

# Spatial properties of fundus reflectance and red–green relative spectral sensitivity

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Received 15 May 2015; revised 3 August 2015; accepted 7 August 2015; posted 11 August 2015 (Doc. ID 240961); published 31 August 2015

We investigated the spatial characteristics of the diffuse light in the eye at two different wavelengths and the extent to which this may affect red–green relative spectral sensitivity. The fundus reflectance of six subjects was measured for different field sizes ranging from a  $0.18^\circ$  to  $7.28^\circ$  radius and for two different wavelengths, 560 and 650 nm. The experimental setup consisted of having an instrument project uniform disks on the fundus and recording their retinal images after a double pass through the eye. Additionally, the relative spectral sensitivity for the same wavelengths was measured using heterochromatic flicker photometry for a stimulus of a  $0.4^\circ$  radius with and without the presence of a synchronously flickering concentric annulus. We concluded that although light backscattered from the fundus contributes to vision, the effect is not strong and can only be observed under appropriate laboratory conditions. This suggests that diffuse light from deeper fundus layers is unlikely to have a practical relevance to vision. © 2015 Optical Society of America

**OCIS codes:** (330.1720) Color vision; (330.4595) Optical effects on vision; (290.1350) Backscattering; (290.1990) Diffusion; (290.2648) Stray light.

<http://dx.doi.org/10.1364/JOSAA.32.001723>

## 1. INTRODUCTION

The optical quality of an optical system is usually characterized by its point spread function (PSF) and/or the modulation transfer function [1]. For the human eye, a common technique to determine those functions is the double-pass technique, where images are projected onto the retina and the reflection is recorded at a camera or detector, conjugated to the retinal plane [2–5]. In these types of measurements, the retina is assumed to be a reflector (a screen like surface), while diffusion phenomena are generally ignored. The recorded double-pass retinal image is the autocorrelation of the PSF of the eye [3]. The reflected light is backscattered at the various layers of the fundus and represents a small fraction of the incident light. This reflectance exhibits a characteristic wavelength dependence of approximately 1% for the green part of the visible spectrum and increasing up to 10% for red and near-IR light [6–8].

By using the optical integration method for the measurement of stray light in the human eye [9], it was observed that for wavelengths longer than 600 nm, diffusion at the fundus contributes significantly to the measurements [10]. Although the shape of the central part of the PSF (pertaining to aberrations) is not affected by diffusion phenomena, the latter may represent an artifact when the skirts of the PSF are analyzed to estimate stray light [9]. This effectively

means that for longer wavelengths there is a much stronger spatial dependence on the overall light in the eye than for shorter wavelengths; the size of an object matters more for longer wavelengths than for shorter wavelengths in terms of how much light is propagating in the eye. Consequently, this light can go through the photoreceptors again, stimulating them again. Therefore, according to our hypothesis, a change in the size of an object would affect its effective luminance more in red than in green light due to increased backscatter.

The objective of this work was twofold: first, to investigate the spatial and spectral characteristics of diffuse light in the fundus and, second, to investigate its visibility, i.e., its effect on color sensitivity. For this purpose we used an optical method to measure the increase in diffuse light at a central retinal patch when uniform bright disks of different field sizes were projected onto the fundus for two different wavelengths (560 and 650 nm). Since diffusion depends on wavelength, changing the field size results in changes in the relative intensity of the central patch when measured optically. Additionally, for the visual test, we used heterochromatic flicker photometry (HFP) at the same spectral and spatial conditions in order to determine if this relative increase in diffuse light is associated with a proportional increase in sensitivity.

## 2. METHODS

Two experimental setups were used: one based on the double-pass principle, for the optical measurement of the reflected light from the fundus [9–11], and another based on HFP, for the study of color sensitivity. Both setups were laboratory prototypes specifically developed for the present study.

### A. Subjects

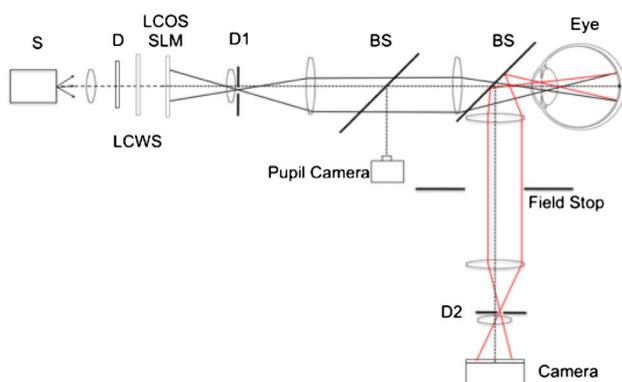
Six subjects, all Caucasians between 28 and 52 years old, with normal trichromatic vision, participated in the study. Their mean age was 36.3, and the standard deviation was 10. Three of the subjects were classified as dark-eyed (two dark brown and one light brown) and three as light-eyed (two blue and one green). This classification was done by simple iris observation. The intraocular scattering of the subjects was measured to be within the normal range and was similar between all subjects. The experiment followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects after they were fully informed about the nature of the measurements.

### B. Optical Test

A schematic diagram of the setup for optical measurements is depicted in Fig. 1. It consisted of a xenon lamp (S) as the light source and a liquid crystal spatial light modulator (LC2002, Holoeye, Berlin-Adlershof, Germany) conjugate with the retina (LCOS SLM), which was used to produce uniform white disks. A selectable bandwidth tunable optical filter (LCWS; Meadowlark Optics, Inc., Frederick, CO) was used to select the two different wavelengths: 560 nm (FWHM = 45) and 650 nm (FWHM = 30). The image of each disk projected onto the retina was recorded after a double passage through the eye through a beam splitter (BS), by an electron-multiplying CCD camera (EMCCD Luca, Andor, Belfast, UK). The camera's response to light was taken into account based on calibration performed in a separate experiment.

The subject's head was stabilized at the appropriate position using a bite bar, and the alignment of the pupil was done using an auxiliary camera through a BS.

Two diaphragms, D1 and D2, with a 1.8 mm diameter, both conjugated to the pupil plane, were displaced transversely



**Fig. 1.** Schematic of the optical setup used for the measurement of the fundus reflectance. D, diffuser.

to each other so that the upper part of the pupil was used for illumination (first pass, illumination pathway) and the lower part for imaging (second pass, imaging pathway). This permitted us to eliminate the contribution of backscattered light from the cornea and the lens. Moreover, the small size of the diaphragms kept aberrations to a minimum. An artificial eye with a spectrally flat diffuse reflector with 99% reflectivity between 350 and 1500 nm (SM05CP2C, Thorlabs Inc., Newton, NJ) acting as the retina was used for the calibration, and all reflectance measurements were normalized with respect to that.

In the optical experiment, a series of 40 disks, ranging uniformly from a  $0.18^\circ$  to  $7.28^\circ$  radius, was projected onto the retina, and the light reflected from the fundus was collected. The fixation point of the target was at about  $7^\circ$  nasally, chosen so that no large retinal blood vessels appeared at or near the center of the disk. This way it was ensured that the measured reflectance was that of the fundus and it was not affected by the presence of a highly absorbing vessel, whose position could vary during the projection of the different size disks. The pupil was dilated by instilling a single drop of tropicamide 1%. The power measured at the corneal plane was between 0.4 and  $0.6 \mu\text{W}$ .

After each image was recorded, its intensity at the center of each projected disk was calculated by averaging the central  $3 \times 3$  pixel area, approximately 7 arcmin. The total light available to the photoreceptors was calculated from the relation

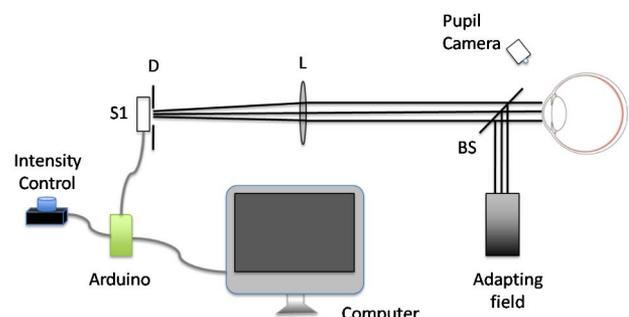
$$I_p = I + rI, \quad (1)$$

where  $I$  is the intensity of the light passing from the photoreceptors before interaction with the deeper layers of the fundus and  $r$  is the measured relative reflectance for the specific wavelength and field size.

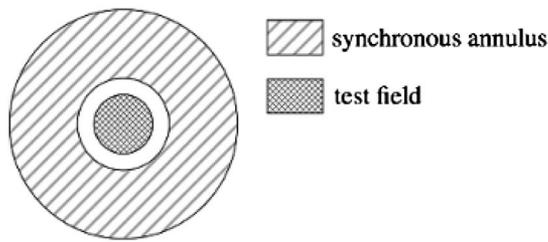
Previous studies with a similar optical setup [5,9,10] have shown that under these illumination and imaging conditions, fundus reflectance is not affected by photoreceptor bleaching.

### C. Psychophysical Test

The psychophysical method was based on HFP, which is proven to be consistent with the CIE standard observer [12]. A schematic of the setup is presented in Fig. 2. The system consisted of a uniform source of 560 and 650 nm LEDs (S1) controlled by an Arduino single-board microcontroller, an adjustable aperture (D), a plano-convex lens (L), a pupil



**Fig. 2.** Schematic of the psychophysical setup used to measure relative color sensitivity.



**Fig. 3.** Schematic of the test field used in the psychophysical experiment.

camera for subject alignment, and a white flat-spectrum source that served as the adapting field, following previous studies [13,14]. The LED source operated in two channels in opposite phases at a frequency of 25 Hz. The red light intensity was kept fixed, whereas the green light intensity was variable, controlled by the subject by means of a potentiometer. The 16° adapting field was superimposed on the flickering field with an illuminance of 3 log photopic trolands. The total average illuminance of the test field and the adapting background was 3.2 log trolands. The test field consisted of a 4° radius surrounded by a dark ring of less than 0.1° thickness, which was surrounded by a concentric annulus flickering in phase with the test field, as seen in Fig. 3. The aperture could take two distinct values, thus blocking or allowing the view of the annulus. The radius of the entire disk, i.e., the test field, dark ring, and annulus, was 6.5°.

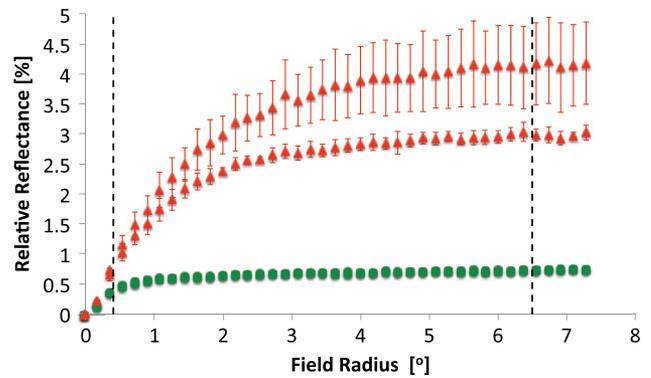
The psychophysical test was performed monocularly, and the task of the subject was to determine the green light intensity where the flicker becomes minimal. The subject was asked to find the minimum flicker intensity controlled by a potentiometer, and the examiner registered the value. At the beginning of the session the subject was given 3 min to get familiar with the experiment and the control of the knob. This time also ensured the adaptation of the subject to the background field.

The procedure was repeated 10 times for each condition, i.e., with the annulus present and without it. It is important to underscore here that although the annular field was flickering in phase with the test field, the procedure is not equivalent to testing at a larger field size. The dark ring surrounding the test field provided a clear demarcation of the test field and the annulus. The subjects, all familiar with the procedure, were instructed to judge flicker minimization based on the modulation solely of the central test field, assuring that the area under investigation was always the same; i.e., only central foveal vision was used in both field sizes.

### 3. RESULTS

Retinal reflectance depended on field size, wavelength, and the subject’s iris color. Figure 4 shows reflected light as a function of field size for red and green light and for the two groups of subjects. The errors are the standard deviation from the mean.

This shows a clear difference between reflected light at 560 and 650 nm for both groups, with the red light showing a maximum reflectance about 3 to 4 times more than that of the green light. The dependence of the reflectance on the field size is, as



**Fig. 4.** Fundus reflection for two different wavelengths and for two different subject groups with respect to field size. In green (circles) the two groups are indistinguishable, whereas in red (triangles) the light-colored iris group exhibits a much higher relative reflection.

expected, stronger for the long wavelength due to its weaker absorption and higher scattering in the retinal pigment epithelium (RPE) and the choroid [15]. Furthermore, although reflectance at red was higher for the subjects in the light-colored iris group, there was no difference at green, since RPE melanin and choroid hemoglobin absorb too much of the green light for the choroid melanin to play any significant role. One needs to note here that iris color is only related to the amount of melanin in the choroid, since the choroid and the iris comprise the same anatomical layer, the uvea, whereas RPE melanin seems to be independent of the iris color [6,16,17]. Nevertheless, there is still a significant difference in spectral reflectivity in longer wavelengths between dark iris subjects and light iris ones, which shows the importance of choroidal melanin in the backscattered light.

In order to calculate the change in the available light to the photoreceptors for two different field sizes at two different wavelengths, we used the following ratio:

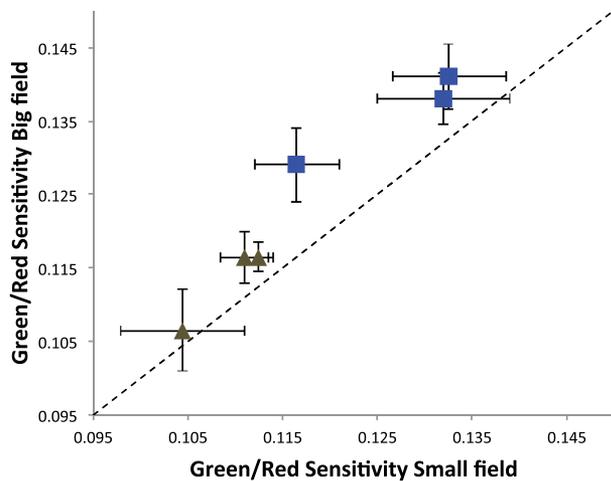
$$R = \frac{J_{p,650}^{big}}{J_{p,560}^{big}} \cdot \frac{J_{p,650}^{small}}{J_{p,560}^{small}} \tag{2}$$

Substituting the intensities from Eq. (1), we get

$$R = \frac{1 + r_{650}^{big}}{1 + r_{560}^{big}} \cdot \frac{1 + r_{650}^{small}}{1 + r_{560}^{small}} \tag{3}$$

The above ratio gives the increase in the red to green light available to the photoreceptors for a large field compared to a smaller field. The above ratios for two field sizes that match the conditions investigated in the psychophysical setup (see below), namely, a 0.4° and 6.5° radius, were 3.3% (SD = 0.6) and 2.02% (SD = 0.22) for light- and dark-eye subjects, respectively. The standard deviation refers to the group standard deviation. The individual deviation between measurements was significantly low and was not taken into account.

In the psychophysical experiment, the respective relative green to red sensitivity increases between the different



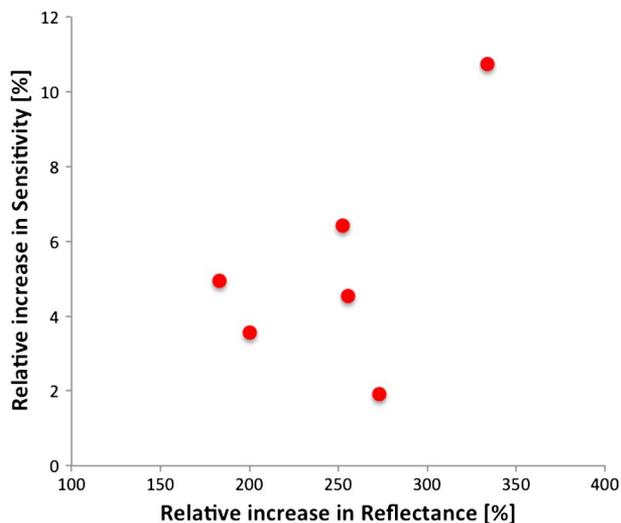
**Fig. 5.** Relative green to red sensitivity without annulus versus relative green to red sensitivity with annulus for all subjects. Subjects with a dark iris are depicted with triangles and subjects with a light iris with squares.

conditions, namely, without and with the annulus, are given by the relation

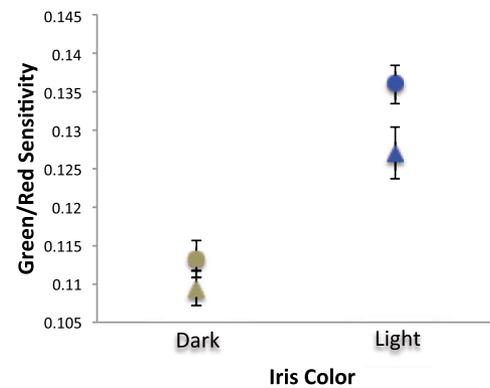
$$R_s = \frac{S_{GR}^{annulus}}{S_{GR}^{noannulus}}, \quad (4)$$

and were calculated to be 7.2% (SD = 2.6) and 3.4% (SD = 1.24) for the light group and dark group, respectively. All subjects demonstrated an increase in green to red relative sensitivity (ranging from 1.9% to 10.7%) with increasing field size. Figure 5 shows green to red sensitivity without the annulus compared to green to red sensitivity with the annulus, for all subjects. Finally, Fig. 6 shows the relative sensitivities for the two groups and for the two different conditions.

Figure 7 shows the relative increase in the red to green reflectance when the field size increases from a  $0.4^\circ$  to  $6.5^\circ$  radius with respect to the relative increase in green to red sensitivity



**Fig. 6.** Relative increase in the red to green reflectance for two field sizes with respect to relative increase in green to red sensitivity with the presence of the annulus.



**Fig. 7.** Relative green to red sensitivity for the two groups for the test field without the annulus (triangles) and with the annulus (circles).

with the presence of the synchronous annulus. The Pearson correlation coefficient was 0.5946.

#### 4. DISCUSSION

The effect of the backscattering component on the photoreceptors was previously studied through the Stiles–Crawford (SC) effect by Berendschot *et al.* [18]. The study showed that the wavelength dependence of the SC effect is caused partially by the backscattered light, and therefore the backscattered light is to some extent visible. Our findings are in line with this observation, showing an increase in sensitivity with the amount of backscattered light. Nevertheless, as shown in previous studies [19,20], the impact of the backscattered light on vision is suppressed due to the directionality of the photoreceptors and the nondirectionality of the backscattered light. This means that of the total light available at the photoreceptors, only a fraction is guided from the outer segments of the photoreceptors and contributes to vision.

There are two main underlying mechanisms that could affect the HFP test: the variability of the L:M ratio between individuals and the change in the optical density of photopigments with eccentricity.

There is a great individual variability in the L:M cone ratio, ranging from as low as 0.4 to as high as 13 [21], which has been shown to affect the individual sensitivity [13]. Therefore, while deeper fundus layers absorb and scatter light depending on the choroid melanin (thus enabling us to naturally classify objects according to the melanin pigmentation), the visual effect could in principle depend also on the L:M ratio of the individual subject. In our study we are interested solely in the change in the sensitivity between two distinct conditions (with an annulus surrounding the test field and without one), or else we are interested in the relative difference in the sensitivity ratios for the two conditions [see Eq. (4)]. This effectively means that the results are normalized, and thus any difference in the L:M ratio between subjects, however great, would not affect the quantities under investigation. Furthermore, since the psychophysical test is done at the same site for both conditions (see Section 2, Methods), changes in the L:M ratio with eccentricity, if any, would not be an issue.

Previous studies have shown that the optical density of the outer segments of the photoreceptors changes with eccentricities, and more specifically it decreases for all types of cones when moving away from the fovea [22–24]. This is believed to be a direct result of the change in length of the outer segments of the cones with eccentricity [25]. It has been shown that this change leads to a change in the width of the absorption spectrum throughout the retina that can effectively affect color vision [26–30] and can even affect macular pigment density measurements using HFP [31]. Nevertheless, this effect, also known as self-screening, did not affect our psychophysical test, since for both conditions (with the annulus and without the annulus) the same patch of foveal photoreceptors was used for the flicker canceling (see Section 2).

It needs to be noted that while the psychophysical task was performed foveally, the diffuse reflectance properties of the fundus were measured at 7° nasally. Optical measurements in the periphery have some advantages; namely, they are more stable, as the subject can see the fixation target better and it is easier to avoid vessels. However, this may have some effect on the measurements due to higher melanin density at the fovea [32,33].

Therefore, the discordance between the psychophysical and the optical results can be attributed to the nonuniform distribution of melanin at the retina and/or large errors in the psychophysical task, associated mostly with the subjective nature of the test. Moreover, regarding the psychophysical test, it is worth noting here that for the light-colored iris subjects, the variation between measurements was larger than that for dark-colored iris subjects, but the results were still consistently better when the annulus was present.

On the other hand, the optical test has been proven to be quite precise, showing very high repeatability of the results. Therefore, direct comparison of the two experiments might be naïve. However, and despite the quantitative discrepancies, a qualitative comparison of the results from the two experiments shows that the trend is the same, indicating that the optically observed excess light can be detected up to a degree psychophysically.

## 5. CONCLUSION

The qualitative comparison of the two different tests revealed two main points: there is an overall increase in the sensitivity due to diffuse light in the deeper fundus layers, which is consistent in all subjects, and this increase depends on the choroidal melanin of the subject, even for a simple classification by iris color. Therefore, there seems to be a contribution from the backscattered choroidal light to vision. Nevertheless, the effect can only be observed under appropriate laboratory conditions. Its small magnitude suggests that diffuse light from deeper fundus layers is unlikely to have practical relevance to vision.

**Funding.** European Commission (EC) (PITN-GA-2010-264605); European Research Council (ERC) (ERC-2013-AdG-339228); SEIDI, Spain (FIS2013-41237-R).

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