

# **Visual field indices and patterns of visual field deficits in mesopic and dark-adapted two-color fundus-controlled perimetry in macular diseases**

*Maximilian Pfau<sup>1</sup>, Moritz Lindner<sup>1,2</sup>, Julia S. Steinberg<sup>1</sup>, Sarah Thiele<sup>1</sup>, Christian K. Brinkmann<sup>1</sup>, Monika Fleckenstein<sup>1</sup>, Frank G. Holz<sup>1</sup>, Steffen Schmitz-Valckenberg<sup>1</sup>*

1. Department of Ophthalmology, University of Bonn, Ernst-Abbe-Str. 2, Bonn, Germany
2. The Nuffield Laboratory of Ophthalmology, Sleep and Circadian Neuroscience Institute, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

*Running head:* Visual field deficits in dark-adapted perimetry  
*Key words:* microperimetry, rod function, cone function, dark adaptation, macular disease  
*Number of words:* 2986 (excluding title page, abstract, legends, and references)  
*Number of figures:* 5  
*Number of tables:* 0  
*Number of supplements:* 4 supplementary figures, 1 supplementary table

## *Correspondence:*

Prof. Dr. Steffen Schmitz-Valckenberg, FEBO  
Department of Ophthalmology  
University of Bonn  
Ernst-Abbe-Str. 2  
53127 Bonn  
Germany  
Tel.: +49 228 287 16826  
Fax: +49 228 287 11470  
E-Mail: [steffen.schmitz-valckenberg@ukbonn.de](mailto:steffen.schmitz-valckenberg@ukbonn.de)

*Contributors:* Conception/design of the study: MP, ML, MF, FGH, SSV. Data acquisition: MP, ML, JSS, ST, CKB. Data analysis: MP, ML, JSS, ST. Data interpretation: MP, ML, MF, FGH, SSV. Drafting the manuscript: MP, ML, SSV. Revising the manuscript critically for important intellectual content: MP, ML, JSS, ST, CKB, MF, FGH, SSV. Final approval of the version to be published: MP, ML, JSS, ST, CKB, MF, FGH, SSV. Agreement to be accountable for all aspects of the work: MP, ML, JSS, ST, CKB, MF, FGH, SSV.

*Funding:* This study was supported by the BONFOR GEROK Program of the Faculty of Medicine, University of Bonn, Grant No O-137.0022 to MP, Grant No O-137.0020 to ML and by the German Research Foundation (DFG), Grant No 658/4-1 and 658/4-2 to MF, Grant No 2846/1-1 to ML.

*Financial Support:* CenterVue SpA, Padova, 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.

**Precis**

Dark-adapted two-color fundus-controlled perimetry provides additional diagnostic information and allows for refined structure-function correlation in macular diseases. Hereby, the variability-weighted mean-deviation and pattern-standard-deviation exhibited the lowest retest-variability of the tested visual field indices.

## Abstract

**Background/Aims:** To analyze the retest-reliability of visual field indices and to describe patterns of visual field deficits in mesopic and dark-adapted two-color fundus-controlled perimetry (FCP) in macular diseases.

**Methods:** Seventy-seven eyes (30 eyes with macular diseases and 47 normal eyes) underwent duplicate mesopic and dark-adapted two-color FCP (S-MAIA, CenterVue). Non-weighted (mean-defect, loss-variance), variability-weighted (mean-deviation, pattern-standard-deviation) and graphical (cumulative defect [Bebie] curves) indices were computed. Reproducibility (coefficient of repeatability, CoR) of these indices was assessed. Cluster analysis was carried out to identify patterns of visual field deficits.

**Results:** The intra-session reproducibility was lower for the mean-defect as compared to the mean-deviation (CoR [dB] 2.67 vs. 2.57 for mesopic, 1.71 vs. 1.45 for dark-adapted cyan, 1.94 vs. 1.87 for dark-adapted red testing) and lower for the square-root loss-variance as compared to the pattern-standard-deviation (CoR [dB] 1.48 vs. 1.34, 0.77 vs. 0.65, 1.23 vs. 1.03). Hierarchical cluster analysis of the indices disclosed six patterns of visual field deficits (approximately unbiased  $p$ -value  $> 0.95$ ) with varying degrees of global versus focal defect and rod versus cone dysfunction. These were also reflected by the cumulative defect curves.

**Conclusion:** FCP with mesopic and dark-adapted two-color testing allows for reproducible assessment of different types of retinal sensitivity, whereby mean-deviation and pattern-standard-deviation exhibited the better retest-reliability of the tested indices. Distinct patterns of retinal dysfunction can be identified using this setup, reflecting variable degrees of rod- and cone-dysfunction in different macular diseases. Dark-adapted two-color FCP provides additional diagnostic information and allows for refined structure-function correlation in macular diseases.

## Introduction

Dark-adapted chromatic perimetry and adaptometry have been used previously to study the spectral sensitivity and dark adaptation kinetics of the rod- and cone-photoreceptors as well as the effect of macular pigment and the aging lens on light sensitivity.[1–4] Clinically, dark-adapted two-color perimetry has been applied for the assessment of rod- and cone-function in retinal diseases including age-related macular degeneration (AMD), the most common cause of blindness in developed countries.[5,6] The technique has also been used as an outcome measure for gene augmentation therapy.[7–9] The commonly focal treatment effects of gene augmentation therapy in the setting of subretinal gene delivery underscore the need for refined testing with high spatial resolution and the need for visual field indices that reflect both, global and focal visual field deficits.[7–9] Recently, the S-MAIA (Scotopic Macular Integrity Assessment, CenterVue, Padova, Italy) device has become available that allows for dark-adapted two-color fundus-controlled perimetry (FCP) even in patients without foveal fixation through simultaneous confocal scanning laser ophthalmoscopy (cSLO) combined with eye-tracking.[10–12] For the interpretation of dark-adapted two-color FCP data, the normal data must be considered.[10] Briefly, with the current stimulus luminance settings of the S-MAIA device, a cyan-red difference close to 0 dB (as observed at eccentricities of 5° and 7° in fully dark-adapted normal eyes) would be indicative of normal rod-function.[10] Isolated rod-dysfunction would result in a decrease of the cyan sensitivity. The red sensitivity would only change insignificantly, since the rod and cone thresholds for the red stimulus are very close. Thus, the cyan-red difference would become more negative. At fixation, isolated cone-dysfunction would lead to a reduction of the red sensitivity, while the cyan sensitivity would not change significantly (due to the marked floor effects of the device for cyan testing). Thus, isolated cone-dysfunction at fixation would lead to less negative cyan-red difference values.[11] The pointwise retest-reliability was shown to be low, homogeneous across eccentricities and various degrees of scotoma depth.[11]

Spatially resolved perimetry data is a prerequisite for accurate structure-function correlation assessments. Yet, spatially resolved data generally represent an analytical challenge. It is difficult to (1.) separate true small local deviation from imperfect reproducibility of threshold determination, (2.) separate small defects from interindividual variability, (3.) compare test results over time, and (4.) compare test results with other psychophysical tests or structural biomarkers (e.g. drusen volume).[13] In the setting of glaucoma, this has been addressed thoroughly by Flammer and associates as well as Heijl and associates, who both have introduced global indices that allow for condensation of visual field data.[14,15] Hereby, the mean-deviation and pattern-standard-deviation are variability-weighted (i.e. account for the variability of each test location in normal eyes), whereas the mean-defect, loss-variance are non-weighted.[14,15] Further, plots that condense visual field data and allow for graphical differentiation between global and focal deficits have been developed including the cumulative defect (Bebie) curve.[16] However, these analysis strategies have not been – to the best of our knowledge – systematically tested in macular diseases and FCP.

The aim of this study was to evaluate systematically the applicability and reproducibility of previously published visual field indices[14–16] in the setting of mesopic and dark-adapted two-color FCP for the assessment of macular diseases. Further, we hypothesized that cluster analysis of dark-adapted two-color FCP visual field data would yield heterogeneous, yet disease-specific groupings since dark-adapted two-color FCP allows for partially photoreceptor specific testing. Presence of such patterns would underscore the additional clinical information provided by dark-adapted two-color FCP.

## Materials and methods

### *Subjects*

Thirty eyes from 30 patients were recruited at the Department of Ophthalmology, University of Bonn, Germany. At least one eye of the subjects needed to present with a known macular disease. Exclusion criteria included refractive errors  $\geq 5.00$  diopters of spherical equivalent and  $> 1.50$  diopters of astigmatism assessed by autorefraction (ARK-560A, Nidek, Gamagori, Japan) as well as a history of glaucoma or relevant anterior segment diseases with media opacities including cataract. Eyes with blue-light filtering intraocular lenses were not included in this study. If both eyes met the inclusion criteria, the eye with better visual acuity was included. Apart from taking the medical history, all subjects underwent routine ophthalmological examinations including best-corrected visual acuity (BCVA), slit-lamp and fundusoscopic examinations. Spectral-domain optical coherence tomography (SD-OCT) raster scanning was performed using a  $30^\circ \times 25^\circ$  scan field (121 B-scans, automated real time (ART) 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 explanation of 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. From 30 patients included in this study, 28 eyes of 28 patients had been previously included in a pointwise retest-reliability analysis.[11]

### *Normative data*

A dataset of 47 normal eyes of 30 subjects (age  $42.7 \pm 21.08$  years, range 12.8–80.1, 14 females) with duplicate mesopic, dark-adapted cyan and dark-adapted red FCP was used as normative data.[10] The hierarchical nature of the data (stimulus position nested in eye

nested in patient) was taken into account using mixed-effects models to obtain the normative data.

### *Fundus-controlled perimetry*

A short mesopic practice examination was performed in patients with no prior perimetry or FCP experience. All patients underwent duplicate mesopic (achromatic stimuli, 400-800 nm) FCP, followed by 30 min of dark adaption (light level < 0.1 lux) and then duplicate dark-adapted cyan (505 nm) FCP and duplicate dark-adapted red (627 nm) FCP. The data of 49 Goldmann III stimuli covering 14° of the central retina with the S-MAIA were analyzed. Mesopic testing (background of 1.27 cd/m<sup>2</sup>, achromatic stimuli ranging from 0.08 cd/m<sup>2</sup> to 318 cd/m<sup>2</sup>) was performed with the preset 4-2 dB staircase strategy, while dark-adapted testing was performed with the preset 2-1 dB staircase strategy. For dark-adapted testing (background of <0.0001 cd/m<sup>2</sup>), the minimum and maximum luminance of the cyan and red stimuli were 0.0025 scotopic (scot.) cd/m<sup>2</sup> and 0.25 scot. cd/m<sup>2</sup>. The conversion of radiant energy into luminous energy was based on the scotopic luminosity function  $V'(\lambda)$  as adopted by the CIE (*Commission Internationale de l'Éclairage*) in 1951.[4]

### *Outcome measures and statistical analyses*

Statistical analyses were performed using the software environment R. Visual acuity measurements (Snellen fractions) were converted to the base-10 logarithm of the minimum angle of resolution (logMAR). The mean defect, loss variance (LV), mean deviation, pattern standard deviation (PSD), short-term fluctuation and the corrected pattern standard deviation (cPSD) were calculated for all three types of testing for all of the 30 eyes with macular disease as proposed by Flammer et al. and Heijl et al., respectively.[14,15] Pearson's  $r$  was used to determine the correlation of the mean defect and mean deviation values and of the LV and PSD. The retest-reliability of the indices was assessed using the Coefficient of



Repeatability (CoR, the value under which the difference between two measurements on the same patients should fall with 95% probability) and Bland-Altman plots.[17] Cumulative defect curves (Bebie curves) were plotted for all patients.[16] The Bebie curves show the data of the patient (black curve) and the normative data with the 95% confidence interval (grey, cyan and red curve in the background). These may allow for differentiation of global defect (all data points shifted downwards, no change in shape) and focal defect (bending downwards).[16] Dot plots (grouped by eccentricity, all angular positions pooled) were created, that show the deviation of the cyan-red sensitivity difference as compared to the retinotopic normative value for each measurement ( $Deviation = Cyan-Red-Diff_{patient} - Cyan-Red-Diff_{normal}$ ). A cyan-red difference close to 0 dB would be indicative of normal rod-function, whereas more negative values would indicate rod dysfunction.[10] The dashed lines indicate the 95% confidence interval of the retinotopic normative cyan-red difference. Measurements that exceeded the 95% confidence interval were colored red.

Hierarchical cluster analysis using Ward's method and Euclidean distance was performed on standardized data (i.e., Z-scores) to account for differences in the dynamic range (36 dB vs. 20 dB). Hierarchical (agglomerative) cluster analysis successively merges similar clusters starting with each observation as its own cluster. The distances between the objects indicating their similarity may be visualized as a dendrogram (tree) as shown in Figure 2. Multiscale bootstrap resampling (10,000 replications) was used for assessing the uncertainty in the hierarchical cluster analysis.[18] It provided approximately unbiased p-values for each cluster in the dendrogram. Clusters with an approximately unbiased p-value greater than 95% were considered as strongly supported by data.[18]

## Results

### *Cohort characteristics*

Perimetry data of 30 eyes with retinal diseases (30 eyes of 30 patients, age [mean±SD] 58.3±18.5 years, range 18.9–85.6 years, 15 female) with duplicate mesopic, dark-adapted cyan and dark-adapted red FCP were included in the analysis (see online supplementary table S1). The average BCVA was 0.26±0.31 logMAR (Snellen equivalent approximately 20/30).

### *Comparison of visual field indices*

The mean defect and mean deviation of the first test were highly correlated for all three types of testing (Pearson's  $r$  of -0.999 for mesopic, -0.991 for dark-adapted cyan and -0.999 for dark-adapted red testing,  $p<0.01$ ) and the cyan-red sensitivity difference (-0.955,  $p<0.01$ ), respectively. Likewise, the correlation among the LV (square-root transformed) and PSD was very high among all three types of testing (Pearson's  $r$  of 0.997, 0.953 and 0.993,  $p<0.01$ ) and the cyan-red sensitivity difference (0.954,  $p<0.01$ ).

The intra-session reproducibility (first versus second test) of the mean defect was slightly lower as compared to the intra-session reproducibility of the mean deviation for all three types of testing (CoR values of 2.67 dB vs. 2.57 dB for mesopic, 1.71 dB vs. 1.45 dB for dark-adapted cyan, 1.94 dB vs. 1.87 dB for dark-adapted red testing and 2.21 dB vs. 2.20 dB for the cyan-red sensitivity difference, Figure 1) Hereby, the largest difference in the CoR was observed for dark-adapted cyan testing. . Similarly, the intra-session reproducibility of the (square-root) LV appeared to be slightly lower as compared to intra-session reproducibility of the PSD (CoR values of 1.48 dB vs. 1.34 dB for mesopic, 0.77 dB vs. 0.65 for dark-adapted cyan, 1.23 dB vs. 1.03 dB for dark-adapted red testing and 1.54 dB vs. 1.42 dB for the cyan-red sensitivity difference). Again, the largest difference in the CoR was observed for dark-adapted cyan testing (Figure 1).

### *Patterns of visual field deficits*

For all of the following analysis, the variability-weighted indices (mean deviation and PSD) were used, since these exhibited slightly better intra-session reproducibility as shown above. Further, to separate real small local deviation from imperfect reproducibility of threshold determinations, the short-term fluctuation was computed to determine the corrected PSD (cPSD) for each type of testing for each patient (see online supplementary table S1). Hierarchical cluster analysis disclosed six distinct patterns of visual field deficits with approximately unbiased p-values greater than 95% among the patients (Figure 2). Of note, (despite of not being included as variable in the cluster analysis) reduced best-corrected visual acuity was associated with the clusters 1, 3 and 4. These clusters were characterized by either a high mean deviation or high cPSD for mesopic or dark-adapted red testing (Figure 3).

Examination of the resultant groupings disclosed that pattern 1 was characterized by high mean deviation and comparatively low cPSD across all three types of testing. Diseases covering large parts of the macula such as geographic atrophy secondary to age-related macular degeneration or Stargardt's disease exhibited pattern 1 (Case 1, Figure 4). The cumulative defects curves typically demonstrated in these cases visual field deficit for all test points in all three types of testing. The dot-plots showed no significant deviation of the cyan-red sensitivity difference due to floor effects of the device (*cf. Discussion*). In contrast, pattern 2 was characterized by low mean deviation but high cPSD among all three types of testing. Patients in this group suffered from diseases that resulted in deep focal scotomata – yet, without affection of the surrounding retina such as branch retinal artery occlusion (Case 2, Figure 5). The cumulative defects curves typically lay within the normative confidence interval for a proportion of the test points and then bended downwards indicating focal defects. The other patterns of visual field deficits exhibited various combinations of mean deviation and cPSD. Pattern 3 was characterized by comparatively low mean deviation

among all three types of testing in conjunction with high cPSD for mesopic and dark-adapted red testing. It encompassed patients with diseases affecting primarily the fovea or parafoveal regions like subfoveal choroidal neovascularization (Case 3, see online supplementary figure S1). Pattern 4 was characterized by moderate mean deviation for dark-adapted cyan testing in association with moderately elevated cPSD for dark-adapted red testing. It encompassed patients with diseases affecting mostly the periphery and mid-periphery of the macula such as central retinal vein occlusion (Case 4, see online supplementary figure S2). Pattern 5 was characterized by moderate mean deviation for dark-adapted cyan testing in association with moderately elevated cPSD for dark-adapted cyan testing. Typically, the dot plots indicated a reduced cyan-red sensitivity difference (i.e. rod dysfunction). Of note, all patients with reticular pseudodrusen secondary to age-related macular degeneration clustered within this group (Case 5, see online supplementary figure S3). Patients in the pattern 6 subgroup exhibited overall almost normal visual field indices among all three types of testing. However, in some cases detailed examination of the cumulative defect curves and of the dot plots could reveal visual dysfunction (Case 6, see online supplementary figure S4).

## Discussion

This study demonstrated (i) the applicability of numerical visual field indices and Bebie curves in the setting of mesopic and dark-adapted two-color FCP, (ii) the superiority of variability-weighted versus non-weighted indices with regard to intra-session reproducibility and (iii) that distinct patterns of visual field deficits were identifiable using dark-adapted two-color FCP.

Based on our results, differences of the mean deviation greater than 2.57 dB (or 2.67 dB for the mean defect) may be interpreted as clinically significant. This is very much in line with previous data for the Nidek MP1 funds perimeter.[19] For dark-adapted testing with a 2-1 staircase strategy, the coefficients of repeatability were even lower (<2 dB). To the best of our knowledge, this is the first study that applied and compared systematically visual field indices including mean defect, mean deviation, loss variance, (corrected) pattern standard deviation and short-term fluctuation in the setting of macular diseases and FCP. Previous studies with FCP (including treatment trials) were mainly limited to the evaluation of the mean sensitivity as global outcome measure.[20–23] However, mean sensitivity does not allow for detection of relative changes as compared to the normative sensitivity profile and overweights test locations with high variability. Especially in dark-adapted cyan testing, the variability-weighted indices (that represent maximum likelihood estimators by taking into account the variability of each test location in normal eyes) performed slightly better than the non-weighted indices with regard to reproducibility. This is most likely attributable to the uneven variability across the visual field for dark-adapted cyan testing.[10] In the setting of glaucoma and standard automated perimetry, an increase of the retest-variability (short-term fluctuation) for test points with low sensitivity has been observed, suggesting that it is not only representative of imperfect reproducibility of threshold determinations but also of disease severity.[24] Thus, the cPSD (corrected for short-term fluctuation) might have less diagnostic value than the PSD in that setting.[24] However, in the setting of FCP and macular diseases we were previously able to show, that the retest-variability is independent of retinal

sensitivity (homoscedastic) and therefore not representative of disease severity.[11] Correction of PSD for short-term fluctuation seems therefore reasonable.

Overall, patterns of visual field deficit for all patients of the current study were in line with previous reports including histological, electroretinography and psychophysical data on macular telangiectasia type 2,[25,26] rod-cone dystrophies[27] and age-related macular degeneration.[6,28,29] The cumulative defect (Bebie) curves in the combination with the dot plots for the cyan-red difference were especially useful in patients with an overall visual field sensitivity superior to the normative data. Examination of these plots could yield interpretable results, while the numerical indices did not provide interpretable information (cf., Patient 6 in Supplementary Figure S4).

### *Limitations*

Patients within the last group (pattern 6) did not show significantly increased mean deviation or corrected pattern standard deviation for all three types of testing. This could be attributable to very subtle deficits in some diseases, but also to the normative data, which was not age-corrected for the individual patients. The currently limited dynamic range (maximal attainable cyan-red difference of approximately -11 dB) does not allow for quantification of severe rod-dysfunction, which could theoretically result in cyan-red differences of up to -22 dB.[4,10] This limitation was especially visible in the cyan-red sensitivity difference dot plots for patients exhibiting the visual field deficit pattern 1. Since the floor of the dynamic range (i.e. 0 dB) was reached for both, the cyan and red stimulus, the cyan-red sensitivity difference appeared to be within normal limits. A further limitation of this study was that a radial grid instead of a rectilinear grid with uniform spacing was used in this study. Due to the central condensation and unequal spacing of test points, the indices in this study must be interpreted as spatially-weighted averages. Last, disease-specific studies comparing dark-adapted two-color FCP against mesopic FCP or standard automated perimetry will be needed to proof its diagnostic utility in a disease-specific context.

### *Conclusions*

In summary, this study demonstrated that traditional visual field indices including graphical indices like the Bebie curve are applicable in the setting of mesopic and dark-adapted two-color FCP in eyes with macular diseases. While the mean defect and mean deviation as well as the loss variance and pattern standard deviation yielded similar results, the reproducibility of the variability-weighted indices (mean deviation and pattern standard deviation) was slightly better suggesting that upcoming studies and therapeutic trials should preferentially use variability-weighted indices. Last, this study demonstrated using a data-driven approach that dark-adapted two-color FCP provided additional clinical information that allowed for a more detailed differentiation of patterns of visual field deficits in macular diseases.

### **Acknowledgements**

We are grateful for the technical support of Carlo Pellizzari (CenterVue SpA, Padova, Italy).

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## Figure captions

### Figure 1. Bland-Altman plots for the perimetry indices

The first row shows the Bland-Altman plots for the mean deviation (Mean Dev.) for mesopic, dark-adapted cyan and dark-adapted red testing as well as the cyan-red sensitivity difference. The second row shows the corresponding Bland-Altman plots for the mean defect (Mean Def.). The x-axis indicates the mean value of the index of the first and second test and the y-axis the difference of the index between the first and second test. Please note, the retest-reliability as indicated by the 95% limits of agreements (LoA, dashed lines) was similar for both indices across all three types of testing.

### Figure 2. Results of the cluster analysis (dendrogram and heatmap)

The dendrogram shows the result of the cluster analysis (Ward's method, Euclidean distance) of the mean deviation (MD) and corrected pattern standard deviation (cPSD) for all three types of testing. The heatmap indicates the value of each variable for each eye in the cluster analysis. Prior to cluster analysis each variable was standardized to obtain Z-scores. For this graphic, the MD values were multiplied by -1 to ensure that the red color represents functional deficit for both, the MD and cPSD. Six resultant clusters exhibited an approximately unbiased p-value greater than 0.95 (multiscale bootstrap analysis with 10,000 replications) indicating that these clusters were strongly supported by the data.

### Figure 3. Boxplots for the visual field indices of the resultant groups

The boxplots show the mean deviation (MD, **A-C**) and corrected pattern standard deviation (cPSD, **D-F**) for all three types of testing in dependence of the resultant groups. The whiskers extend to a maximum of 1.5 IQR. Data beyond the end of the whiskers were considered as outliers and plotted as points (as specified by Tukey). The mean deviation (**G**) and cPSD (**H**) for the cyan-red sensitivity difference were also plotted. Please note, that these variables were not included in the cluster analysis (to avoid redundancy). Reduced visual acuity (**I**), which was also not included in the cluster analysis, exhibited associations with certain patterns as well.

### Figure 4. Exemplary case of pattern 1

The figure shows the left eye of a 59-year-old male patient who was referred with unilateral central serous chorioretinopathy in the left eye. The choroid appeared to be thick with densely packed choroidal vasculature in both eyes (i.e., pachychoroid pigment epitheliopathy). All of the Bebie curves (**A**) exhibited from the first rank onwards manifest deviation indicating global defect. The mesopic testing (**B**) showed a relative scotoma across the whole visual field. The mean deviation and cPSD were -11.5 dB and 4.4 dB for mesopic, -11.7 dB and 2.6 dB for dark-adapted cyan and -12.2 dB and 2.2 dB for dark-adapted red testing. Fluorescein angiography (2 minutes 55 seconds, **B**) showed hyperfluorescence indicating leakage and indocyanine green angiography revealed thickened choroidal vasculature (**D**). The green arrows indicate the position of the spectral domain optical coherence tomography B-scans. The spectral domain optical coherence tomography (**C**) revealed a choroidal excavation in combination with pachychoroid vasculature. The dot plot (grouped by eccentricity, all angular positions pooled) shows the deviation of the cyan-red sensitivity difference as compared to the retinotopic normative value for each measurement (**E**). The dashed lines indicate the 95% confidence interval of the normative data. Measurements that exceeded the 95% confidence interval were colored red.

**Figure 5. Exemplary case of pattern 2**

The figure shows the left eye of a 31-year-old female patient who was admitted due to cilioretinal artery occlusion. All of the Bebie curves (**A**) ran initially roughly parallel to the normative curves and then exhibited manifest deviation from the 30<sup>th</sup> rank onwards indicating focal defect. The mean deviation and cPSD were -3.3 dB and 7.4 dB for mesopic, -2.6 dB and 4.3 dB for dark-adapted cyan and -4 dB and 4.2 dB for dark-adapted red testing. The dark-adapted red testing (**B**) showed a scotoma in the temporal visual field (nasal retina), which was correlated to the area of non-perfusion disclosed by fluorescein angiography (**D**). The green arrows indicate the position of the spectral domain optical coherence tomography B-scans. Spectral domain optical coherence tomography showed hyperreflectivity of the inner retinal layers ranging from the nerve fiber layer to the outer plexiform layer (**C**). This resulted in reduced signal of the outer retina. The dot plot (grouped by eccentricity, all angular positions pooled) shows the deviation of the cyan-red sensitivity difference as compared to the retinotopic normative value for each measurement (**E**).

**Supplementary Figure S1. Exemplary case for pattern 3**

Multimodal imaging of the right eye of this 55 year-old male patient revealed presence of choroidal neovascularization (CNV). The mean deviation and cPSD were -7.2 dB and 6.3 dB for mesopic, -4.9 dB and 3.2 dB for dark-adapted cyan and -7.8 dB and 4.7 dB for dark-adapted red testing. The Bebie curves (**A**) were more severely affected for mesopic and dark-adapted red testing as compared to dark-adapted cyan testing. The dark-adapted red testing (**B**) showed a central scotoma, which was correlated to the presence of CNV with intra- and subretinal fluid as seen in the spectral domain optical coherence tomography (**C**). There were areas with increased and decreased signal in fundus autofluorescence imaging (**D**). The green arrows indicate the position of the spectral domain optical coherence tomography B-scans. Please note, the area of hyperautofluorescence (**D**) was correlated with alterations of the outer retinal SD-OCT bands 2-4 (**C**) and associated with reduced dark-

adapted red sensitivity, especially when comparing the inferior with the superior retina (**B**). The dot plot (grouped by eccentricity, all angular positions pooled) shows the deviation of the cyan-red sensitivity difference as compared to the retinotopic normative value for each measurement (**E**).

### **Supplement Figure 2. Exemplary patient for pattern 4**

A 73-year-old female patient with so-called ‘quiescent’ occult choroidal neovascularization (CNV) secondary to age-related macular degeneration in OD (no previous injections of an anti-VEGF agent) was seen for routine follow-up. BCVA was 20/40. Examination of the macula revealed pigmentary changes and a flat pigment epithelial detachment. Signs of active choroidal neovascularization (CNV) such as intra- or subretinal fluid or retinal hemorrhage were neither detectable by funduscopy nor by spectral domain optical coherence tomography (SD-OCT). Yet, SD-OCT showed an irregularly slightly elevated retinal pigment epithelial detachment (**C**). Further, the outer retinal band 2 was not delimitable above the inferior parts of the retinal pigment epithelial detachment. Swept-source OCT angiography (**D**) showed a well demarcated type 1 CNV. The mean deviation and corrected pattern standard deviation were -7.2 dB and 1.6 dB for mesopic, -8.8 dB and 2.4 dB for dark adapted cyan and -5.5 dB and 3.1 dB for dark-adapted red testing. Likewise, the Bebie curve (**A**) deviated the most from its normative shape for dark-adapted red testing. The dot plot (grouped by eccentricity, all angular positions pooled) shows the deviation of the cyan-red sensitivity difference as compared to the retinotopic normative value for each measurement (**E**). The dashed lines indicate the 95% confidence interval of the normative data. Measurements that exceeded the 95% confidence interval were colored red.

**Supplement Figure 3. Exemplary case for pattern 5**

A 85-year-old female patient complained about night vision problems. BCVA was 20/25 (logMAR 0.10) in her left eye. Funduscopy revealed yellowish-pale lesions with punctate appearance across the macula. Fundus autofluorescence (FAF) and infrared reflectance (IR) confocal scanning laser ophthalmoscopy (cSLO) imaging as well as spectral domain optical coherence tomography (SD-OCT) confirmed the presence of reticular drusen (**B, C, D**). The cumulative defect curves (**A**) showed a parallel shift of the cumulative defect curve for mesopic (and dark-adapted red testing) without a distinct change in shape. The cumulative defect curve for dark-adapted cyan testing showed pronounced kinking as compared to the normative curve. In accordance, the mean deviation and corrected pattern standard deviation were -3.5 dB and 0 dB for mesopic, -5.3 dB and 2.9 dB for dark-adapted cyan and -5.55 dB and 1.8 dB for dark-adapted red testing. The dot plot (grouped by eccentricity, all angular positions pooled) shows the deviation of the cyan-red sensitivity difference as compared to the retinotopic normative value for each measurement (**E**). The dashed lines indicate the 95% confidence interval of the normative data. Measurements that exceeded the 95% confidence interval were colored red.

**Supplement Figure 4. Exemplary case for pattern 6**

A 57-year-old female patient presented in fall 2016 stating that she noticed minimal chromatic distortion close to the point of fixation in both eyes in her upper visual field. There was no family history of retinal dystrophies. The patient took 200 mg hydroxychloroquine (Quensyl®) daily for 13 year. The medication was discontinued in fall 2015. Best-visual acuity (BCVA) was 20/20 in both eyes. In the slit-lamp examination and in funduscopy, all findings were within normal limits. Fundus autofluorescence (FAF) confocal scanning laser ophthalmoscopy (cSLO) imaging as well as infrared reflection (IR) cSLO imaging revealed no abnormal findings (**B**). Further, standard automated perimetry (10-2 field) showed no visual field defects. In contrast, the dark-adapted red testing showed abnormal low sensitivity



values in the inferior retina, especially at an eccentricity of 3°. A dense SD-OCT raster scan revealed structural changes at the level of the outer-plexiform-layer/outer-nuclear-layer junction corresponding to the dark-adapted red functional testing (**C**). Indeed, an outer retinal en-face slab foreshadowed that the structural changes were bull's-eye shaped (**D**). The mean deviation and corrected pattern standard deviation were 0.9 dB and 0 dB for mesopic, -0.6 dB and 1.2 dB for dark-adapted cyan and -0.45 dB and 1.7 dB for dark-adapted red testing. Indeed, detailed evaluation of the Bebie curves (**A**) confirmed, that the especially the cumulative defect curve for red testing was not symmetric and exhibited kinking from its 30<sup>th</sup> defect value onwards. The dot plot (grouped by eccentricity, all angular positions pooled) shows the deviation of the cyan-red sensitivity difference as compared to the retinotopic normative value for each measurement (**E**). The dashed lines indicate the 95% confidence interval of the normative data. Measurements that exceeded the 95% confidence interval were colored red.