



# Correlation between color vision, visual acuity, contrast sensitivity and photostress recovery in the visually impaired: a cross-sectional study

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**Background:** To investigate the correlation of colour vision, visual acuity, contrast sensitivity, and photostress recovery time test scores in visually impaired patients.

**Materials and methods:** A total of 133 subjects were enrolled and 133 eyes were examined. The pathological group consisted of 76 (57.1%) males with an average age of 68.0 (SD = 13.2) and 57 (42.9%) females, with an average age of 68.1 (SD = 15.2), Mann–Whitney U test was used to evaluate the differences in K-colour tests, HRR, visual acuity, Contrast Sensitivity test and photostress recovery time test between two different groups of severity.

**Results:** Correlations were found among colour vision tests, visual acuity, contrast sensitivity, and photostress recovery time scores in eyes with age-related macular degeneration, with diabetic retinopathy, with optic nerve diseases, and various other retinal diseases ( $P < 0.05$ ). In patients with moderate-visual impairments.

**Conclusions:** The colour vision test scores correlate with the scores of visual acuity, contrast sensitivity, and photostress recovery time test. It may be a useful clinical surrogate for functional vision.

**Keywords:** colour vision test, contrast sensitivity, K-colour test, PSRT test, visual impairment

## Introduction

The assessment of an individual abilities requires the consideration of both visual function and functional vision, especially in the case of visual impairment<sup>[1]</sup>. Visual function depicts how the basic visual system can detect a target stimulus by changing a single parameter of the target. Furthermore, the necessity to characterize accurately visual function performance is crucial in order to generate an appropriate management strategy that best suits the needs and developmental goals of an individual<sup>[2]</sup>.

Functional vision refers to how well an individual performs whereas collaboration with the visual environment<sup>[3]</sup>.

Characterizing functional vision involves the assessment of multiple and varying parameters captured under complex, real-life conditions and everyday activities. Observing a patient's

## HIGHLIGHTS

- Most LV-causing diseases tend to have a progressively course and therefore, early detection of signs of their deterioration is of great importance.
- The assessment of an individual abilities requires the consideration of both visual function and functional vision, especially in the case of visual impairment.
- The colour vision tests are correlated with the visual manifestations in low vision patients that include visual acuity, contrast sensitivity, and photostress recovery time test.
- The combination of all these techniques can provide a holistic understanding regarding an individual's visual abilities.

functional vision can signal which test of visual function should be carried out in the clinical setting.

Various disorders regarding the visual function associate with colour vision abnormalities. Colour vision is of high importance for the management of the patients, since the majority of diseases are progressive and the prognosis is significantly dependent on early detecting and treatment. Changes in colour perception are considered one of the early signs of the onset or evolution of the underlying ophthalmic disease<sup>[4]</sup>. Hence, the detection of any colour vision disorder/deficiency is a significantly important diagnostic element of the progression of the disease<sup>[5,6]</sup> in a wide variety of acquired vision deficiencies (11 plates with decreasing intensity). In clinical practice the most usual colour test are the Ishihara and the HRR. The K-colour test is a digital test and examines the thresholds of light intensity required for hue recognition which may be disrupted in patients with ocular diseases<sup>[7–9]</sup>.

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article

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Annals of Medicine & Surgery (2024) 86:742–747

Received 18 January 2023; Accepted 9 November 2023

Published online 20 November 2023

<http://dx.doi.org/10.1097/MS9.0000000000001522>

Recently, various digital colour vision tests have been developed. The Mollon-Reffin minimalist test in which the participant has to identify a coloured cap among four grey distractors with various brightness<sup>[10]</sup>. Several different versions of the Cambridge Colour Test with dynamic chromaticity contrast adjustment according to the answers given by the patient, providing a quantification of the discrimination performance along the three confusion axes<sup>[11]</sup>. A new test for colour vision assessment, called Optopad is based on the measurement of colour discrimination thresholds using an iPad<sup>[12]</sup>. The Seoul National University colour vision test uses pseudoisochromatic plates on a computer screen to assess the red, green, blue, and yellow colour vision abnormality<sup>[13,14]</sup>.

Recent studies show that contrast sensitivity (CS) function seems to be more sensitive in depicting early signs of vision impairment for a vast number of ocular diseases<sup>[10,15]</sup>. The principal advantage of letters instead of gratings is that letters are familiar and important in everyday life. Certain mobile electronic devices (e.g. iPad test, Mobile app Aston, PsyPad test etc.) have been developed already to assess visual function and especially CS evaluation<sup>[16–18]</sup>. However, Pelli-Robson used in the present study is the most usual test in clinical practice.

Furthermore, photostress recovery time test is an objective quantitative measure of macular function. Various instruments and techniques have been used to conduct photostress testing. Light sources have included the ophthalmoscope, penlight, and others. However, the lack of standardization with respect to the intensity and duration of the bleaching light is the major limitation of the technique. Various diseases are influencing central vision, including age-related macular degeneration (AMD)<sup>[19]</sup> central serous retinopathy (CSR)<sup>[20]</sup>, retinal detachments (RD)<sup>[21]</sup>, and retinitis pigmentosa (RP)<sup>[22]</sup> that may affect the recovery time. In the study an electronic photostress recovery time (PSRT) test was used<sup>[23]</sup>.

In this study we assess the correlations of colour vision with visual acuity, contrast sensitivity and PSRT values in different causes of visual impairment.

## Methods

This is a cross-sectional study. Approval for this study was granted by the bioethical committee (Ethical Approval code#1.60/21.11.2018), and adhered to the principles embodied in the Declaration of Helsinki Code of Ethics of the World Medical Association. Consent forms for the research were acquired by all subjects before their participation. The General Data Protection Regulation GDPR in a research context, and the Greek Law of Data Protection were respected through the confidentiality and anonymity of the data.

### Participants

All participants were recruited prospectively from our outpatient unit. The work has also been reported in line with the STROCSS criteria<sup>[24]</sup>.

The group of pathological eyes enrolled in this study, consists of 133 patients (133 eyes).

Visual impairment was categorized in accordance to WHO classification (WHO):

Mild-visual acuity worse than 6/12 to 6/18

Moderate-visual acuity worse than 6/18 to 6/60

### Clinical Examination

All subjects underwent a comprehensive eye examination including medical history. Only the eye with the Best-corrected visual acuity (BCVA) was included from each subject, and in the case of similar BCVAs, the right eye was chosen.

The exclusion criteria were as follows: eye disorders that may potentially affect colour vision such as high refractive error, corneal opacity, acute uveitis, acute glaucoma. Patients were free of significant lenticular changes according to WHO classification<sup>[25,26]</sup>. Subjects taking pharmacological treatment that may affect vision were excluded too. We have also excluded all patients with a visual impairment that could be attributed to more than 1 etiologies.

### Visual acuity test

Measurement of best corrected visual acuity BCVA determined with the Early Treatment Diabetic Retinopathy Study (ETDRS-4m) chart (Precision Vision, USA, Chart 1) under standard clinical conditions. BCVA was converted to logarithm of the minimal angle of resolution (logMAR) visual acuity

### Colour vision testing

Colour vision assessment was performed monocularly by a single-blind researcher using (1) the H-R-R (edition 2002; Richmond Products Inc, Albuquerque, New Mexico, USA) The six screening plates were used<sup>[27]</sup>; (2) The Ishihara Test for Colour Blindness, 38 plates (edition 2018; Kanehara & Co, Ltd, Tokyo, Japan). The number “12” had to be identified by all subjects. In case of 4 or more errors in the first 21 plates, the test was classified as abnormal<sup>[28]</sup>. Each missing number or symbol was counted as an error with an interval time of 3 seconds between plates, in both tests. (3) The K-colour test<sup>[29]</sup>. The K-colour test examines the thresholds of light intensity required for hue recognition which may be disrupted in patients with ocular diseases<sup>[7–10]</sup>. Each plate (11 plates) had a saturation of 100%, relatively fixed hue values as aforementioned, and gradually decreasing brightness. The individual looks at the screen on the front panel of the smartphone. The test starts from the easiest colour threshold and reaches the most difficult colour threshold for each colour with decreasing levels of intensity. The brightness of the colour targets was reduced step by step while the other colour properties (hue and saturation) were relatively stable. The test continues to present colour targets in the following turn: red, green, and blue colour targets. The plates were changed randomly at 3-S intervals. Each plate corresponding to a particular brightness value was shown four times with shapes changing randomly and the response was considered correct if the subject recognized the shape accurately three times, using a forced-choice method.

### Contrast sensitivity test

The Pelli-Robson contrast sensitivity test is comprised of black Sloan letters arranged in 16 groups of 3 letters and is performed on a white chart measured 59 × 84 cm. The Pelli-Robson chart has a range of contrast, from 0.00 log units (100%) to 2.25 log units (0.56%), and each triplet decreases by 0.15 log units<sup>[30]</sup>. All letters occupy a spatial frequency depending on the testing distance. The chart was performed, in order to attain a spatial frequency of 1.5 cpd<sup>[31]</sup>, and 3.0 cpd as well<sup>[32]</sup>.

## Pelli-Robson

The Pelli-Robson test with optotypes (letters) only measures ~0.9–1 cpd at the recommended distance of 1 m, and the examination must be done at different distances if more cycles per degree are needed.

The PR chart was used at approximately 1.67 m rather than the 1.0 m standard distance to achieve an approximate spatial frequency of 1.5 cpd and thus be comparable to the other test.

We employed the following relation between the viewing distances of the PR optotypes and the respective cpd values:

$$\text{distance} = \frac{\text{size\_letter}}{\tan(\text{cycles\_letter/cpd})}$$

Where: *size\_letter* is the size of the optotype (e.g. 4.85 cm for the standard PR chart), *cycles\_letter* is the number of cycles of the optotype (e.g. ~2.5 cycles for the standard PR chart), and *cpd* is the number of cycles per degree of subtended angle at a distance equal to *distance*.

Testing on the Pelli-Robson, subjects pronounced the letters across and down the chart. They were directed to start reading from the top left corner of the chart through the right side and perform it for each row. Firstly, at 1.67 m and then at 3.34 m or vice versa to evaluate contrast sensitivity at 1.5 cpd and 3.0 cpd, respectively.

## K-PSRT test (glare test)

Subjects completed measurement of BCVA at 40 cm. At ~5 cm distance from the eye (self-fixation was confirmed), the light produced by the smartphone camera filled the pupil for up to 10 sec. The user turns back the smartphone to a horizontal position at a distance of 40 cm. The photostress recovery time was assessed immediately by asking the subjects to read correctly three successive letters of size corresponding to the previous line of the BCVA line on the screen, with the opposite eye shielded. Photorecovery testing was performed with the pupils undilated. PSRT was recorded in seconds<sup>[23]</sup>.

The K-PSRT application as well as the K-colour test has been performed using a Samsung Galaxy A30S, the features of which are the following: platform: Android OS 9.0, Mali-G71 MP2; display: Super AMOLED, size: 6.4 inches, 100.5 cm<sup>2</sup>, resolution: 720 × 1560 pixels – 19.5:9 ratio (~268 ppi density); running Android OS 9.0. with maximal brightness of 489 cd/m<sup>2</sup> (personal communication with Samsung Technical Support). The device was switched on approximately 5 minutes before each experimental session to permit its output to stabilize. The screen was standardized at full brightness.

## Statistical analysis

Continuous variables were presented with mean and standard deviation, whereas for categorical variables frequencies and percentages were used. Shapiro-Wilk test was used to evaluate the assumption of normal distribution of the continuous variables. Mann-Whitney U test were used to evaluate the differences in K-colour tests, HRR, visual acuity, Contrast Sensitivity test and PSRT test between the 2 different groups of severity of visual impairment due to our continuous variables were not normally distributed across the different severity groups. To assess the correlation between contrast sensitivity test, logMAR VA and

PSRT test with all colour vision tests (K-colour tests, H-R-R, Ishihara), Spearman's correlation coefficient was calculated. Statistical analysis was performed using SPSS 28.0. Statistical significance level was set at *P*-value ≤ 0.05.

## Results

A total of 133 subjects were enrolled and 133 eyes were examined. The sample consists of 76 (57.1%) males with an average age 68.0 (13.2) and 57 (42.91%) females with an average age of 68.1 (15.2). The average best corrected visual acuity (logMAR) for pathological eyes was 0.70 (SD = 0.24) and ranged from 0.94 to 0.46 log units (Table 1).

Patients with mild-visual impairment have significantly higher scores than moderate impairment, in K-Colour tests (Red, Green, Blue), HRR, Ishihara and Contrast Sensitivity tests for the examined spatial frequencies 1.5 c/d and 3.0 c/d. Regarding the PSRT, there was no significant difference among (Table 2).

In 52 patients with AMD (Table 3), there was a moderate and positive significant correlation between contrast sensitivity test at 1.5 c/d and K-colour tests (Red, Green, Blue), and a strong and positive significant correlation between contrast sensitivity test at 1.5 c/d with the H-R-R and Ishihara test. Contrast sensitivity test at 3.0 c/d had a moderate and positive significant correlation with K-colour tests (Red, Green, Blue) and H-R-R test, and a strong and positive significant correlated with Ishihara test. Moreover, a weak and positive significant correlation was observed between logMAR VA and K-colour tests (Red, Blue) and H-R-R test, and a moderate and positive had significant correlation between logMAR VA and Ishihara test. Finally, PSRT-test had significant moderate and positive correlation with K-colour test (Red, Blue) and H-R-R. No other significant correlations were observed.

Regarding 27 patients with Diabetic retinopathy (Table 4), contrast sensitivity test at 1.5 c/d had a weak and positive significant correlation with H-R-R, a moderate and positive significant correlation with K-colour tests (Red, Blue) and Ishihara test, and a strong and positive significant correlation with green scores in K-colour test. There was also a weak and positive significant correlation between contrast sensitivity test at 3.0 c/d

**Table 1**  
Characteristics of 133 patients

Characteristics	
No. eyes, <i>N</i> (%)	
Right	71 (53.4)
Left	62 (46.6)
Sex, <i>N</i> (%)	
Male	76 (57.1)
Female	57 (42.9)
Age (years), mean (SD)	
Male	68.0 (13.2)
Female	68.1 (15.2)
Causes, <i>N</i> (%)	
AMD	52 (39.1)
DR	27 (20.3)
Other causes of low vision	54 (40.6)
logMAR, mean (SD)	0.70 (0.24)

Distribution of the clinical/demographic characteristics of the study population (age, sex, causes).

AMD, age-related macular degeneration; DR, diabetic retinopathy, Other causes of low vision: retinitis pigmentosa, stargardt disease, macular holes, epiretinal membrane, retinal vein occlusion, retinal detachment). Optic nerve diseases (OND) logMAR = logarithm of the minimum angle of resolution.

**Table 2**  
Median (range) between mild and moderate impairments

	Visual impairment		P
	Mild (n=37)	Moderate (n=96)	
K-Colour Red	3 (0, 11)	1 (0, 11)	<0.001
K-Colour Green	4 (0, 11)	2 (0, 11)	<0.001
K-Colour Blue	5 (0, 11)	4 (0, 11)	0.002
HRR	3.5 (0, 6)	0.5 (0, 6)	<0.001
Ishihara plates	30.5 (1, 38)	4.5 (0, 38)	<0.001
CS 3 c/d	1.35 (0.6, 1.65)	0.90 (0.15, 1.50)	<0.001
CS 1,5 c/d	1.35 (0.75, 1.65)	1.05 (0.45, 1.65)	<0.001
PSRT <sup>a,b</sup>	30 (10, 180)	28 (10, 80)	0.907

PRST, photostress recovery test.  
<sup>a</sup>n = 18 patients with glare.  
<sup>b</sup>n = 32 patients with glare.  
 \*Statistically significant at level 0.05.

and blue scores in K-colour test, and a moderate and positive significant correlation between contrast sensitivity test at 3.0 c/d and K-colour test for Red and Green. As regards the HRR and Ishihara test there was a moderate and positive significant correlation with the CS at 3.0 c/d. Finally, the PSRT test had significant moderate and negative correlation with H-R-R and Ishihara test. No other significant correlations were observed.

**Discussion**

Most LV-causing diseases tend to have a progressively course and therefore, early detection of signs of their deterioration is of great importance for medical support and significantly affects the maintenance of a functional level of vision. Acquired deficiency changes in severity, vary between eyes and is frequently more difficult to be classified. Moreover, unlike congenital deficiency, acquired colour deficiency is often associated with reduced visual acuity and the occurrence of visual field defects<sup>[33]</sup> This study intended to explore if apparent colour vision defects measured by H-R-R, Ishihara tests and K-colour tests indicate changes in other psychovisual parameters, such as contrast sensitivity, visual acuity as well as PSRT.

Screening tests, such as pseudoisochromatic plates, are designed to quickly and reliably identify colour defects. Grading tests, particularly arrangement tests are meant to assess the severity of the defect. Diagnostic tests, like the anomaloscope, can precisely classify the defect<sup>[34]</sup> Anomaloscope permits the differentiation of red-green colour vision defects. However, the instrument is relatively complex and requires training to use and interpret properly.

**Table 3**  
Comparisons of novel colour test scores, Ishihara and HRR test with Contrast sensitivity, visual acuity, PSRT in AMD patients (n = 52)

	K-colour test				
	Red	Green	Blue	HRR	Ishihara plates
CS 3 c/d	0.59 <sup>a</sup>	0.48 <sup>a</sup>	0.42 <sup>a</sup>	0.54 <sup>a</sup>	0.77 <sup>a</sup>
CS 1.5 c/d	0.55 <sup>a</sup>	0.55 <sup>a</sup>	0.48 <sup>a</sup>	0.57 <sup>a</sup>	0.71 <sup>a</sup>
logMAR VA	-0.31 <sup>a</sup>	-0.25	-0.29 <sup>a</sup>	-0.38 <sup>a</sup>	-0.55 <sup>a</sup>
PSRT	-0.24	-0.34 <sup>a</sup>	-0.40 <sup>a</sup>	-0.33 <sup>a</sup>	-0.05

AMD, age-related macular degeneration; PRST, photostress recovery test.  
<sup>a</sup>Statistically significant at level 0.05.  
 (Spearman's correlation coefficient).

**Table 4**  
Comparisons of novel colour test scores, Ishihara and HRR test with Contrast sensitivity, visual acuity, PSRT in DR patients (n = 27)

	K-colour test				
	Red	Green	Blue	HRR	Ishihara plates
CS 3 c/d	0.63 <sup>a</sup>	0.55 <sup>*</sup>	0.38	0.65 <sup>a</sup>	0.49 <sup>a</sup>
CS 1,5 c/d	0.60 <sup>a</sup>	0.55 <sup>a</sup>	0.53 <sup>a</sup>	0.77 <sup>a</sup>	0.65 <sup>a</sup>
LogMAR VA	-0.26	-0.16	-0.17	-0.29	-0.29
PSRT	0.29	0.27	0.29	0.56 <sup>a</sup>	0.66 <sup>a</sup>

DR, diabetic retinopathy; PRST, photostress recovery test.  
<sup>a</sup>Statistically significant at level 0.05.  
 (Spearman's correlation coefficient).

In the present study, the HRR, the Ishihara tests and the K-Colour test were selected for comparisons due to their general acceptance as effective colour tests in clinical practice. The Ishihara test, is known for its high sensitivity and excellent specificity for the detection of congenital protan and deutan deficiencies. Ishihara tests are often combined with the American Optical Company Hardy, Rand, and Rittler (H-R-R) plates, which, provide screening and diagnostic plates and for tritan defects and correctly classified protans and deutans on 80 to 90 per cent of occasions<sup>[35-38]</sup>. The smartphone-based K-colour test evaluates colour vision defects in patients with acquired deficiencies. The K-colour test was found to be reliable and it is not affected by illumination like the traditional pseudoisochromatic plates within a range of lighting conditions since the colours are viewed on a smartphone screen<sup>[39]</sup>. It has been stated that the minimum visual acuity 20/200 is required for the performance of HRR, Ishihara test as well as the K-colour vision test<sup>[40]</sup>.

Test of Cone Contrast was created to evaluate each axis' colour contrast sensitivity and to identify the different types of dyschromatopsia. The test is conducted using a computer and displays coloured letters that gradually lose contrast with the background in order to determine the threshold for letter recognition. A letter that must be recognized and named verbally by the participant during the test appears in the centre of the screen. The monocular exam has a voice processing system that advances the tester to the following screen if the correct response is made. The patient must respond "no" if he cannot see any letters. 20 letters are displayed during the test, which lasts for 1-1.6 seconds<sup>[41]</sup>.

The most widely used colour vision test in everyday practice is the Ishihara test, which has to be combined with another test e.g. the HRR test for a more comprehensive colour examination<sup>[41]</sup>. Thus, sometimes multiple tests are required to diagnose acquired defects and to follow the evolution of colour vision loss.

Either YB or RG thresholds, or both, were found to be abnormal in eyes with macular degeneration This observation is consistent with reports by Verriest *et al.*<sup>[41]</sup>. Though, many aspects of functionality in vision remain normal, the loss of both RG and YB colour vision may be surprisingly severe in AMD<sup>[42]</sup>. In the early stages of the disease process, most patients will have (blue-yellow) defects. Other studies have found that either B-Y or R-G thresholds, or both, were abnormal in eyes with AMD, and the B-Y loss seems to be on average greater than R-G loss<sup>[43]</sup>. S cones appear to be more vulnerable to alterations in the metabolic environment of the retinal pigment epithelium (RPE)—photoreceptor complex in the early stages of the disease<sup>[44]</sup>.

The impaired red/green discrimination and blue-yellow loss in diabetic patients suggest the existence of different causes of the

functional defect. Our results are in accordance with a previous study, where diabetic retinopathy, were evaluated in colour vision with the use of HRR and Ishihara test<sup>[45]</sup>. In our study, the HRR and the Ishihara test scores significantly correlated with the K-colour test scores in red, green, and blue.

Furthermore, in maculopathies particularly in the earlier stages of the disease process, most patients exhibit BY defects, despite preserving a relatively good visual acuity. Optic nerve diseases more frequently cause a RG deficiency, but in cases of well-preserved visual acuity, the predominant deficiency is on BY axis. The most frequent chromatic anomaly related to early primary open-angle glaucoma is a blue-yellow defect<sup>[41]</sup>. Concerning patients with retinitis pigmentosa, previous studies reported that they demonstrate either BY or RG deficiencies<sup>[46]</sup>. In Stargardt's disease out of 12 carriers of ABCA4 mutations, 6 (50%) had a normal colour vision, 3 (25%) had M-L mechanism deficiencies, 2 (17%) had S-mechanism deficiency, and 1 (8%) had combined S- and M-L mechanism deficiency<sup>[47]</sup>.

The results presented herein, together with the outcome of other studies, indicate that VA and CS do not always show the same progression in visual function loss, although they show in general a moderate correlation<sup>[48]</sup>. The results support the fact that contrast sensitivity evaluation on a routine ophthalmological examination along with visual acuity assessment is crucial. Previous studies of the Pelli-Robson test have stated that mean values at 1.5 c/d in normally sighted subjects range between 1.70 and 1.98 whereas in the visually impaired population vary between 1.01 and 1.57<sup>[49,50]</sup>. Thus, in the present study the performance of the PR test is highly comparable to previous studies. Additionally, at 3 c/d existing literature shows that controls' contrast range approximately between 1.68 to 1.85.

Prolonged PSRT was seen in AMD patients irrespective of the type of AMD<sup>[51]</sup>. Photostress recovery time can be prolonged in other diseases affecting the macula like diabetes and drug-induced maculopathy<sup>[52,53]</sup>. Recovery time is more prolonged in cases of AMD than in diabetic retinopathy as the pathology lies at the level of the RPE photoreceptor complex. In diabetic retinopathy, it is in the inner retinal layers. There is a statistically significant prolongation of photostress recovery in the presence of diabetic macular oedema, but to a less pronounced degree than with AMD or subretinal fluid<sup>[52]</sup>. In our study, the results are in agreement. This study attempted to establish differentiating between normal from abnormal macular diseases<sup>[23]</sup>. The recovery time from normal individuals ranged from 10 to 50 sec<sup>[54]</sup>. Normal PSRTs were 27 s up to the age of 60 years, and 30 s over the age of 60 years<sup>[55]</sup>. Various instruments and techniques have been used to conduct photostress testing. Light sources have included the ophthalmoscope, penlight, and computer monitor. However, the lack of standardization for the intensity and duration of the bleaching light is the major limitation of the technique. This new testing modality, designed to be an easily implemented measurement in clinical ophthalmic practice, can expand our understanding of macular function. The above findings support the usefulness of a reproducible photostress application as an indicator of macular pathology.

## Conclusions

There are the same limitations to the present study. The comparison with the anomaloscope, which is the gold standard

evaluation for colour vision defects might be useful. Moreover, the lack of standardization in the PSRT test. Our findings need further support and further studies are needed to analyse the relations concerning colour vision, contrast sensitivity, PSRT-test, and visual acuity.

In conclusion, the combination of all these examinations can provide a holistic understanding regarding an individual's visual abilities.

## Ethical approval

Approval for this study has been obtained by the Bioethical Committee (Ethical Approval, code#1.60/21.11.2018), School of Medicine, Medical Department of the Aristotle University of Thessaloniki. Its conduction was following the rules and regulations of the Declaration of Helsinki.

## Consent

Consent to use data for scientific purposes was obtained from all patients.

## Sources of funding

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness Entrepreneurship and innovation under the call Research-Create-Innovate.

## Author contribution

D.A.: data collection, draft preparation, writing, measurements. S.A.: writing, measurements. T.C.: writing, measurements. A.N.: bibliographic support, draft preparation. P.T.: data analysis. V.K.: design innovation, scientific responsible, moderator, writing.

## Conflicts of interest disclosure

The authors declare that there is no conflicts of interest regarding the publication of this paper.

## Research registration unique identifying number (UIN)

Officially registered at ClinicalTrials.gov. The number of the study is NCT05184036 (<https://www.clinicaltrials.gov/ct2/show/NCT05184036?cond=NCT05184036&draw=2&rank=1>).

## Guarantor

Prof. Vasileios Karampatakis.

## Data availability statement

Data available upon reasonable request.

## Provenance and peer review

Not commissioned, externally peer-reviewed.

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