Ophthalmologica

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Short title (Running head)	Repeatability of Scotopic Fundus-Controlled Perimetry
Section title	Original Paper
Issue ID	000
Citation line	Ophthalmologica
DOI: 10.1159/000	453079
Article title	Test-Retest Reliability of Scotopic and Mesopic Fundus-Controlled Perimetry Using a Modified MAIA (Macular Integrity Assessment) in Normal Eyes
Subtitle	

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Received	Received: September 5, 2016
Accepted	Accepted after revision: November 3, 2016
Revised	
Published Online	Published online: ■■■

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Keywords	Microperimetry Rod function Cone function Dark adaptation Bland-Altman plots Healthy eyes

Abstract

Purpose: To assess the intrasession test-retest reliability of scotopic cyan and scotopic red fundus-controlled perimetry (FCP) in normal subjects using a modified MAIA "microperimeter" (macular integrity assessment) device. **Methods:** Forty-seven normal eyes of 30 subjects (aged 33.8 years) underwent duplicate mesopic (achromatic stimuli, 400-800 nm), scotopic cyan (505 nm), and scotopic red (627 nm) FCP, using a grid of 49 stimuli over 14° of the central retina. Test-retest reliability for pointwise sensitivity (PWS), stability of fixation, reaction time and test duration were analyzed using mixed-effects models. **Results:** PWS test-retest reliability was good among all 3 types of retinal sensitivity assessments (coefficient of repeatability of 4.75 dB for mesopic, 5.26 dB for scotopic cyan, and 4.06 dB for scotopic red testing). While the mean sensitivity decreased with eccentricity for both mesopic and scotopic red testing, it was highest at 7° eccentricity for the scotopic cyan assessment ($p < 0.001$). **Conclusions:** The modified MAIA device allows for reliable scotopic FCP in normal subjects. Our findings suggest that testing of scotopic cyan sensitivity largely reflects rod function.

Body

Introduction

Tremendous progress has been achieved since the first description of fundus-controlled perimetry (FCP) in 1851 by Hermann von Helmholtz [1]. Early approaches based on direct ophthalmoscopy or direct slit-lamp ophthalmoscopy were highly examiner dependent and allowed only photopic testing due to high levels of light necessary for retinal illumination [1–5]. Today, using infrared light and scanning laser ophthalmoscopy (SLO), it has become possible to reduce the background illumination for FCP to a degree

which has opened the door for a topographic point-by-point assessment of scotopic function [6–8].

Multiple retinal diseases are associated with rod photoreceptor functional impairment including pseudoxanthoma elasticum [9], macular telangiectasia type 2 [10], retinitis pigmentosa [11–13], and rod-cone dystrophies [14]. Moreover, histological evidence suggests that rod loss precedes cone loss in both exudative and nonexudative age-related macular degeneration (AMD) [15, 16] – the leading cause of legal blindness in developed countries [17]. In addition to rod loss, cone degeneration has also been observed histologically in AMD [16]. Psychophysical data in patients with early and late AMD have further underlined that rod dysfunction exceeds cone dysfunction, whereby the underlying mechanisms for the greater vulnerability of rods versus cones are largely unknown [18–24]. Cone-specific dysfunction is also detectable in AMD both psychophysically and with objective methods such as multifocal pupillographic perimetry or multifocal electroretinography [25–29].

The first commercially available device to perform automated dark-adapted perimetry was a modified first-generation Humphrey Field Analyzer (HFA, Carl Zeiss Meditec Inc., Dublin, CA, USA) [30]. This device did not correct for fixation (i.e., not fundus-controlled), which was likely the reason for the inconclusive results for structure-function correlation analyses in patients with AMD [18]. Later, it was demonstrated that scotopic sensitivity loss exceeded photopic sensitivity impairment in areas with increased levels of fundus autofluorescence [31]. Secondly, the no longer commercially available scanning laser ophthalmoscope (SLO 101, Rodenstock, Ottobrunn, Germany) has been used for scotopic FCP; however, only a single congress abstract has been available so far to the best of our knowledge [Van de Velde FJ, et al. IOVS 1993;34:ARVO Abstract 3542]. Thirdly, a modified version (MP-1S) of the well-established MP-1 microperimeter (Nidek Technologies, Padua, Italy) has become available in the meantime.

While many reports on mesopic function with the MP-1 device are available, only a few reports have been published on scotopic FCP using the MP-1S [20, 21, 24, 32–35]. For example, we have recently shown that the presence of reticular drusen is spatially confined to outer retinal thinning and impairment of scotopic function [21]. Moreover, a correlation of the extent of outer retinal thinning and the extent of scotopic sensitivity loss was shown. Another modified version of MP-1 has also been used for fundus-controlled two-color perimetry and dark adaptometry [36, 37]. However, the dynamic range of the stimulus-presenting LCD display of the MP-1 (20 dB) is considered to be low and prone to

ceiling and floor effects in both mesopic and scotopic examinations [20, 32, 33]. Moreover, the quality of the reference image (infrared fundus camera) has been reported to be low, making reliable detection of retinal pathologies and test pattern placement challenging [38].

Hence, there is an unmet need for a reliable scotopic SLO-based FCP system in both clinical and research settings. A modified version of the well-established macular integrity assessment (MAIA, CenterVue, Padua, Italy) device with two additional projection LEDs and the possibility to reduce the SLO laser power for scotopic testing has recently become available. It allows for mesopic FCP testing with achromatic stimuli as well as scotopic testing with both cyan (505 nm) and/or red (627 nm) stimuli. While the scotopic cyan testing is intended to be largely derived from rod photoreceptor-mediated function, also excluding S-cone activity, the scotopic red would be more influenced by cone-mediated function, rather reflecting a mixture of both rod- and cone-mediated responses [39–41].

The aim of the study was to assess the test-retest reliability of scotopic cyan and scotopic red FCP in normal subjects using a modified MAIA device. Specifically, we evaluated pointwise sensitivity (PWS), fixation stability and reaction time with regard to reliability. The results were also compared to mesopic testing performed during the same session using the same device. Furthermore, the differences in retinal sensitivity and reaction time among the types of testing were analyzed with regard to photoreceptor mediation (cone vs. rod). Finally, we discuss the results of test-retest reliability in comparison to previous reports of mesopic testing using the MAIA device.

Methods

Subjects

This study was designed to enroll 30 subjects with at least 1 eye with no known retinal disease at the Department of Ophthalmology, University of Bonn, Germany. Subjects were recruited from inpatients, family members of inpatients, medical students and colleagues. The inclusion criterion for study eyes was a best-corrected visual acuity of 6/6 as assessed by autorefraction (ARK-560A, Nidek, Gamagori, Japan). Exclusion criteria were refractive errors >5.00 dpt of spherical equivalent and >1.50 dpt of astigmatism as well as a history of glaucoma, retinal diseases, uveitis, diabetes, a history of prior vitreoretinal surgery or retinal laser treatment. If both eyes met the inclusion criterion, both eyes underwent the full imaging and functional testing protocol in random

order. Apart from taking the medical history, spectral domain optical coherence tomography raster scanning was performed using a 30 × 25° scan field (61 B-scans, automated real-time mode 20 frames, centered on the fovea, Spectralis OCT2, Heidelberg Engineering, Heidelberg, Germany).

The study was approved by the Institutional Review Board of the University of Bonn (ethics approval ID: 191/16). After having explained the nature and possible consequences of the study, informed written consent was obtained from all subjects. The protocol followed the tenets of the Declaration of Helsinki.

Fundus-Controlled Perimetry

All study eyes underwent 2 mesopic as well as 2 scotopic cyan and 2 scotopic red FCP tests of the central retina using a modified MAIA device (software version 2.0.4). The basic design of the MAIA platform has been described previously [42]. Briefly, it employs confocal SLO with a central wavelength of 850 nm (36.5 × 36.5°, 25 frames per second) for fundus tracking. In the current study, a testing grid with 49 stimuli was used. The stimuli grid distribution contained 4 concentric rings with 12 evenly distributed stimuli (angular distance of 30° to each other), separated at 1, 3, 5 and 7° from a central stimulus (Fig. 1). Prior to testing, pupil dilatation was performed using 1.0% tropicamide.

A 645-nm red ring with 1° diameter size was used as target of fixation (with the preset luminance for mesopic or the preset luminance for scotopic testing). Goldmann size III stimuli were applied, directly projected on the retina by means of LED projectors and each presented for 200 ms. For mesopic testing, achromatic stimuli (400--800 nm) were presented using a 4--2 staircase threshold strategy, while patients observed the fixation target against a background of 1.27 cd/m^2 (4 asb) [43, 44]. The minimum and maximum luminance of the stimuli were 0.08 cd/m^2 (0.25 asb) and 318 cd/m^2 (1,000 asb), respectively, for a dynamic range of 36 dB. For scotopic testing, 2 different LED types were used -- cyan (505 nm) and red (627 nm) -- and projected with a 2-1 staircase threshold strategy, while patients observe the fixation target against a background of <0.0001 cd/m^2 [43, 44]. The minimum and maximum luminance of the stimuli were 0.0025 cd/m^2 (0.008 asb) and 0.25 cd/m^2 (0.800 asb), representing a dynamic range of 20 dB. The emission spectrum produced by the 3 types of LEDs is shown in online supplementary Figure 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000453079).

Irrespective of the type of testing (mesopic, scotopic cyan, scotopic red), the MAIA started examinations with 1 paracentral test stimulus in each quadrant (eccentricity of 3°, angular position of 0, 90, 180, 270°, respectively). These 4 threshold values were then used to adjust the initial brightness levels for measuring the remaining test loci in each of the corresponding quadrants. For mesopic function, the testing sensitivity started with a level of 2 dB brighter of the respective initial threshold value using a 4--2 dB full-threshold strategy. For scotopic testing, the testing sensitivity started with a level of 2 dB dimmer than the respective initial threshold, particularly to avoid bleaching, and using a 2--1 dB full-threshold strategy. These differences between mesopic and scotopic FCP were not modifiable as they were included in the presetting of the device. After the first test and for each of the 3 types of functional testing, subjects were asked to sit back and rest until they were ready to proceed to the second test. The second test was performed using the follow-up mode. For mesopic function, the starting brightness for each stimulus was automatically set to 2 dB brighter than the previously determined threshold, while the threshold sensitivity for scotopic testing was preset as the initially determined brightness.

FCP was performed by a single experienced examiner in a darkened room. At room light, a brief instruction regarding the testing procedure was given and the correct operation of the subject response trigger was practiced. This was followed immediately by the first mesopic test. The sequence of testing is illustrated in online supplementary Figure 2. For mesopic testing, the room light was switched off during the examination and briefly turned on between the different mesopic tests (in case of bilateral inclusion, the 2 eyes first underwent one after the other the 2 mesopic tests). Following mesopic and prior to scotopic testing, the eyes underwent 30 min of dark adaptation (while waiting in the examination room, lights off; light level <0.1 lx). The 4 scotopic tests were then consecutively performed in 1 eye and finally -- if both eyes were included -- in the other eye. The fellow eye was always covered with an opaque eye patch. Test reliability was assessed by measuring the frequency of false-positive responses (measured by presentation of suprathreshold stimuli to the optic nerve head). Participants with a false-positive rate greater than 33.3% in any of the tests were excluded from the analysis.

Outcome Measures and Statistical Analyses

Statistical analyses were performed using software environment R [45]. Graphs were created using the ggplot2 library [46].

The primary outcome measure was the PWS intrasession test-retest reliability for the scotopic cyan and scotopic red retinal sensitivity testing in comparison to mesopic FCP in normal eyes, assessed by 95% coefficient of repeatability (CoR) as recommended by Bland and Altman [47] and previously applied by Chen et al. [52] for the assessment of test-retest variability of mesopic FCP using the Nidek MP-1.

Prior to the evaluation of the primary outcome measure, the data were screened for possible systematic learning curve and bleaching effects. The frequency of stimuli with a dense scotoma (failure to respond to the brightest stimulus, i.e. <0 dB) was computed and compared with regard to the 3 types of testing, the stimulus eccentricity, and the test number (2nd vs. 1st test) using mixed-effects logistic regression considering the hierarchical nature of the data (test locations nested within eyes).

For all of the following analyses, sensitivity measurements “<0 dB” were incorporated as “0 dB” in analogy to the calculation of the different visual field indices [48]. The data were interpreted as equidistant (interval scaled) data according to the Weber-Fechner law [49–51]. The effect of stimulus eccentricity, angular position, and test number (2nd vs. 1st test) on the sensitivity in all 3 types of testing was assessed using linear mixed-effects models. Hereby, the eccentricity, angular position, and test number (2nd vs. 1st test) were considered as fixed effects, while considering stimulus position nested within the eyeas a random effect.

The primary outcome measure (95% CoR) was determined by multiplying the within-subject standard deviation by 2.77 (1.96 times $\sqrt{2}$). A mixed-design analysis of variance was used to determine the within-subject standard deviation. Additionally, the frequencies of change in 5 different categories (no change, <±2 dB, <±4 dB, <±6 dB, >±6 dB) were computed as absolute count and cumulative percentage [52]. For visualization of the test-retest reliability, Bland-Altman plots with the mean difference and the 95% limits of agreement (LoA) were provided [47].

The effective dynamic range and the number of discriminable steps were analyzed as proposed by Wall et al. [53]. The ceiling was defined as a value above which less than 0.5% of the values fall in normal eyes. The floor was defined as the frequency of 0 dB on the retest (first definition: 5% of retest values equal to 0 dB, second definition: 0 dB most frequent retest value). The definition of the number of discriminable steps from normal to the floor is described in detail in the above-mentioned publication [53].

Furthermore, the stability of fixation was assessed by the area of an ellipse (in degree^2) that covered 63 and 95% of fixation points, respectively. After log transformation, the

test-retest reliability of the fixation assessment (normally distributed according to the Shapiro-Wilk test) was evaluated using intraclass correlation (ICC). The test-retest reliability of the average reaction time (normally distributed according to the Shapiro-Wilk test) was assessed using the CoR and differences in average reaction times among the types of testing were compared using a one-way analysis of variance followed by a post hoc Tukey test.

Results

A total of 32 subjects were recruited between June and July 2016. FCP testing was not possible in 2 myopic subjects because ocular media opacities (vitreous floaters) interfered with the automated fundus tracking at the beginning of scotopic testing. These 2 subjects were excluded from the analysis. The remaining 30 individuals (median age 33.8 years, range 12.8--80.1, 14 females) underwent the complete standardized protocol, including duplicate mesopic, scotopic cyan and scotopic red FCP, and were included in the following analysis. In a subset of 17 subjects (median age 25.1 years, range 12.8--70.2, 8 females), both eyes were tested.

To avoid invalid inference due to intereye (intraindividual) correlation, only the first examined eye of each subject is included in the analysis shown below ($n_{subjects}$ = 30, n_{eyes} = 30). The stratified analysis (for the second eyes) for subjects with bilateral enrollment is shown in online supplementary Table 1 ($n_{subjects}$ = 17, n_{eyes} = 17).

Frequency of Stimuli Indicating Dense Scotomata (i.e., <0 dB)

For mesopic testing, no stimulus indicating a dense scotoma (i.e., <0 dB) was observed, while for scotopic red testing, there was a single stimulus with a dense scotoma (Table 1). The number of stimuli with a dense scotoma for scotopic cyan testing was 72 (4.89%) for the first and 80 (5.44%) for the second test, respectively. Further analysis revealed that the majority of these dense scotomata was spatially confined to an eccentricity of 0--1° (first test: 58 of 72 [80.6%], second test 78 of 90 [86.7%]). A mixed-effects logistic regression analysis revealed that in scotopic cyan testing the likelihood of nonseen stimuli was significantly higher towards the fovea (p < 0.001). The test number (2nd vs. 1st test) exhibited no statistically significant effect in this regard.

Predictors of PWS

Linear mixed-effects models were used to examine the influence of stimulus location (eccentricity and angular position), test number (2nd vs. 1st test) and possible interactions for all 3 types of testing thoroughly (Table 2). For mesopic testing, the sensitivity was highest at 1° and decreased towards the periphery ($p < 0.001$). Sensitivity along the horizontal meridian was slightly higher than along the vertical meridian (nasal ±0.00 dB, temporal +0.24 dB, superior --0.55 dB, inferior --1.19 dB, $p < 0.001$, online suppl. Fig. 3). A slight improvement between the first and second test was observable (+0.39 dB, $p < 0.001$). Of note, this test-retest improvement was not observable in the second eyes (online suppl. Table 1).

In scotopic cyan testing, the sensitivity increased significantly towards the periphery (1° +0.47 dB, 3° +5.76 dB, 5° +6.25 dB, 7° +6.65 dB, $p < 0.001$). Overall, the superior hemifield exhibited a significantly higher sensitivity than the inferior hemifield (Fig. 2). The observation was most pronounced in the vertical meridian (superior +1.85 dB, inferior --0.75 dB). There was a significant decrease in sensitivity between the first and second test (--3.1 dB, $p < 0.001$) that exhibited a significant interaction with the eccentricity, i.e., the decrease was most pounced at 0 and 1° and minor at 3 and 7° ($p < 0.001$). At 5°, no relevant effect was observable.

In scotopic red testing, the measured sensitivity was highest at 1° and decreased towards the periphery. The horizontal meridian exhibited a slightly higher sensitivity than the vertical meridian (nasal ±0.00 dB, temporal --0.18 dB, superior --0.74 dB, inferior --1.35 dB, $p < 0.001$; Fig. 2). The sensitivity was slightly decreased in the second test (--0.4 dB, $p < 0.001$).

CoR and Frequency of Change in PWS

The CoR, i.e., the value below which the absolute differences between 2 measurements would lie with 0.95 probability, was 4.75 dB for mesopic, 5.26 dB for scotopic cyan, and 4.06 dB for scotopic red testing (Table 3). For mesopic and scotopic red testing, the CoRs were in the same range across all eccentricities. The CoRs for scotopic cyan testing were lower at 3, 5 and 7° than at 0--1° (4.27 dB, 4.24 dB, 4.26 dB vs. 7.36 dB). Table 4 lists the frequency of change in PWS. For all 3 types of testing, the cumulative percentage of stimulus points within ±2 dB repeatability was >75%.

Bland-Altman Plots and 95% LoA

The 95% LoA according to Bland-Altman statistics ranged from --5.08 dB (95% CI, --5.29 to --4.86) to 4.29 dB (95% CI, 4.08–4.50) for mesopic testing. Graphical analysis showed no ceiling or floor effects (Fig. 3a). For scotopic red testing, similar LoA, ranging from --3.59 dB (95% CI, --3.77 to --3.41) to 4.38 dB (95% CI, 4.20–4.56), were calculated. As opposed to mesopic testing, a marked ceiling effect was detectable for scotopic red FCP (Fig. 3c). Finally, the widest limits of agreement as well as signs for both floor and ceiling effects were found for scotopic cyan testing with LoA ranging from --4.72 dB (95% CI, --4.96 to --4.49) to 5.65 dB (95% CI, 5.41–5.88) and also showing several outliers (Fig. 3b).

Effective Dynamic Range

Last, the effective dynamic range and the number of discriminable steps were analyzed as proposed by Wall et al. [53]. The ceiling (defined as a value above which less than 0.5% of the values fall in normal eyes) was 32 dB for mesopic, 20 dB for scotopic cyan, and 20 dB for scotopic red testing (online suppl. Table 4). The floor was not determinable using normal eyes for mesopic and scotopic red testing due to the absence of scotomata. In scotopic cyan testing, the fovea and parafovea served as physiologic scotoma allowing the assessment of floor effects. The floor for scotopic cyan testing was depending on the definition at 9 dB (5% of retest values 0 dB) or at 3 dB (0 dB most frequent retest value). The number of discriminable steps was 4 (online suppl. Fig. 2).

Stability of Fixation

The analysis of the test-retest reliability for stability of fixation (after log transformation to reduce skew) disclosed moderate to high agreement between the 2 tests for all 3 types of retinal sensitivity testing with ICCvalues of 0.891 for mesopic, 0.671 for scotopic cyan, and 0.879 for scotopic red testing, respectively (online suppl. Table 3). The difference in fixation stability between the mesopic tests and the scotopic cyan (p = 0.029) and scotopic red (p = 0.077) tests was found to be statistically significant and almost statistically significant, respectively. No overall difference in fixation stability was observed between the scotopic cyan and red tests (p = 0.920) (Fig. 4a; online suppl. Table 4).

Reaction Time

The test-retest reliability for the average reaction times was high with CoR values of 126 ms for mesopic, 146 ms for scotopic cyan, and 137 ms for scotopic red testing (online

suppl. Table 5). Mesopic testing showed the shortest reaction time (mean ± SD: 486 ± 46 ms, pooled data of the first and second test), followed by scotopic red (619 ± 50 ms) and then scotopic cyan (712 ± 52 ms) retinal sensitivity assessment ($p < 0.001$; Fig. 4b; online suppl. Table 6).

Testing Duration

The mean duration for the first test was 6 min and 24 s for mesopic, 8 min and 8 s for scotopic cyan, and 7 min and 45 s for scotopic red testing (Table 5). For both scotopic cyan and scotopic red testing, the test duration was significantly shorter for the second as compared to the first test (Table 5). No reduction in test duration was observed between the 2 mesopic tests. As outlined above, the strategy with regards to the initial brightness levels for test loci in the second test and using the follow-up mode was different between mesopic (2 dB above the value determined in the first test) and scotopic assessments (initial brightness equal to value determined in the first test).

Discussion

The current study demonstrates that the recently introduced modified MAIA device allows for a practical, reliable and meaningful assessment of scotopic FCP in normal subjects. Compared to mesopic testing that has been available for several years and which was also evaluated in this study, the assessment of scotopic function is obviously more complex and more time-consuming since it requires dark adaptation and examination in a complete darkened room and, thus, is more prone to confounding factors. The inability to ensure a meaningful tracking of the fundus under dark-adapted conditions (due to the reduced SLO laser power) in 2 subjects with vitreous floaters (who had to be excluded) indicates that the assessment of scotopic function may not be feasible in some normal subjects. On the other hand, the number of false-positive answers was below 33% in all subjects. Furthermore, the average test duration for each of the 2 scotopic tests was below 9 min -- without the time for dark adaptation – and, therefore, on average only approximately 30% longer than mesopic testing.

The more elaborated protocol with 2 different types of scotopic testing (cyan and red) has been employed in the device for a more accurate discrimination of specific photoreceptor-mediated function [39–41]. Indeed, several results of this study are in accordance with the view that the scotopic cyan test condition largely reflects rod function,

while the scotopic red condition derives from both rod and cone photoreceptors. First, the reported anatomical distribution of rod cells corresponds to the topographic differences of median sensitivity values for scotopic cyan testing (Fig. 2) that showed the highest median sensitivity at 7°, a centripetal decrease, including a sharp decline between 3 and 1° and slightly but consistently higher values towards the superior retina [54, 55]. By contrast, this topographic pattern was not detectable by scotopic red testing. Secondly, the decrease in sensitivity in the second as compared to the first scotopic cyan test at 0 and 1° was most likely related to mild bleaching effects on the sensitive rods due to the fixation target. The third line of evidence comes from differences in reaction time among tests. Although no direct instruction was given to the subjects to respond as quickly as possible, reaction time variability was generally small (Fig. 4b). Longer reaction times for both scotopic as compared to mesopic test conditions are likely to reflect the considerably lower stimulus intensities employed and the increasing recruitment of rod photoreceptors in those tests [56]. For scotopic red, subjects commonly reported disparate color percepts depending on the degree of eccentricity. In line with the retinal distribution of L-cone and rod distribution, the stimuli appeared to be “red” at 0--5° and “white” at 7° [54, 55]. It would be conceivable that this difference in perception caused a “degree of uncertainty” and thus longer reaction times. For scotopic cyan, the even longer reaction times at similar levels of luminance most likely additionally reflect the so-called slow rod pathway [57]. It is well known that rod latency is depending on the degree of dark adaptation. While under conditions of equal cone and rod adaption, the cone-rod latency has been shown to be 8--20 ms in humans, rod signaling is delayed at higher levels of luminance [57–61]. This so-called slow rod pathway is presumably mediated via rod bipolar and AII amacrine cells [62]. The published cone-rod latency of 60--80 ms under these conditions is of a similar magnitude as the difference of 96 ms of average reaction time between scotopic cyan and scotopic red testing [57].

Several results of the statistical analysis of the intrasession duplicate testing suggest that a reliable assessment of both types of scotopic function is feasible using this instrument. These include the strong agreement for the PWS retest reliability and that >75% of threshold values were within ±2 dB of repeatability. No major learning effect was found in the current protocol, involving a brief instruction of the procedure and practice of the response trigger function. A minor intersession learning effect might have been the reason for the higher count of 10 versus 2 dense scotomata (out of 564 stimuli) in the first as compared to the second scotopic cyan testing at an eccentricity of 3°.

The slightly higher CoR for mesopic testing in this study (4.75 dB) as compared to the previous reports by Wu et al. [63] (3.74 dB, using the MAIA device) might be caused by differences in the testing protocol. While the former included training with presenting of test stimuli prior to actual testing and used a grid of 37 loci, the current study -- in addition to no dedicated training (see above) -- used a grid with 49 stimuli (longer duration). In fact, the 12 most eccentric test points (in areas that were not included by Wu et al.) showed the lowest overall CoR for mesopic testing.

The measures for reliability for both scotopic testing were mostly within the same range as compared to the duplicate assessment of mesopic testing in this study. One exception was the assessment of scotopic cyan testing at 0--1°. In this area, very-high-threshold values were consistently detected which would indicate a floor effect. A likely reason for this phenomenon is the absence or relatively small amount of rods in this area and/or bleaching effects of the fixation target interfering with the detection of low luminance levels [64]. The latter hypothesis is supported by the reduction in sensitivity between the first and second scotopic cyan test, which was confined to 0 and 1° (i.e. the location of the fixation target). Moreover, the CoR for scotopic cyan testing at 0--1° has to be interpreted with caution, since it is most likely an overestimation of the repeatability due to the floor truncation and the inclusion of nonseen stimuli “<0 dB” as “0 dB.” Therefore, assessment and interpretation of rod function based on scotopic cyan sensitivity would be challenging in the central retina. In fact, the central area is excluded in the preset grid of the device and was only included in the customized grid of this study to specifically assess scotopic testing in this area as well. Compared to mesopic testing, the LoAs and their graphical analysis suggest that scotopic red is more prone to ceiling effects, while both floor and ceiling effects must be particularly considered for scotopic cyan testing.

Indeed, the physical dynamic range of a perimetric test does not necessarily represent the effective dynamic range, which can be truncated due to the limits of perception (floor) and due to the test-retest reliability (floor) [53]. The analyses in analogy to Wall et al. [53] disclosed that the effective ceiling for mesopic testing was 32 dB instead of 36 dB. The floor was not quantifiable in normal eyes for mesopic and scotopic red testing due to the lack of scotomata. In scotopic cyan testing, the floor was estimable; however, precaution must be taken due to the observed bleaching effects at 0 and 1°. With re-dark adaption between the test-retest, the floor of the effective dynamic range for scotopic cyan testing would have been most likely lower. The number of discriminable steps for scotopic cyan testing was equivalent in comparison to previously published data for standard automated

perimetry using the Humphrey Field Analyzer (24-2, Goldmann III) and frequency-doubling technology perimetry [53].

The high ICC for the bivariate contour ellipse area indicates high test-retest repeatability for the assessment of fixation stability. The lower stability of fixation compared to mesopic testing is in accordance with several previous publications that have consistently reported about the difficulties of ensuring a stable fixation under scotopic condition [20, 32]. Some subjects state that the fixation target disappeared during scotopic testing and reappeared after blinking or short fixation movements. This observation could be explained by the Troxler effect (especially for low-contrast targets) [65].

The current study is limited by inclusion of only normal subjects and the limited number of subjects. In addition, longer test-retest intervals (e.g., days or weeks) were not assessed. Furthermore, the test-retest reliability would be most likely higher with repeated dark adaption in between the tests.

In summary, we performed a systematic analysis of scotopic retinal sensitivity assessment in normal subjects using the recently introduced modified MAIA device. We demonstrated a strong agreement for intrasession PWS reliability for both scotopic cyan and scotopic red testing. Results for the assessment of reliability were mostly comparable and in the same range as mesopic testing. In accordance with anatomical and functional differences between rods and cones, including the topographic distribution, the unequal signaling pathway and the different range of luminance sensitivity, the results indicate that particular attention to various phenomena must be considered when interpreting scotopic test results. This includes the increased susceptibility for floor and ceiling effects and the occurrence of severe outliers. Although scotopic testing was more demanding, it was feasible in almost all subjects in the study. Finally, several observations strongly suggest that the testing of scotopic cyan sensitivity largely reflects rod function. Further hardware and software updates of the device might become available and may improve the performance, reliability and the ability to interpret testing results. This may offer the opportunity for more refined structural-functional analyses in a broad spectrum of retinal diseases and the potential use as a functional outcome measure in future interventional clinical trials.

Acknowledgements

We are grateful for the technical support of Carlo Pellizzari from Centervue SpA. CenterVue SpA, Padua, Italy has provided research material (S-MAIA) for the conduct of this study. CenterVue had no role in the design or conduct of the experiments.

Disclosure Statement

The disclosure codes of the ARVO (Association for Research in Vision and Ophthalmology) Commercial Relationships Policy (April 13, 2015) were used in the following:

M. Pfau: Heidelberg Engineering, Optos, Zeiss, Centervue F, Heidelberg Engineering, R; M. Lindner: Heidelberg Engineering, Optos, Zeiss, Centervue, Genentech F; M. Fleckenstein: Heidelberg Engineering, Optos, Zeiss, Centervue F, Heidelberg Engineering, R; R.P. Finger: Heidelberg Engineering, Optos, Zeiss, Centervue F; G.S. Rubin; W.M. Harmening: Heidelberg Engineering, Optos, Zeiss, Centervue F; M.U. Morales: Centervue I; F.G. Holz: Heidelberg Engineering, Optos, Zeiss, Centervue F, Heidelberg Engineering, Zeiss, Centervue C, Heidelberg Engineering, Centervue R; S. Schmitz-Valckenberg: Heidelberg Engineering, Optos, Zeiss, Centervue F, Heidelberg Engineering, R.

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Appendix after References (Editorial Comments)

Legend(s)

Fig. 1. An exemplary report for all three different types of testing. Each image depicts the local retinal sensitivity of the proband superimposed on the SLO fundus photo. The numeric value represents the measured threshold in dB (**A**. mesopic, **B** scotopic cyan, **C** scotopic red). (**D**) The point-wise dB difference between the scotopic cyan (**B**) and red (**C**) tests.

Fig. 2. The pooled (first and second test, i. e. each locus represents 60 measurements) sensitivity values for each stimulus location are illustrated as median dB value (**A**. scotopic cyan and **B.** scotopic red testing). Across all tested eyes, the data of left eyes was transformed so that the stimuli match up with the pattern of the right eye. The horizontal (**C**) and vertical (**D**) retinal sensitivity profiles are shown in analogy to Wentworth 1930 and Sloan 1939. The dots indicate the mean value, the error bars the standard error of the mean (SEM).

Fig. 3. Bland Altman plots for mesopic (**A**), scotopic cyan (**B**) and scotopic red (**C**) testing (after pair-wise exclusion of dense scotomata in at least one of the two tests; see Table 2 for frequency). The x-axis shows the mean point-wise sensitivity (PWS) for each pair of repeated tests. The PWS difference between two tests (first minus second test) is indicated on the y-axis. The overall mean difference is illustrated by the solid line; the 95% limits of agreement are marked by the two dashed lines. The size of individual circles illustrates the count of overlapping data points. Please note instrument limitations in maximum and minimum stimulus intensity that manifest as converging point spread at both ends of mean PWS (≤ 4 dB and/or ≥ 16 dB) are visible for scotopic cyan and scotopic red testing.

Fig. 4. (**A**) Illustration of the variability of fixation stability (pooled data of first and second test). Box plots show the distribution of the area of the ellipse encompassing 63% of fixation points (in degree^2). The upper and lower whiskers extend from the hinge to the highest/lowest value that is within 1.5 * interquartile range (IQR). Data beyond the end of the whiskers are interpreted as outliers and plotted as points. The mean values are indicated by the rhombs. (**B**) Illustration of the variability of the average reaction in each subject (in milliseconds, pooled data of first and second test).

Table(s)

Footnote(s)

Table 1. Frequency of non-seen stimuli (<0 dB)

Eccentricity	Mesopic		Scotopic cyan		Scotopic red	
	1st test	2nd test	1st test	2nd test	1st test	2nd test
Overall (*n* = 1,470)	0	0	72	80	1	0
0° (*n* = 30)	0	0	10	15	0	0
1° (*n* = 360)	0	0	48	63	0	0
3° (*n* = 360)	0	0	10	2	0	0
5° (*n* = 360)	0	0	2	0	0	0
7° (*n* = 360)	0	0	2	0	1	0

Table 2. Linear mixed-effects model analysis of sensitivity

Variable	Mesopic		Scotopic cyan		Scotopic red		*p* value[b]
	estimate, dB	95% CI	estimate, dB	95% CI	estimate, dB	95% CI	
Intercept[a]	25.22	24.45 to 25.99	6.27	5.04 to 7.49	13.88	13.08 to 14.68	
Eccentricity							<0.001
1°	2.32	1.78 to 2.86	0.47	−0.54 to 1.48	1.51	1 to 2.02	
3°	1.34	0.8 to 1.88	5.76	4.76 to 6.77	0.41	−0.1 to 0.93	
5°	0.06	−0.48 to 0.6	6.25	5.24 to 7.25	−1.12	−1.63 to −0.61	
7°	−0.95	−1.49 to −0.41	6.65	5.64 to 7.65	−1.89	−2.4 to −1.37	
Angular position							<0.001
30°	−0.07	−0.4 to 0.27	0.73	0.27 to 1.18	−0.34	−0.66 to −0.03	
60°	−0.37	−0.7 to −0.04	1.14	0.68 to 1.6	−0.65	−0.97 to −0.33	
90° (superior)	−0.55	−0.89 to −0.22	1.85	1.39 to 2.31	−0.74	−1.05 to −0.42	
120°	−0.43	−0.77 to −0.1	1.72	1.26 to 2.18	−0.95	−1.26 to −0.63	
150°	−0.19	−0.53 to 0.14	1.33	0.87 to 1.78	−0.58	−0.9 to −0.26	
180° (temporal)	0.24	−0.09 to 0.58	0.24	−0.22 to 0.7	−0.18	−0.49 to 0.14	
210°	−0.15	−0.49 to 0.18	−0.23	−0.69 to 0.23	−0.29	−0.61 to 0.02	
240°	−0.71	−1.04 to −0.37	−0.58	−1.04 to −0.12	−0.92	−1.23 to −0.6	
270° (inferior)	−1.19	−1.52 to −0.85	−0.75	−1.21 to −0.29	−1.35	−1.67 to −1.04	
300°	−0.88	−1.22 to −0.55	−0.75	−1.21 to −0.29	−1.11	−1.43 to −0.8	
330°	−0.4	−0.73 to −0.06	−0.23	−0.69 to 0.23	−0.29	−0.6 to 0.03	
Test number (2nd vs. 1st test)	0.39	0.26 to 0.53	−3.1	−4.4 to −1.8	−0.4	−0.52 to −0.27	<0.001
Test number × eccentricity[c]							<0.001[c]
2nd test – 1°			2.04	0.69 to 3.39			
2nd test – 3°			2.84	1.49 to 4.19			
2nd test – 5°			3.17	1.82 to 4.52			
2nd test – 7°			2.73	1.37 to 4.08			

[a] The intercept indicates the mean measured sensitivity at the foveal center point (eccentricity 0°, angular position 0°) in the first test run. [b] *p* values were obtained from the likelihood ratio tests. [c] A likelihood ratio test indicated a significant interaction between test number and eccentricity for scotopic cyan testing. No significant interaction was observed for mesopic and scotopic red testing.

Table 3. Coefficients of Repeatability (CoRs)

Eccentricity	CoR, dB		
	mesopic	scotopic cyan	scotopic red
Overall	4.75	5.26	4.06
0–1°	4.84	7.36	4.08
3°	4.35	4.27	4.07
5°	4.61	4.24	3.69
7°	5.14	4.26	4.36

Table 4. Frequency of change in point wise sensitivity (PWS)

Type of testing	Change, dB	Frequency	Cumulative percentage
Mesopic	no change	603	41
	±1 to ±2	552	78.6
	±3 to ±4	265	96.6
	±5 to ±6	36	99.0
	>±6	14	100
Scotopic cyan	no change	297	20.2
	±1 to ±2	829	76.6
	±3 to ±4	225	91.9
	±5 to ±6	75	97
	>±6	44	100
Scotopic red	no change	269	18.3
	±1 to ±2	920	80.9
	±3 to ±4	233	96.7
	±5 to ±6	34	99
	>±6	14	100

Table 5. Differences in test duration

Type of testing	Duration of 1st test (mean ± SD), s	Duration of 2nd test (mean ± SD), s	Paired t test with Bonferroni correction
Mesopic	383.5±18.1	380.0±23.4	n.s.
Scotopic cyan	488.6±36.9	436.4±32.2	$p_{adjusted} < 0.05$
Scotopic red	465.2±42.4	419.5±39.9	$p_{adjusted} < 0.05$

The testing strategy of the initial brightness levels for the second test was different for mesopic as compared to scotopic testings. While the former started at each location with a value of 2 dB above the previously determined threshold, the latter applied the brightness level of the results of the first test.

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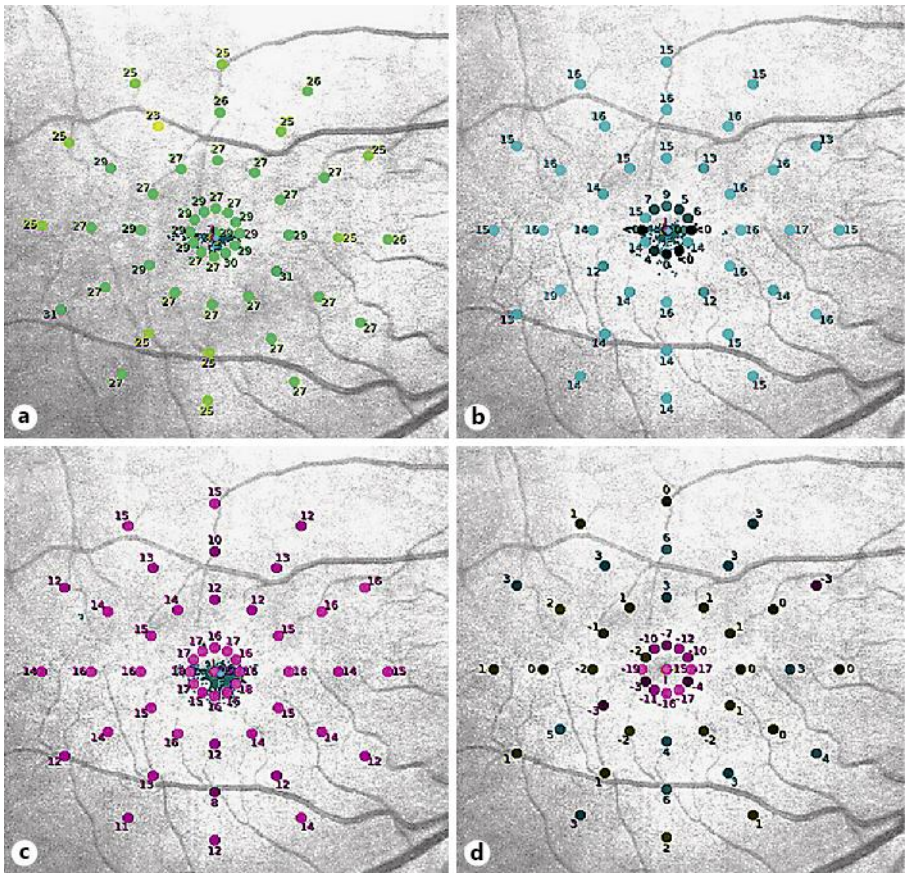

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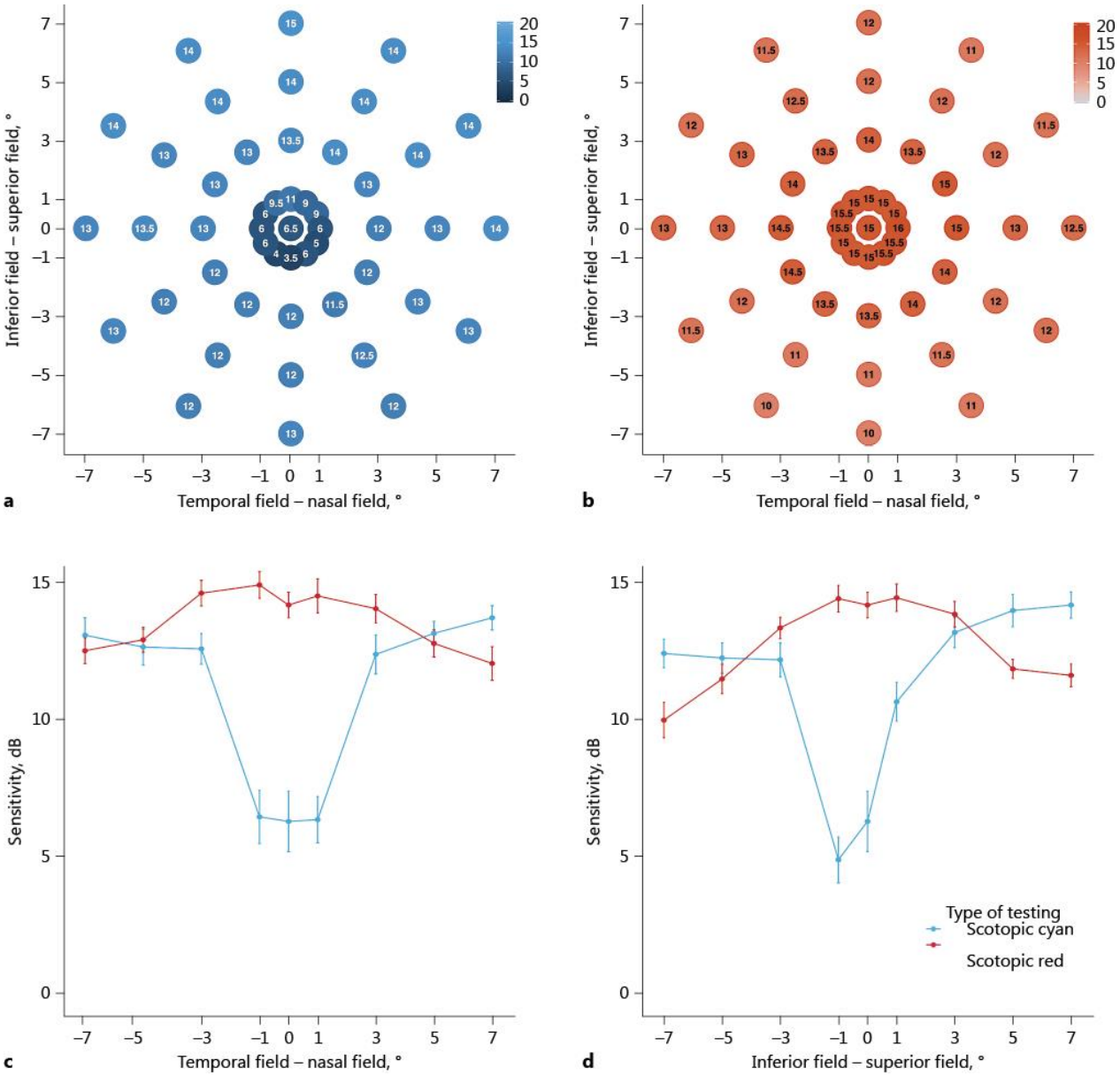

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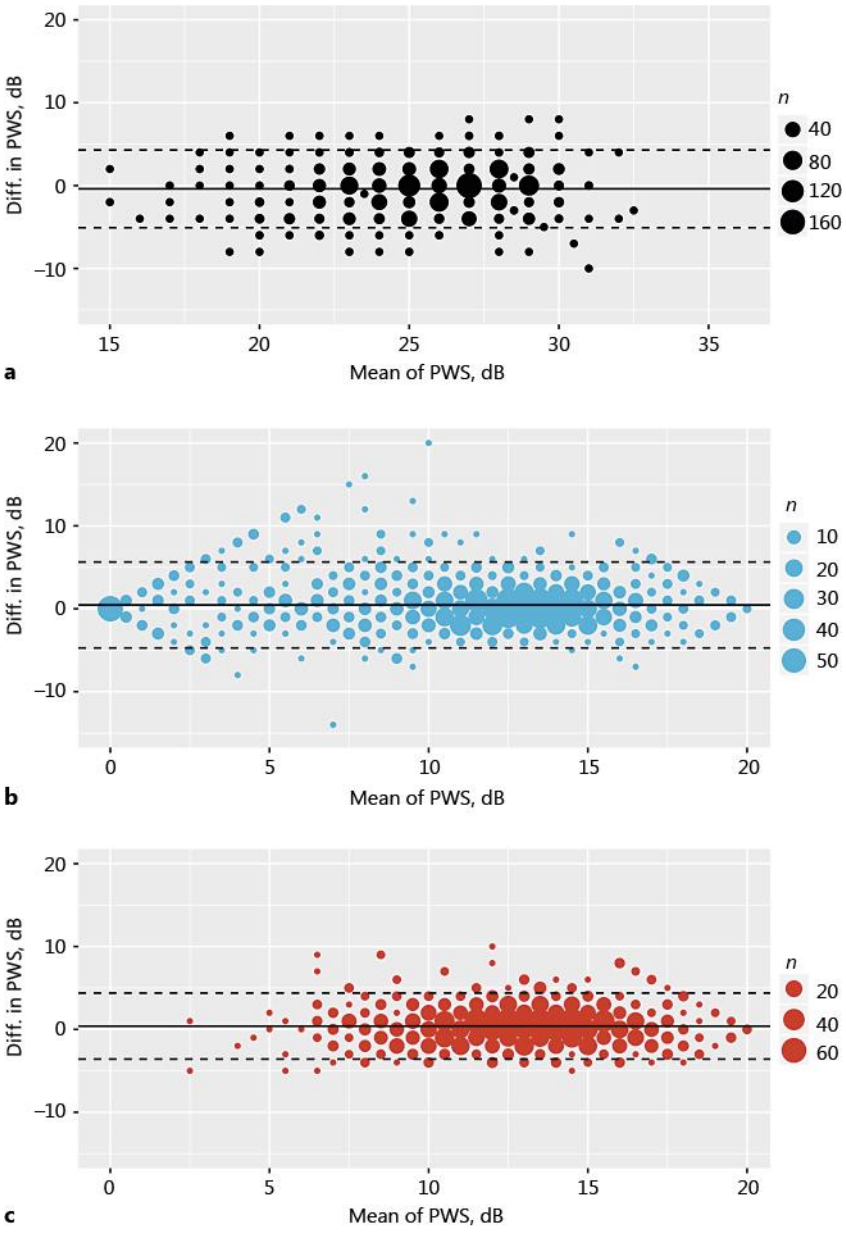

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