Chromatic Multifocal Pupillometer for Objective Perimetry and Diagnosis of Patients with Retinitis Pigmentosa

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Purpose: To assess visual field (VF) defects and retinal function objectively in healthy participants and patients with retinitis pigmentosa (RP) using a chromatic multifocal pupillometer.

Design: Cross-sectional study.

Participants: The right eyes of 16 healthy participants and 13 RP patients.

Methods: Pupil responses to red and blue light (peak, 485 and 625 nm, respectively) presented by 76 light-emitting diodes, 1.8-mm spot size at different locations of a 16.2° VF were recorded. Subjective VFs of RP patients were determined using chromatic dark-adapted Goldmann VFs (CDA-GVFs). Six healthy participants underwent 2 pupillometer examinations to determine test–retest reliability.

Main Outcome Measures: Three parameters of pupil contraction were determined automatically: percentage of change of pupil size (PPC), maximum contraction velocity (MCV; in pixels per second), and latency of MCV (LMCV; in seconds). The fraction of functional VF was determined by CDA-GVF.

Results: In healthy participants, higher PPC and MCV were measured in response to blue compared with red light. The MLCV in response to blue light was relatively constant throughout the VF. Healthy participants demonstrated higher PPC and MCV and shorter LMCV in central compared with peripheral test points in response to red light. Test–retest correlation coefficients were 0.7 for PPC and 0.5 for MCV. In RP patients, test point in which the PPC and MCV were lower than 4 standard errors from the mean of healthy participants correlated with areas that were indicated as nonseeing by CDA-GVF. The mean absolute deviation in LMCV parameter in response to the red light between different test point was significantly higher in RP patients (range, 0.16–0.47) than in healthy participants (range, 0.02–0.16; P < 0.0001) and indicated its usefulness as a diagnostic tool with high sensitivity and specificity (area under the receiver operating characteristic curve (AUC), 0.97, Mann–Whitney–Wilcoxon analysis). Randomly reducing the number of test points to a total of 15 points did not significantly reduce the AUC in RP diagnosis based on this parameter.

Conclusions: This study demonstrates the feasibility of using a chromatic multifocal pupillometer for objective diagnosis of RP and assessment of VF defects. Ophthalmology 2016;123:1898-1911 © 2016 by the American Academy of Ophthalmology.
white and chromatic stimuli arranged in a dartboard pattern was developed (nuCoria Field Analyzer; nuCoria Pty. Ltd., Acton, Australia). This method analyzes both eyes simultaneously, and although it is promising, it cannot differentiate between the rod and cone systems.

Retinitis pigmentosa encompasses a group of progressive retinal degeneration diseases that predominantly affect the rod photoreceptor system, resulting in night blindness in the early phase of the disease and loss of peripheral vision that progresses to tunnel vision. In later stages of RP, degeneration of cone photoreceptors causes progressive decline of visual acuity. Disease progression is monitored by electroretinography and perimetry. However, poor test–retest repeatability in patients with RP, specifically in areas with VF deficits, limits the ability to assess disease progression and particularly to design and interpret clinical trials of potential therapeutic agents.

We recently demonstrated a proof of concept for using a chromatic multifocal pupillometer for detection of VF defects in RP patients. The pupil responses of healthy volunteers and RP patients were recorded at 13 different locations of the 30° VF in response to blue- and red-light stimuli (peak, 485 and 640 nm, respectively; light intensity, 40 cd/m²; target size, 64 mm²). Retinitis pigmentosa patients demonstrated a significantly reduced percentage of pupil constriction (PPC) compared with healthy participants in testing conditions that emphasized rod contribution (blue light) in nearly all VF locations. By contrast, the PPC in responses to red light (which emphasizes cone contribution) was reduced significantly in RP patients compared with healthy participants, mostly in peripheral locations. In central locations, there was no significant difference between the PPC of RP patients and healthy participants in response to red light. In a second study, we demonstrated that RP patients demonstrated significantly lower PPC in response to blue light in peripheral locations of the central VF than healthy participants. Furthermore, in both studies, minimal PPC was recorded in RP patients in areas that were not detected in dark-adapted chromatic Goldmann perimetry. In these studies, we evaluated only a single parameter of the pupil response, the PPC. These studies suggested that VF defects as well as rod and cone function may be assessed in RP patients using a chromatic multifocal pupillometer.

In the current study, we examined the dynamics of the pupil response in the central VF of RP patients and healthy participants. We analyzed additional parameters of the pupil light response, the maximal contraction velocity (MCV), and the latency of MCV (LMCV) to determine the effect of retinal degeneration on these parameters. To the best of our knowledge, this is the first report of evaluation of the LMCV parameter in pupillometry studies. The central VF of RP patients was assessed using a chromatic multifocal pupillometer and was compared with the patients’ chromatic dark-adapted Goldmann VF (CDA-GVF) results as well as the pupillometry results of healthy participants. We report that RP patients demonstrated significantly lower PPC and MCV in areas that were reported as nonseeing by CDA-GVF and that the mean absolute deviation in the LMCV parameter between different test point locations was significantly higher in RP patients and may present a valuable parameter as a diagnostic tool for RP.

**Methods**

**Participants**

The Sheba Medical Center Institutional Review Board Ethics Committee approved this trial. The study was conducted according to the tenets of the Declaration of Helsinki and was registered at www.clinicaltrials.gov (identifier, NCT02014389). Informed consent was obtained from all participants. Sixteen healthy volunteers, age matched with patients (see below; 6 men and 10 women; mean age ± standard deviation, 38.4±15.6 years; range, 26–77 years) were included in the study. Inclusion criteria were normal eye examination results, best-corrected visual acuity of 20/20, normal color vision, no history of or current ocular disease, no use of any topical or systemic medications that could adversely influence effluent pupil movements, and normal 24-2 Swedish interactive threshold algorithm results, developed for the Humphrey standard perimeter (Humphrey Field Analyser II, Swedish interactive threshold algorithm 24-2; Carl Zeiss Meditec, Inc., Jena, Germany).

The study patient group comprised 13 patients with RP (3 women and 10 men; mean age ± standard deviation, 36.1±14.6 years; range, 20–65 years). Inclusion criteria for RP patients were typical abnormal fundus appearance and previously recorded electroretinography results that were abnormal under scotopic or photopic conditions or both (in compliance with the protocol of the International Society for Clinical Electrophysiology of Vision) and typical abnormal kinetic chromatic Goldmann test results (loss of VF that is either concentric or that began superiorly and subsequently demonstrated an arcuate scotoma that progressed either from the nasal or the temporal side; incomplete midperipheral ring scotoma that broke through into the periphery; or a residual central VF, with blue and red isopters that were either superimposed or in which the isopter in response to the red stimulus was larger than that in response to the blue stimulus of 1°).

Exclusion criteria were a concurrent ocular disease and any other condition affecting the pupill response to light. Data recorded for all patients included gender, diagnosis, and electroretinography responses. Patients were tested for best-corrected visual acuity and for color vision by the Farnsworth D15 test. The right eyes of both healthy and RP participants were examined.

**Light Stimuli**

Light stimuli were presented using a Ganzfeld dome apparatus (Accutome, Inc, Malvern, PA; Fig 1) placed 330 mm from the patient’s eye. All tests were performed in a dark room. The untested eye was covered. Participants were asked to fixate on a white-light fixator (0.9 cd/m²; Fig 1B, white arrow) at the center of the dome. Stimuli were presented from 76 targets (light-emitting diodes) with diameter of 1.8 mm² in a VF of 16.2°. The wavelength and intensity of light stimuli selected for this study were 625±5 nm and 1000 cd/m² for long-wavelength stimuli (red light) and 485±5 nm and 200 cd/m² for short-wavelength stimuli (blue light). The light intensities were chosen after preliminary calibrations that enabled us to identify the minimal stimulus intensity that yielded a substantial pupil response in 5 healthy participants. Background luminance was 0.05 cd/m². Light intensities were determined by measurement with the LS-100.
luminance meter (Konica Minolta, Tokyo, Japan). Stimulus duration was 1 second and the interstimulus interval was 4 seconds.

Pupil Measurement

Pupil diameter was recorded in real time by a computerized infrared high-resolution camera (the camera pinhole is marked with a black arrow in Fig 1B) that recorded the pupil diameter at a frequency of 30 Hz. The software (Accutome, Inc.) searched for the pupil in every image. Participants were dark adapted for 3 minutes before testing. Tests in which the subject blinked during the first 2.5 seconds after stimulus onset were excluded automatically, and the targets were retested.

Analysis of Pupil Responses

During the recording of the pupil diameter in response to each light stimulus, 5 parameters were calculated within the software using the change in pupil diameter over time: the initial diameter of the pupil (in pixels), the minimum pupil diameter (in pixels), PPC, MCV (in pixels per second), and LMCV (in seconds). The PPC was determined using the following formula, as described previously:\(^2\):

\[
PPC = \left( \frac{\text{Initial Pupil Diameter} - \text{Minimum Pupil Diameter}}{\text{Initial Pupil Diameter}} \right) \times 100
\]

The MCV was determined by calculating the maximum rate at which the pupil contracted from the light stimulus between the initial pupil diameter measurement and the minimum pupil diameter measurement. The LMCV was determined by calculating the time point for each pupil response at which the maximum rate of pupil contraction (MCV) occurred from each light stimulus.

Chromatic Dark-Adapted Visual Field

Retinitis pigmentosa patients were tested for kinetic VF by CDA-GVF.\(^2\) Briefly, a Goldmann perimeter (940-ST; Haag-Streit AG, Liebefeld, Switzerland) was used to map patients’ conventional and 2-color dark-adapted VFs. Patients were dark adapted for 30 minutes before testing. The setting used for stimuli were II3c for the long-wavelength stimulus and 2 log units lower in luminance (II3c) for the short-wavelength stimulus. For quantification of functional CDA-GVF, a schematic representation of the pupillometer target points was overlaid on the CDA-GVF test record, and pupillometer targets that were withinseeing areas by the CDA-GVF were scored as 1. Pupillometer targets that were in nonseeing areas by the CDA-GVF were scored as 0. The fraction of functioning VF was calculated as the sum of scores divided by 76.

Statistical Analysis

Statistical analyses were performed using Excel (Microsoft, Redmond, WA) and R software version 3.0.1 (The Free Software Foundation [FSF]). Results were presented as mean ± standard error (SE). Student t test was used to evaluate demographic differences between patients and controls. Test–retest reliability of the pupil response measurements was calculated using Pearson correlation, and the correlation between pupillometer recordings and CDA-GVF testing were calculated with Spearman \(\rho\) test. Variability in LMCV recordings was measured by the mean absolute deviation, and this measure was compared between the study group and the control group using a 2-sided Mann–Whitney–Wilcoxon test. The effects of using fewer test points and the robustness of the LMCV for discrimination was examined via simulation by randomly selecting \(n\) test points \((n = 5, 10, 15, \ldots, 75)\) and calculating the area under the receiver operating characteristic curve (AUC) obtained using the LMCV.
based on these $n$ points. This was done for 200 repeats, and the mean AUC for each $n$ thus was obtained.

**Results**

**Characterization of Pupil Responses of Healthy Participants**

First we characterized the pupil response parameters in each test point for red- and blue-light stimuli in control participants. Figure 2 demonstrates color-coded maps of mean PPC (Fig 2A, B), MCV (Fig 2C, D), and LMCV (Fig 2E, F) recorded from control participants in each test point location in response to red-light stimuli (Fig 2A, C, E) and in response to blue-light stimuli (Fig 2B, D, F). The mean PPC recorded in response to blue-light stimuli ranged from 13% to 28% at different test point locations (mean ± SE, 19.4±0.22%) and was significantly higher compared with the red-light stimuli (range, 7%–22%; mean ± SE, 12±0.2%; $P < 0.0001$, $t$ test), even though the red-light stimuli were presented at a 5-fold higher intensity than the blue-light stimuli. Similarly, the mean MCV was significantly higher in response to blue-light stimuli (range, 28–43 pixels/second; mean ± SE, 37±0.3 pixels/second) compared with the red-light stimuli (range, 18–36 pixels/second; mean ± SE, 23.17±3 pixels/second; $P = 2\times10^{-7}$, $t$ test). There was no significant difference in the mean LMCV between the red and blue light (range, 0.6–0.8 seconds; $P = 0.11$, $t$ test).

Higher PPC was recorded in central locations of the VF compared with peripheral locations in response to both red and blue light (Fig 2A, B). A similar pattern of faster mean MCV in central locations compared with peripheral locations was demonstrated clearly in response to red-light stimuli (Fig 2C). The central–peripheral gradient pattern was less evident in
response to blue light (Fig 2D). The LMCV parameter was relatively constant throughout the VF field in response to both red and blue light, with an interquartile range of 0.6 to 0.7 seconds. The longest LMCV values were measured at peripheral test points in response to both wavelengths (Fig 2E, F).

Test reliability was assessed by retesting 6 healthy controls. Good linear correlation between the test and the retest was demonstrated for the parameter PPC for both colors (blue: \( R^2 = 0.762; P < 0.0001 \); red: \( R^2 = 0.721; P < 0.0001 \); Fig 3A, B). The parameter MCV demonstrated lower but still reasonable correlation between test and retest (blue: \( R^2 = 0.513; P < 0.0001 \); red: \( R^2 = 0.522; P < 0.0001 \); Fig 3C, D). The lowest correlation between test and retest was recorded for the LMCV parameter (blue: \( R^2 = 0.419; P < 0.0001 \); red: \( R^2 = 0.208; P < 0.0001 \); Fig 3E, F).

Retinitis Pigmentosa Patients Demonstrated Diminished Pupil Responses in Correlation with Disease Severity

Thirteen RP patients were divided into 2 groups based on their CDA-GVF testing results. Group A consisted of 5 patients with some functional CDA-GVF in response to red and blue light, whereas group B included 8 patients with a severe disease with no detection of either blue or red light by CDA-GVF (Table 1). Figure 4 demonstrates grey-scale maps of mean PPC (Fig 4A–C), MCV (Fig 4D–F), and LMCV (Fig 4G–I) recorded in each test point location in response to blue light in healthy participants (Fig 4A, D, G) and in patients with intermediate retinal degeneration (group A; Fig 4B, E, H) and severe retinal degeneration (group B; Fig 4C, F, I). Color coding was set to resemble the Humphrey perimeter’s output, with a white color for normal values and darker colors for values that were less than normal. Normal values were set as the mean of healthy participants in each test point location (Figs 2 and 4). Deviation from normal values was determined based on the SEs calculated for each parameter in each target point in the healthy participants. Thus, for PPC and MCV, the darkest color was used for test points in which the mean of patients was less than 5 SEs from the mean of healthy participants in those points. For LMCV, the darkest color was used for test points in which the mean of patients was more than 5 SEs from the mean of healthy participants in those points. In group B, mean PPC and MCV in response to the blue light were less than 5 SEs from the mean of healthy participants in nearly all test point locations (Fig 4C, F).

Figure 3. Scatterplots showing the test versus retest of (A, B) percentage of change of pupil size (PPC), (C, D) maximum contraction velocity (MCV), and (E, F) latency of maximum contraction velocity (LMCV) parameters in serial testing of 6 healthy participants in response to (A, C, E) blue and (B, D, F) red light. sec = second.
Similarly, the mean LMCV was more than 5 SEs from the mean of healthy participants in 68 of 76 test points (89% of the VF; Fig 4I).

By contrast, the mean PPC recorded in patients in group A was equal to or was lower by less than 2 SEs from the mean of healthy participants in most of the VF test points (50 test points, 66% of the VF; Fig 4B). In 26 test points (34% of the VF) that were located mostly in the periphery of the VF, the mean PPC of group B patients was less than 2 SEs from the mean of healthy participants. The MCV and LMCV parameters also demonstrated an intermediate defect in pupil response. Thus, in 33 test point locations (43% of the VF), the mean MCV was less than 3 SEs from the mean of healthy participants (Fig 4E), and in 25 test point locations (33% of the VF), the LMCV was less than 3 SEs from the mean of healthy participants (Fig 4E, H).

Figure 5 demonstrates the pupil responses to red light in healthy participants and RP patients. Retinitis pigmentosa patients from both groups demonstrated lower PPC and MCV and longer LMCV compared with healthy participants, but to a smaller extent than the response to the blue light. Thus, in group B, the mean PPC and MCV were less than 5 SEs from the mean of healthy participants in 35 (46% of the VF) and 57 (75% of the VF) test points, respectively (Fig 5C, F). Similarly, the mean LMCV was more than 5 SEs from the mean of healthy participants in 50 test points (66% of VF) compared with 68 points (89% of the VF) in response to blue light (Fig 5I). Group A demonstrated a milder decline in pupil responses to red light compared with group B, with only 4 (5% of the VF) and 9 (12% of the VF) test points in which the PPC and MCV were less than 5 SEs from the mean of healthy participants, respectively (Fig 5B, E). The mean LMCV was more than 5 SEs from the mean of healthy participants in 35 test points (46% of the VF; Fig 5H).

**Variability in Latency of Maximum Contraction Velocity as a Diagnostic Tool for Retinitis Pigmentosa**

As shown in Figures 2 and 4, LMCV was relatively constant in healthy participants in response to blue and red light in most test point locations, ranging from 0.6 to 0.8 seconds. By contrast, this parameter was highly variable between different test point locations in RP patients, ranging from 0.6 to 1.7 seconds and from 0.6 to 1.4 seconds in response to the blue and red light, respectively (Figs 4 and 5). To evaluate the extent of the variability in LMCV between different test point locations of the VF, the mean response for each subject was determined (i.e., the mean LMCV among the subject’s 76 test points). Then, the mean absolute deviation was calculated as the mean of the absolute difference between the mean and the measurements in each of the test points. Figure 6A demonstrates for each participant a boxplot depicting the distribution of the LMCV parameter for all test points in response to the red light. The mean absolute deviation in LMCV in response to the red light between different test points of each participant was significantly higher in patients with RP than in healthy participants ($P < 10^{-6}$, Mann–Whitney–Wilcoxon test). In addition, the Mann–Whitney–Wilcoxon statistic test indicated that a classification method based on measurement of LMCV in response to red light would have an AUC of 0.97. Similarly, the mean absolute deviation of LMCV in response to the blue light between different test points of each participant was significantly higher in patients with RP than in healthy participants ($P = 10^{-4}$, Wilcoxon–Mann–Whitney test; AUC, 0.93; data not shown). There was no significant difference in the absolute mean
deviation of the PPC and MCV parameters between RP patients and healthy controls (data not shown).

The mean absolute deviation in LMCV in response to the red light correlated negatively with the fraction of functional subjective VF as determined by CDA-GVF ($\rho_{\text{Spearman}} = -0.45; P = 0.13$; Fig 6B). The highest mean absolute deviation in LMCV (>0.3) was found in patients with no light detection by CDA-GVF. By contrast, lower mean absolute deviation in LMCV (<0.3) was demonstrated in patients with some functional CDA-GVF.

### Clustering Analysis

Next we examined whether fewer test point locations could be used for RP diagnosis in an attempt to optimize the analysis of pupillometer-based perimetry and reduce testing time. Figure 7 shows the mean and standard deviation of the AUC obtained after a random selection of test locations. Randomly reducing the number of test points to a total of 15 points did not significantly reduce the AUC in RP diagnosis based on the absolute mean deviation of LMCV. The AUC of 0.90 (highlighted by a dashed red line) suggests that the probability of LMCV to discriminate successfully a (randomly selected) RP patient from a (randomly selected) healthy subject is 0.9.

### Individual Retinitis Pigmentosa Cases

To illustrate the pattern of recorded pupil response values compared with the results of subjective CDA-GVF testing, the individual reports for 3 RP patients are presented in Figures 8, 9, and 10.

**Patient 30: No Light Detection by Chromatic Dark-Adapted Goldmann Visual Field.** Patient 30 showed no light detection by CDA-GVF (Fig 8A, E). The PPC and MCV parameters were less than 5 SEs from the mean of healthy participants in 75 of the 76 test points (99% of the VF) in response to blue light (Fig 8B, C). The LMCV parameter was more than 5 SEs from the mean of healthy participants in 48 of the 76 test points (63% of the VF; Fig 8D).

The pupil responses to red light also were diminished significantly throughout the VF, with 69 and 74 of the 76 test points (91%...
and 77% of the VF), respectively, presenting PPC and MCV less than 4 SEs from the mean of healthy participants (Fig 8F, G). The LMCV parameter was more than 5 SEs from the mean of healthy participants in 38 test points (50% of the VF; Fig 8H). The mean absolute deviation in LMCV in response to the red light for this patient was the largest recorded in this study (0.47 second; Fig 6A).

Patient 4: Tunnel Vision by Chromatic Dark-Adapted Goldmann Visual Field. Patient 4 demonstrated tunnel vision on CDA-GVF (Fig 9A, E). The map of the PPC parameter in response to blue light correlated well with the CDA-GVF map. Thus, in peripheral test point locations (nonseeing by CDA-GVF), the PPC values were less than 5 SEs from the mean of healthy participants, whereas in central locations, PPC values were only 1 to 3 SEs less than the mean of healthy participants (Fig 9B). The map of MCV demonstrated substantial reduction of MCV throughout the VF, with MCV less than 4 SEs from the mean of healthy participants in all 76 test points (100% of the VF; Fig 9C). The LMCV parameter was 1 to 2 SEs from the mean of healthy participants in 20 test points (26% of the VF), mostly in central locations (Fig 9D).

A similar tunnel vision pattern of pupil responses was obtained in response to the red light. The PPC and MCV parameters were equal to or only 1 SE from the mean of healthy participants in 14 and 8 central test point locations, correlating to 18% and 11% of the VF, respectively. By contrast, the PPC and MCV recorded in nearly all peripheral test points were more than 5 SEs less than the mean of healthy participants (Fig 9F, G). The LMCV parameter was close to normal (1–2 SEs from the mean of healthy participants) in 38 test points (50% of the VF). Most of these test points were located at the center of the VF (Fig 9H). The mean absolute deviation in LMCV in response to the red light for this patient was intermediate (0.26 second; Fig 6A).

Patient 3: Larger Fraction of Functional Chromatic Dark-Adapted Goldmann Visual Field. Patient 3 had a significant functional portion of the 16.2° VF as determined by the subjective CDA-GVF (Fig 10A, E). The pupil responses of this patient to both the red and blue light were equal to or 1 to 2 SEs from the mean of healthy participants in most test points located in functional areas determined by the CDA-GVF. Most areas that demonstrated substantially reduced PPC and MCV and longer LMCV compared with control values (more than 2 SEs from the mean of healthy participants) were in the central 8° VF. The mean absolute deviation in LMCV in response to the red light for this patient was intermediate (0.26 second; Fig 6A).
Discussion

This study demonstrated the feasibility of using a chromatic multifocal pupillometer for objective assessment of VF defects in patients with RP. Test point locations in which PPC and MCV were less than 4 SEs from the mean of healthy participants correlated with areas that were abnormal (nonseeing) by CDA-GVF. The RP patients with severe VF loss presented more testing points that differed substantially from the mean of healthy participants compared with patients with a moderate loss of VF, particularly in response to the blue-light stimuli. Patients with some functional VF demonstrated reduced pupil light response (PLR) particularly in peripheral test points and in response to the blue-light stimuli. The pathologic features of RP are characterized by a loss of rod function that exceeds the reduction of cone function, and the VF loss typically begins with peripheral VF constriction.3,28 Our finding that pupil response to blue light in RP patients was more affected than the pupil response to red-light stimuli strongly suggests that the pupil response to blue light measured by our chromatic pupillometer in response to focal light stimuli is mediated mainly by rods, whereas the pupil response to red light is mediated mainly by cones. Hence, a chromatic multifocal pupillometer may enable objective noninvasive assessment of the function of rods and cones at distinct locations of the VF for the first time. These results are supported by our previous study that demonstrated reduced pupil responses in peripheral VF locations in response to blue light32 and by the studies from Kawasaki et al,35 Kardon et al,36,37 and Lorenz et al38 that demonstrated that transient pupil response to full-field low-intensity blue-light stimulus reflects rod activity and that transient pupil response to red light is driven predominantly by cones. Curcio et al39 reported that the average horizontal diameter of the rod-free zone in the human fovea is 0.350 mm (1.25°).
Because the 4 most central pupillometer test point locations are located at 1.85°, the pupil responses to blue light recorded from these 4 central test points may be mediated by foveal rods. Since the pupil responses recorded in this study were transient and because dim light was used in the current test protocol, it is less likely that intrinsically photosensitive retinal ganglion cells are involved in pupil response to blue-light stimuli presented at the 4 central test points. However, because cones are highly concentrated in the fovea, it is possible that some of the pupil response to blue light in the 4 central test points is mediated by S cones. We plan to examine this possibility in a future study by testing cone–rod dystrophy patients.

The intensity of blue-light stimulus used in our study was 5-fold lower than that of the red-light stimulus. Nevertheless, pupil responses to blue-light stimulus in healthy participants were stronger than the responses to red-light stimuli in the same test point locations (Fig 2). These findings correlate with those of our previous study using the first chromatic pupillometer prototype, and with the findings of Kawasaki et al., Kardon et al., and Lorenz et al., and may be explained by the lower number of cones compared with rods in the human retina and by the smaller receptive fields of cones and their lower sensitivity for light compared with rods.

The LMCV parameter recorded in response to red-light stimuli seems to be a useful tool for noninvasive and objective diagnosis of RP with an AUC of 0.97. Our computer-based random clustering analysis suggested that shortening test duration may be possible with computational clustering, without reducing the sensitivity and accuracy of RP diagnosis. Thus, testing only 15 test points in response to red-light stimuli, which is predicted to take 1 minute, would enable diagnosis of RP with an AUC of 0.9. Importantly, our study group included RP patients at different stages of the disease, some with substantial functional VF (such as patient 3) and some patients who had no light detection (such as patient 30), further emphasizing the high specificity and sensitivity of the LMCV score. In coming clinical trials using a chromatic multifocal pupillometer, we will test a larger VF using a larger cohort of patients and healthy participants. The future study also will include more thorough computational optimization for clustering based on the promising results of random reduction of target locations. In addition, future trials are planned in which the pupillometry testing of patients at early stages and healthy participants will be

![Figure 8. A, E, Chromatic dark-adapted Goldmann visual field (CDA-GVF) results in patient 30 demonstrated no light detection. The (B, F) percentage of change of pupil size (PPC), (C, G) maximum contraction velocity (MCV), and (D, H) latency of maximum contraction velocity (LMCV) parameters of this patient in response to (B–D) blue-light and (F–H) red-light stimuli recorded in each of the 76 points of the 16.2° visual field are presented. Color coding is the same as described in Figure 4. deg = degree.](image-url)
performed in a masked fashion to examine the applicability of pupillometry testing as a diagnostic tool.

Previous studies suggested direct correlation between pupil response amplitude, maximum velocity, and latency in response to full-field white-light stimulus. The authors noted that most of the latency in healthy participants was the result of delay in iris smooth muscle contraction and that a relatively small part is the result of conduction along the pupil reflex pathway. Because apoptosis in the iris muscle increases with age, we included age-matched groups with a wide range of ages (26–77 years). To the best of our knowledge, our study enables mapping different pupil light response parameters in different locations of the VF in response to red and blue light for the first time. Our findings suggest that in most cases, maximum pupil contraction velocity correlated directly with change in pupil diameter. However, some exceptions were noted (for example, in patient 4). A future clinical trial with a larger study group may enable determination of the correlation between the different pupil response parameters in this new setting of response to focal chromatic light stimuli.

One of the major limitations of subjective perimetry is test–retest variability. A good correlation was demonstrated in PPC and MCV in test–retest analysis. The large bin size of the MCV parameter (0.033 second) and outliers may be the cause for the low test–retest correlation of LMCV. A future clinical trial will examine the test reliability in repeated testing of a larger number of healthy participants and patients.

The chromatic multifocal pupillometer device presented here was built to enable mapping of the central VF. We chose to examine the device in RP patients because of their pathologic features that facilitate the differentiation between cone and rod function. Future clinical trials will include patients with other blinding diseases, such as patients with macular degeneration and glaucoma, using a device that enables 30° VF testing.

The major limitation of this study is the size of the study cohorts. This is a preliminary study that is being followed by a larger clinical trial with more types of diseases. Several studies demonstrated a correlation between structural abnormalities on optical coherence tomography (OCT), including decreased outer nuclear layer and outer segment thickness and the loss of local VF sensitivity of RP patients. Wen et al demonstrated strong positive correlation between cone function preservation measured by multifocal electroretinography, VF sensitivity, and the remaining thickness of the photoreceptor layer by spectral-domain OCT in RP patients. Recent adaptive

Figure 9. A, E, Chromatic dark-adapted Goldmann visual field (CDA-GVF) results in patient 3 demonstrating the subjective visual field (VF) for (A) blue and (E) red light. The dashed green line marks the borders of the 16.2° VF. The (B, F) percentage of change of pupil size (PPC), (C, G) maximum contraction velocity (MCV), and (D, H) latency of MCV (LMCV) parameters of the patient in response to (B–D) blue-light and (F–H) red-light stimuli recorded in each of the 76 points of the 16.2° VF are presented. Color coding is the same as described in Figure 4. deg = degree.
optics scanning laser ophthalmoscopy studies demonstrated early detection of reduced cone density and irregular cone mosaic spatial arrangement in the macula of RP patients with best-corrected visual acuity of 20/20.46–48 Future trials will include follow-up pupillometry and spectral-domain OCT imaging that will allow analysis of longitudinal changes in pupillary response with respect to disease progression and examination of the correlation between functional pupillometry findings and spectral-domain OCT structural findings. In addition, it would be interesting to examine the correlation between the chromatic multifocal pupillometry function testing and adaptive optics scanning laser ophthalmoscopy imaging. In the current study, the pupillometer test results were compared with CDA-GVF that enables evaluation of cone and rod responses. In future studies, the pupillometer results will be compared with the more commonly used Humphrey perimetry (Carl Zeiss Meditec, Inc., Jena, Germany).

Taken together, our results suggest direct correlation between seeing and pupil response function of the retina. Different parameters of pupil response can be used for VF determination (PPC and MCV) and disease diagnosis (stimulus color and LMCV). The pupillometer test is predicted to be less stressful for tested subjects because patients are unaware of the test results and the test may facilitate objective perimetry and assessment of function of retinal photoreceptors with minimal patient cooperation and minimal technician training.

Figure 10. Chromatic dark-adapted Goldmann visual field (CDA-GVF) results in retinitis pigmentosa patient 4 demonstrating the subjective visual field (VF) for (A) blue and (E) red light. The dashed green line marks the borders of the 16.2° VF. The (B, F) percentage of change of pupil size (PPC), (C, G) maximum contraction velocity (MCV), and (D, H) latency of MCV (LMCV) parameters of this patient in response to (B–D) blue-light and (F–H) red-light stimuli recorded in each of the 76 points of the 16.2° VF are presented. Color coding is the same as in Figure 4. deg = degree.

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Footnotes and Financial Disclosures

Originally received: January 11, 2016.
Final revision: May 21, 2016.
Accepted: May 23, 2016.

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Financial Disclosure(s): The author(s) have made the following disclosure(s): Y.R.: Financial support (to institution) — Accutome Inc., Malvern, PA; Patent — Sheba Medical Center, Tel-Hashomer, Israel

Supported by Accutome, Inc., Malvern, PA; Accutome, Inc., participated in review of the manuscript but had no role in the design or conduct of this research.

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Obtained funding: Rotenstreich
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Abbreviations and Acronyms:
AUC = area under the receiver operating characteristic curve; CDA-GVF = chromatic dark-adapted Goldmann visual field; LMCV = latency of maximum contraction velocity; MCV = maximum contraction velocity; OCT = optical coherence tomography; PPC = percentage of change of pupil size; RP = retinitis pigmentosa; SE = standard error; VF = visual field.

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