to bright light were performed after the DACP tests. During dark adaptation, participants were interviewed with a standardized questionnaire, including history of systemic and eye diseases, smoking status, medications, vitamins, and supplements being taken to rule out any systemic or eye conditions that may affect vision. Multimodal imaging was performed after DACP in all participants to confirm the clinical classification of AMD.

Dark-Adapted Retinal Sensitivity Measurement

Dark-adapted retinal sensitivity was measured with a Medmont DACP, which is a blackened bowl perimeter with 135-fixed test locations covering a field up to 144° horizontally and 72° vertically (Fig. 1B). In our study, the perimetric test grid consisted of 28 test points spaced within the central 24° of the retina with a stimulus size of 1.73° (Goldmann size V). Test points of 505-nm wavelength stimulus (dynamic range, 0–75 dB) were distributed at 4° , 5.7° , 6° , 8° , 12° , 16.7° , and 24° eccentricity to the fovea, and test points of 625-nm wavelength stimulus (dynamic range, 0–50 dB) were distributed at 2° , 4° , 5.7° , 8° , 12° , 16.7° , and 24° eccentricity to the fovea (see the

polar plots in the Results). Stimuli were presented with a duration of 200 ms and retinal sensitivities were determined using a 4-2 staircase threshold strategy.

Retinal sensitivity was measured monocularly on the study eye, with the fellow eye patched. Pupils were dilated to at least 6 mm with tropicamide 0.5%. Spherocylindrical lens correction was inserted into a lens holder with the refractive correction set-up for a viewing distance of 30 cm. Participants were instructed to fixate at a small dim red fixation light at the center of the test grid, and fixation was monitored throughout testing using an infrared camera. To minimize the intersubject variability, all participants received the same instructions before performing the test. Retinal sensitivity for the 505-nm stimulus was measured first followed by the 625-nm stimulus. For the 505-nm stimulus, measurement of dark-adapted retinal sensitivities commenced at exactly 20 minutes (test 1) after dark adaptation, and the measurements were repeated at exactly 30 (test 2) and 40 (test 3) minutes after the initial dark-adaptation. For the 625-nm stimulus, measurement of dark-adapted retinal sensitivities was performed twice, one after test 1 of the 505-nm stimulus and another after test 2 of the 505-nm stimulus. A short break was provided between measurements to avoid fatigue and to ensure an accurate time frame for the next measurement. Each measurement took approximately 3 to 4 minutes. Retinal sensitivity data of each test within the session were used to determine the intrasession test–retest repeatability.

For the intersession test–retest repeatability retinal sensitivities for the 505-nm stimulus followed by the 625-nm stimulus were measured at 30 minutes after dark-adaptation. The 30 minutes of dark adaptation was chosen because we learned in the intrasession testing that by 30 minutes the retina was dark adapted for both wavelengths to a point that there was no significant difference in sensitivity between the two test time points. Participants were invited to return to the clinic 4 ± 2 weeks after the initial testing, for repeated DACP measurements. Retinal sensitivity data of each visit were used to determine the intersession test–retest repeatability.

Multimodal Imaging and Clinical Grading

Multimodal imaging was performed after the DACP testing was complete. Near-infrared reflectance, short-wavelength fundus autofluorescence, optical coherence tomography (Spectralis HRA-OCT; Heidelberg Engineering, Heidelberg, Germany), and color fundus photography (Canon CR6-

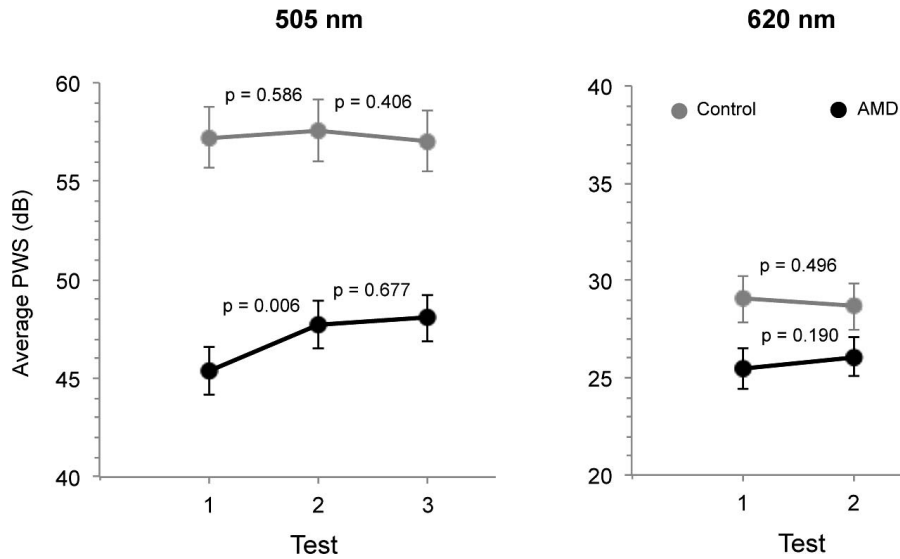


Figure 2. Average PWS of each test for the 505- and 625-nm stimuli. Note that for the 505-nm stimulus, there was a significant increase in the average PWS between test 1 (20 minutes after dark-adaptation) and 2 (30 minutes after dark-adaptation) but not between test 2 and 3 (40 minutes after dark adaptation) in the AMD group. Error bars: 95% confidence interval.

45NM; Canon, Saitama, Japan) were performed to confirm the clinical classification of AMD. Two graders performed the AMD grading according to the Beckman Classification and Grading System.²⁰

Analysis

The outcome parameters were the sensitivity of each individual test point (point-wise sensitivity, PWS). Data of the repeated sensitivity measurements for the 505- and 625-nm stimuli within the test session were used to determine the intrasession test–retest repeatability. Retinal sensitivity data from the first and second visit at 30-minutes dark adaptation were used to determine the intersession test–retest repeatability. Changes in average PWS between the two time points were determined using a linear mixed-effects model, with test number as the fixed effect and test points nested within an eye as a random effect. Bland-Altman plots were used to inspect the test–retest characteristics and the test–retest coefficients of repeatability (CoR) were calculated. The CoR represents a score for which 95% of the test–retest differences are expected and is influenced by the measurement error of the instrument and subjective variability. Due to a small sample size, a bootstrap resampling procedure with 1000 repetitions was also used to evaluate the overall CoR. A larger value of CoR represents a greater degree of test–retest variability. The cumulative percentage of PWS

difference between the tests was also generated to examine the level of repeatability.

Results

Eighteen study eyes of 18 subjects were included in the intrasession test–retest repeatability component. Of 18 eyes, 8 eyes were healthy control eyes, and 10 eyes had AMD (9 eyes with intermediate AMD, 5 had reticular pseudodrusen, and 1 with noncentral geographic atrophy [GA]). The mean age was 70.8 ± 10.0 (range, 58–81) years in healthy control group and 73.1 ± 5.2 (range, 66–80) years in AMD group ($P = 0.561$).

Intrasession Test–Retest Repeatability

The average PWS of each test for the 505- and 625-nm stimuli is shown in Figure 2. For the 505-nm stimulus, there was a significant increase in average PWS between test 1 and 2 ($P = 0.006$) but not between test 2 and 3 ($P = 0.667$) in the AMD group. Thus, the intrasession test–retest repeatability for the 505-nm stimulus was only performed between tests 2 and 3. For the 625-nm stimulus, there was no significant change in the average PWS between test 1 and 2 in either the AMD or control group. Hence, the intrasession test–retest repeatability for the 625-nm stimulus was performed between tests 1 and 2.

Bland-Altman plots for the 505- and 625-nm stimuli of the control and AMD groups are shown

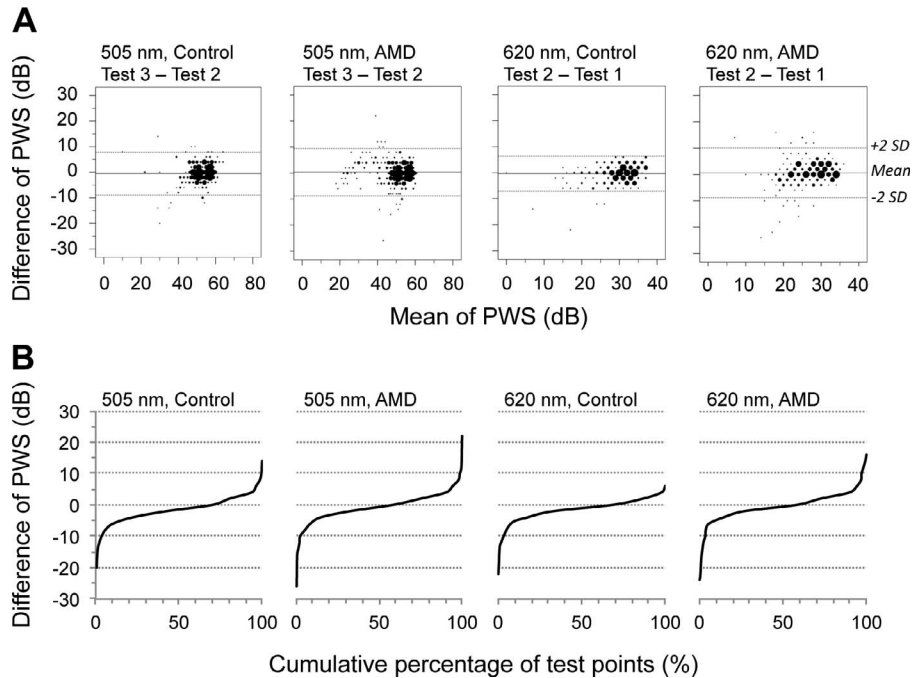


Figure 3. Bland-Altman plots of PWS for the 505- and 625-nm stimuli (A). There was no association between the difference and magnitude of PWS in any of the examination pairs. The AMD group had a greater limit of agreement compared with the control group for both stimuli. Note that the *larger dots* represent greater overlapping of test points. Cumulative percentage of PWS difference (B) showed that approximately 80% of all test points had a PWS difference of ± 5 dB between the two tests.

in Figure 3A. The AMD group had a greater limit of agreement (i.e., more variable) compared with the control group for both stimuli. The cumulative percentage of PWS difference plots showed that approximately 80% of all test points in both the control and AMD groups had a difference of ± 5 dB between the 2 tests (Fig. 3B).

For the 505-nm stimulus, the overall CoR for the intrasession test–retest repeatability was 8.4 dB for the control subjects and 9.1 dB for AMD cases. For the 625-nm stimulus, the CoR was 6.7 dB for the control group and 9.5 dB for the AMD group. When

Table. Coefficient of Repeatability Per Ring Eccentricity

Ring	505 nm		625 nm		
	Control	AMD	Control	AMD	
4	8.5	6.5	2	5.6	7.0
5.7	7.6	8.3	4	4.8	5.9
6	6.7	8.3	5.7	6.2	5.8
8	5.9	8.5	8	5.8	7.1
12	9.2	12.1	12	7.5	11.3
16.7	9.0	10.2	16.7	6.4	9.4
24	10.9	8.9	24	9.8	15.7

applying the bootstrap resampling method with 1000 times repetitions to address issues associated with a small sample size, the overall CoR of both stimuli in both groups was markedly reduced. For the 505-nm stimulus, the CoR of both the control and AMD groups was 2.8 dB. For the 625-nm stimulus, the CoR for the control group was 2.2 dB and for AMD group was 2.7 dB.

To examine the relationship between the level of repeatability and test locations the CoR of each ring eccentricity and each test point was calculated. The CoR of each ring eccentricity for the 505- and 625-nm stimuli is shown in the Table. The CoR was greater in the peripheral rings compared with the central rings. The CoR for each of the 28 test points for the 505 and 625 nm in controls and AMD groups is shown in Figure 4.

Intersession Test–Retest Repeatability

Twenty eyes of 20 participants (13 control and 7 nonneovascular AMD subjects) were included. Of seven AMD eyes, six eyes had intermediate AMD and one had a noncentral GA. There was no significant difference in average age between the control (65.2 ± 6.5 years, range, 59–79) and AMD group (70.7 ± 6.2 years, range, 62–81, $P = 0.083$). The average time

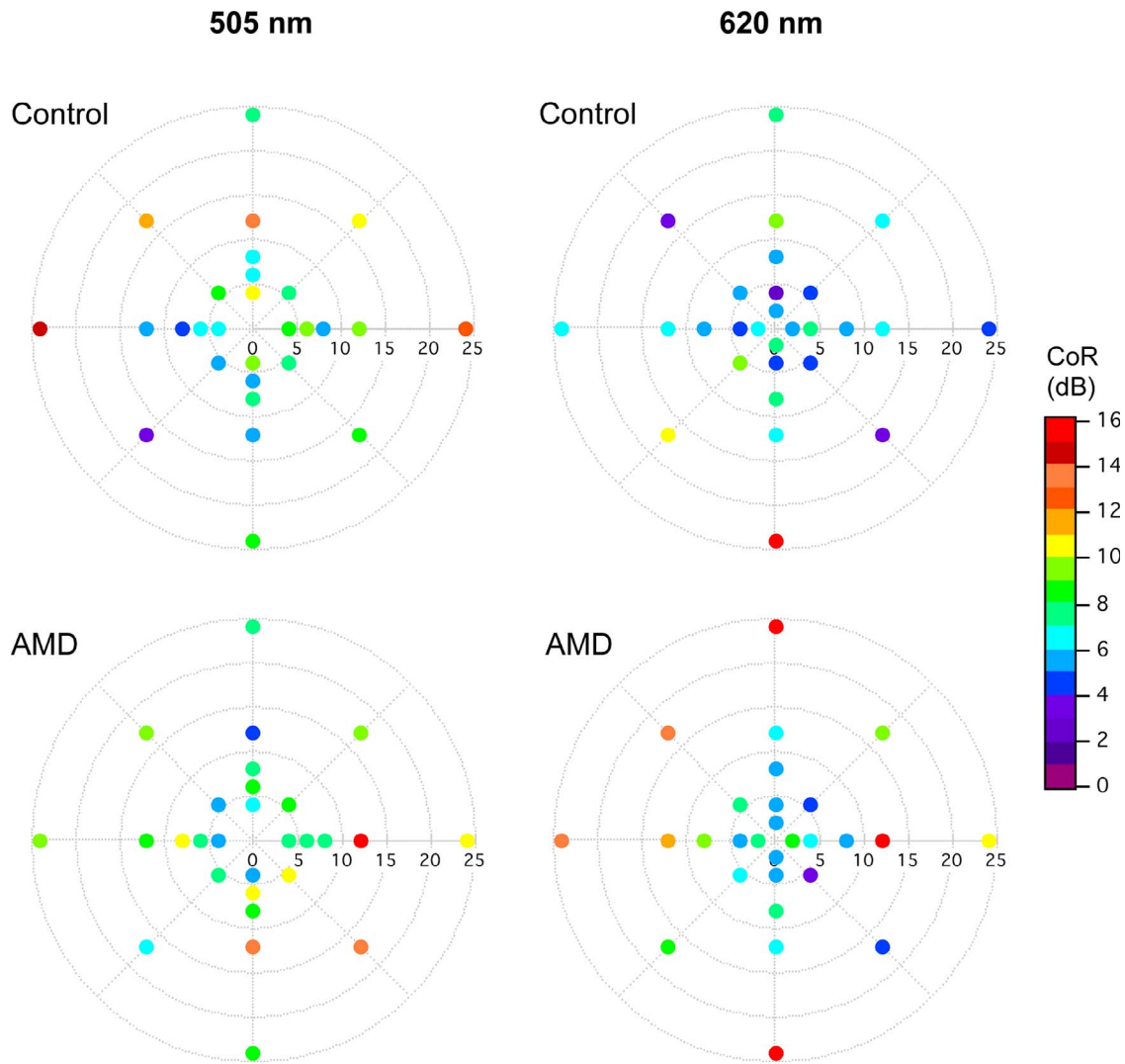


Figure 4. Coefficient of repeatability of each test point for the 505- and 625-nm stimuli. Plots are presented in retinal view and referenced to the right eye. Note that central test points ($<10^\circ$) are more repeatable than the peripheral test points.

between the visits was 4.5 weeks for the control subjects and 4.3 weeks for the AMD subjects ($P = 0.803$).

The average PWS of each visit for the 505- and 625-nm stimuli are shown in [Figure 5](#). There was no significant change in average PWS between the visits for both stimuli and the study groups.

Bland-Altman plots for the 505- and 625-nm stimuli of the control and AMD groups are shown in [Figure 6A](#). The cumulative percentage of PWS difference plots show that approximately 80% of all test points in both the control and AMD groups had a difference of ± 5 dB between the two visits ([Figure 6B](#)).

For the 505-nm stimulus, the overall CoR of the intersession test was 8.2 dB for the control and 11.7 dB for the AMD group. The CoR of the 625-nm

stimulus was 6.2 dB for control subjects and 8.4 dB for AMD cases. When a bootstrap resampling procedure was applied with 1000 repetitions, the CoR for 505-nm wavelength was improved to 2.1 dB for the control group and 4.2 dB for the AMD group. For the 625-nm stimulus, the CoR of the control and AMD groups was improved to 1.7 dB and 2.9 dB, respectively.

To examine the relationship between the level of repeatability and test locations the CoR of each test point was calculated. The CoR of each test point for the 505 and 625nm for the control and AMD group is shown in [Figure 7](#), which again showed that central test points are more repeatable than the peripheral test points. Unlike the intrasession test where the CoR of the AMD and control groups were similar, the

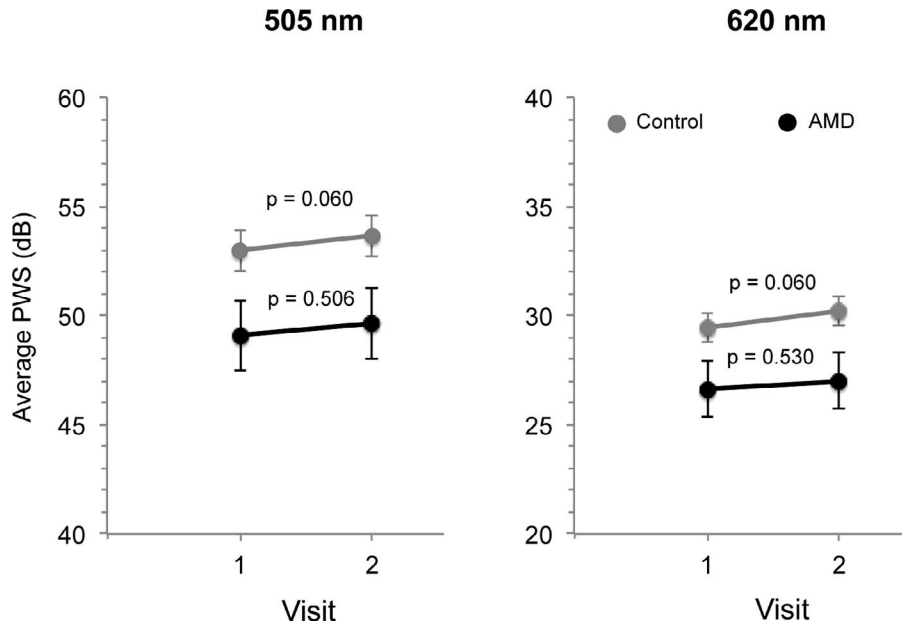


Figure 5. Average PWS of each visit for the 505- and 625-nm stimuli. No significant change in average PWS between the visits was detected for both the 505- and 625-nm stimuli. Error bars: 95% confidence interval.

CoR of the intersession test for the AMD group was much greater than that of the control group. Further examination of the data showed that one's subject drove the high variability. When the data of that subject were removed from the analysis the overall CoR for the AMD group improved to 8.9 dB.

Discussion

Parameters pertaining to the process of dark adaptation may provide important functional markers in evaluating the progression of AMD when vision

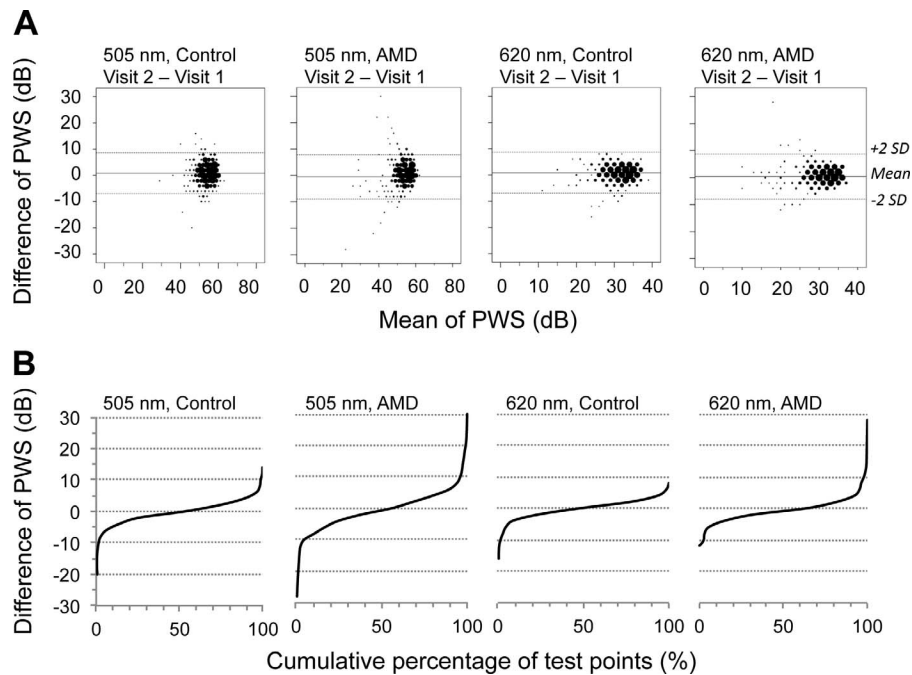


Figure 6. Bland-Altman plots of PWS for the 505- and 625-nm stimuli (A). The AMD group had a greater limit of agreement compared with the control group for both stimuli. Cumulative percentage of PWS difference (B) showed that approximately 80% of all test points had a PWS difference of ± 5 dB between the two visits.

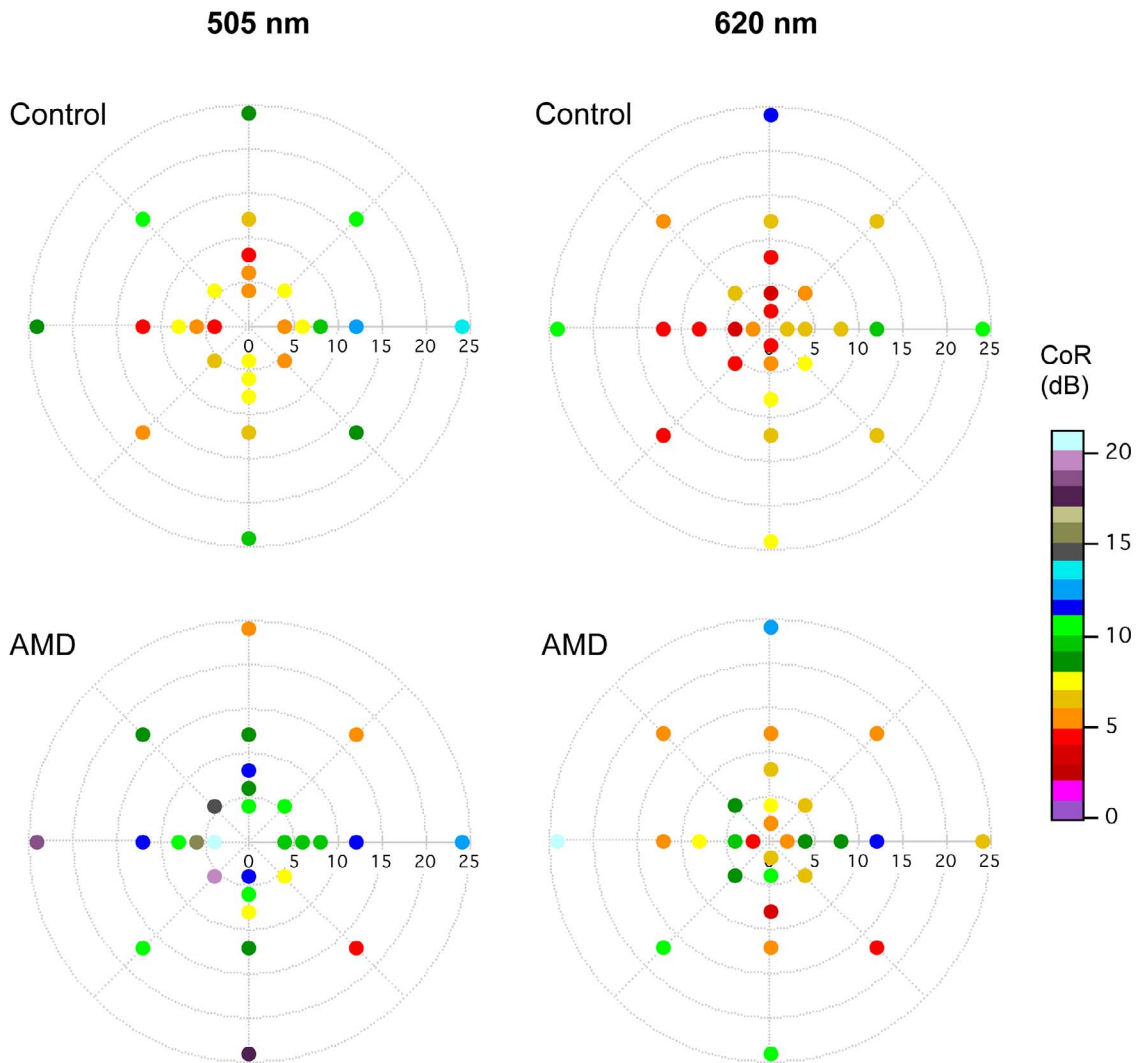


Figure 7. Coefficient of repeatability of each test point for the 505- and 625-nm stimuli. Plots are presented in retinal view and referenced to the right eye. In general, central test points ($<10^\circ$) are more repeatable than the peripheral test points.

is still good. The DACP is a novel and unique perimeter that has two-color stimuli and has the required dynamic range of test locus luminance to measure subtle changes in retinal sensitivity early in the disease process. However, as with all subjective tests, there is variability, and a potential learning effect that reduces the robust nature of the parameters when used as a biomarker. Our aim was to determine the robustness of the sensitivity data collected using this new DACP, by examining the intrasession and intersession test–retest repeatability in AMD patients and aged-matched control participants. We found that approximately 80% of all test points had a PWS difference of ± 5 dB between the two intrasession or intersession measurements for both stimuli and in both study groups. We also found that test points

within the central retina ($<10^\circ$) are more repeatable than test points in the peripheral retina.

We found that there was a significant improvement in retinal sensitivity for the 505-nm stimulus between 20 and 30 minutes of dark-adaptation in the AMD group but not in the control group, suggesting that dark-adaptation was still occurring after 20 minutes of patching in the AMD group. This highlights the importance of the duration of patching, particularly in AMD patients before measuring the scotopic sensitivity. The duration of patching should be taken into account when comparing the scotopic sensitivity data or if dark-adapted retinal sensitivity is considered as a parameter of disease severity.

Pfau et al.¹⁷ assessed the intrasession test–retest repeatability of a scotopic MAIA in young, healthy

controls (median age of 38.8 years) and reported that the CoR for all test points within the central 7° was 5.26 dB for the 505-nm stimulus and 4.06 dB for the 627-nm stimulus. Our data showed that the overall CoR of all test points within the central 24° in healthy control subjects with an average age of 65.2 years was 8.4 dB for the 505-nm stimulus and 6.7 dB for the 625-nm stimulus. When analyzing only test points within the central 8°, the CoR reduced to 7.2 dB for the 505-nm stimulus and 5.6 dB for the 625-nm stimulus. Thus, the CoR of both the 505- and 625-nm stimuli of the DACP was slightly greater than that of the S-MAIA but our cohort was significantly older. However, when comparing CoR difference between these two devices, it should be noted that the dynamic range available in DACP is 2.5 to 3.75 times larger (50 dB for 625-nm and 75 dB for 505-nm wavelengths) than the dynamic range of the S-MAIA (20 dB); therefore, the CoR of the DACP is likely to be intrinsically greater. In addition, the limited dynamic range of the S-MAIA is not sufficient to cover the entire range of dark-adapted rod-mediated function, and thus likely to be affected by a ceiling effect (see Fig. 1A).

In this study, we found that the intersession CoR of the AMD group is much larger than that of the control group, especially for the 505-nm stimulus. Possible explanations for this large CoR in the AMD group include variability in the level of exposure to the ambient light before testing and a higher variability in rod recovery in eyes with AMD. Thus, scotopic retinal sensitivity following patching may not be a robust biomarker for monitoring rod function in longitudinal studies in subjects with AMD. These limitations could be overcome by assessing rod functional dynamics after photobleach.

Previously, it has been reported that a ceiling and floor effect was found when evaluating scotopic sensitivity using a MP-1S or the S-MAIA.^{17,19} In our study, we did not observe any ceiling and floor effects using the DACP either in healthy and non-neovascular eyes due to the large dynamic range of light intensity with the new instrument.

Currently, there is a great interest in developing an effective functional biomarker of AMD severity in the early stages of the disease, as the prospects for early intervention studies become a reality. BCVA is a poor marker of disease severity in the early stages of AMD as BCVA is often unchanged until late in the disease process.^{7,21} Many other visual function tests such as LLVA, contrast sensitivity, reading speed, flicker sensitivity, and retinal photopic or mesopic sensitivity

have been assessed in patients with various stages of AMD^{7,22–25}; however, these tests do not specifically assess rod function, which is known to be abnormal early in the disease process with many patients often describing difficulty seeing in dim light and having delayed ability to see in the dark.^{26,27} Psychophysical studies have demonstrated a decrease in scotopic sensitivities and slow recovery of dark adaptation in AMD cases compared with healthy control subjects.^{3,4,8} These findings are consistent with histologic evidence showing a decline in the number and density of rods in AMD.^{28,29} Given the large dynamic range and the repeatability levels of the DACP, further investigation on the utility of the DACP for monitoring AMD progression is warranted. One way to potentially overcome the effect of measurement variability on detecting longitudinal changes in sensitivity is to examine the data using the cumulative percentage of PWS rather than comparing the change in average retinal sensitivity over time. It is expected that an improvement in sensitivity at follow-up would be associated with an upward shift of the sigmoid curve. Alternatively to minimize the variability in the measurements would be the use of rod dynamic parameters, such as the rod intercept time or the rod recovery rate after photobleaching.

The strength of our study was a strict protocol adherence with regard to time of patching and performing the tests with all participants performing the test at the same time after adaptation. The weakness of the study included a relative small sample size, particularly the AMD cases, which may over estimate the CoR. Although the DACP is not equipped with fundus tracking, the system has an infrared camera to monitor the eye's position. Fundus tracking undoubtedly offers some benefits, particularly in cases where there is worse vision and difficulty in fixating. However, because the use of this test is likely to be more applicable in the early stages where vision is good, fixation should still be steady. Currently, none of the available instruments can offer both fundus tracking capability and a large dynamic range due the design and type of stimulus display. Thus, the DACP offers an exciting addition to other functional tests that need to be further validated in larger longitudinal studies.

In conclusion, AMD eyes have not fully recovered after 20 minutes of patching; thus, the duration of patching needs to be considered when using scotopic sensitivity data. Using the novel DACP, we found that approximately 80% of all test points had a PWS difference of ± 5 dB between the two intrasession or

intersession measurements for both stimuli wavelengths and in both study groups. The CoR of the AMD group was slightly greater than that of the control group and that test points within the central 10° rings had a smaller CoR than test points in the peripheral rings. DACP offers an opportunity to measure rod function at multiple locations in early stages of AMD.

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