Counterphase modulation photometry: Comparison of two instruments

Helen Le Sueur,[1*] John D. Mollon,[2] Jeroen Granzier[1] and Gabriele Jordan[1,]

[1]*Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, UK*
[2]*Department of Experimental Psychology, University of Cambridge, Cambridge, UK*
**Corresponding author: Gabriele.Jordan@ncl.ac.uk*

The ratio of long-wavelength to medium-wavelength sensitive cones varies significantly between people. In order to investigate the possible effect of this variation in large numbers of participants, a quick and efficient method to estimate the ratio is required. The OSCAR test has previously been utilized for this purpose, but it is no longer available commercially. Having access to one of the few remaining OSCAR instruments, we compared observers' mean settings to those obtained with the Medmont C100, a newer but apparently similar device. We also obtained Rayleigh matches for each participant. 102 volunteers took part in the study. Settings on the OSCAR test were highly correlated with those on the Medmont C100. Both tests appear to be influenced not only by L:M cone ratios, but also by the spectral positions of the cone photopigments, since anomaloscope mid-match points accounted for a significant proportion of the variance. We conclude that the Medmont C100 can be used as a suitable replacement for the OSCAR test and has a role in the rapid estimation of L:M cone ratios.

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1.Introduction

Classically, estimates of the ratio of long-wavelength sensitive (L) to medium-wavelength sensitive (M) cone types in the retina were derived by finding the relative heights of L and M cone sensitivities needed to reconstruct the luminosity curve obtained from flicker photometry. Using this method, it was shown that the L to M cone ratio varies substantially between people with normal color vision [1]. Since then several other techniques have been used to estimate the range of this variation, including electroretinography (ERG) [e.g. 2-4] and retinal densitometry [3] yielding a range of L:M cone ratios that extends from 0.4:1 to 13:1 with an average of 2:1 [4]. A similar average estimate has been suggested by the more direct methods of microspectrophotometry [5] and retinal imaging [6].

To compare the outcome of several different methods, Kremers et al. studied the L:M cone ratios in 33 participants using psychophysics, ERG and retinal densitometry [3]. Psychophysical methods included cone modulation thresholds, minimal flicker perception, and heterochromatic flicker photometry. Though individual cone ratios were not given, all measures used indicate that there is substantial variation of L:M cone ratios across normal observers. The ratios obtained with each method were found to correlate highly, with the exception of cone modulation thresholds which were measured at low temporal frequencies.

Flicker photometric settings and the equivalent ERG settings are likely to be influenced not only by L:M cone ratios but also by individual variation in the exact spectral positions of the cone pigments [7]. To allow for this source of variance, Carroll and colleagues [4] explored the variation in cone ratios using ERG in 62 males with normal color vision and estimated subjects' L-cone spectral absorbance curves from their respective L-opsin gene sequences. The corrected estimates of L:M cone ratios were found to vary from 0.4:1 to 13:1, but the majority (80%) of participants exhibited ratios within a much narrower range (1:1 – 4:1).

To explain the substantial variability in the L:M cone ratio, two factors can be considered. Firstly, polymorphisms upstream of the opsin genes may affect transcription factor binding sites and determine which opsin gene is expressed in each photoreceptor, thus influencing the ratio of L to M cones [8, 9]. Secondly, some 15% of women are heterozygotes for dichromacy or anomalous trichromacy and in their case, X-chromosome inactivation will lead to abnormal cone ratios: Protan carriers, for example, will have fewer cones of the long-wave type [10].

Though several different methods for estimating the L:M ratio are available, many of these are impractical for taking quick measurements from large numbers of participants. Both electrophysiological measures (such as ERGs) and many psychophysical ones (such as conventional flicker photometry) require time-consuming procedures.

In 1983 Oscar Estévez and colleagues introduced a flicker photometric-type test (known as the OSCAR test) as a quick screening test for color vision deficiencies [11]. It is a small, portable device that measures the relative sensitivity to red and green light using the method sometimes termed *counterphase modulation photometry*. Relative to conventional flicker photometry, the method has the advantage that the time-averaged luminance and chromaticity remain constant during a participant's settings. Estévez and colleagues showed that their test reliably distinguishes protans and deutans, and this has subsequently been confirmed in a number of studies [12-14]; but the test proved to be unsuitable as a general screening test for color deficiencies, since many deutan subjects are not distinguishable from normal [15]. More recently, the OSCAR test has been used to estimate L:M cone ratios in a substantial cohort of over 1000 participants and has proved to be a reliable and quick estimate of cone ratios [16]. The theoretical basis for the OSCAR's ability to estimate cone ratios lies in the fact that the total strength of the signal from either the L or the M cone depends on the total number of each cone type. For a participant with a lower L:M cone ratio than average, he or she will need a greater depth of modulation of the red LED to balance the modulation of the green LED. For this reason, the OSCAR test can also be used to differentiate between protan and deutan heterozygotes (see [14] for detail). Despite its advantages, unfortunately, the OSCAR test is no longer available commercially.

The Medmont C100 is a newer alternative to the OSCAR test and is claimed to work in the same way. Like the OSCAR test, the Medmont C100 was not originally developed to estimate cone ratios; instead it was

introduced to the market as a test for color vision deficiencies and though it has been shown to be unsuitable for the purpose of separating color-deficient people from color-normal people [17], it has found use in categorizing already diagnosed red/green deficiencies into protan and deutan groups [17]. In addition, the instrument has been used to reliably identify carriers of protan deficiencies [18]. Despite the similarity in design and appearance, the two tests have never been directly compared and the potential of the Medmont C100 for estimating L:M cone ratios has not been exploited.

Those color scientists who aim to test large numbers of observers to gain population statistics would benefit from a quick and reliable method of estimating L:M cone ratios. Our aim was to compare the two instruments and establish whether the Medmont C100 could be a suitable substitute for the OSCAR test. We also obtained anomaloscope settings for each participant.

2.Method

A. Participants

114 participants were originally recruited to this study. Out of these, 103 (44 male, 59 female) completed all measures. One female participant was removed from analysis owing to lack of comprehension of one of the tasks. A total of 102 participants were included in the analyses.

The age range was 7 to 65 years, with a mean age of 31 years. There was no difference in mean age between males and females (mean female age: 31 years, mean male age: 30 years, $t = 1.984$, $p = 0.724$).

Ethical approval for the study was granted by the Faculty of Medical Science (FMS ethical application 00622/2012).

B. Instruments

OSCAR Test:

The OSCAR test is a small instrument, designed to be held in the hand. Inside the device, the outputs of a red (650 nm) and a green (560 nm) LED (see [11]) are mixed in a Perspex rod and are modulated in counterphase at 16Hz. The participant, looking at the other end of the rod, observes a flickering orange light and, using a control wheel, adjusts the relative depth of modulation of the two LEDs. To make a setting, the participant is instructed to stop when the flicker either disappears or is judged to be minimal. The scale is shown on the wheel and ranges from -9 to +5.

Medmont C100 Test:

The Medmont C100 imitates the design of the OSCAR test, except that the scale appears on the rear of the instrument and ranges from -5 to +5. The dominant wavelengths of the red and green LEDs are given as 626- and 569 nm respectively and the rate of flicker is 16Hz. As in the case of the OSCAR test, participants are required to adjust the control wheel until the flicker disappears or appears minimal. A setting of less than -2 should indicate a protan deficiency whereas a setting of more than +2 should indicate a deutan deficiency amongst those already diagnosed with colour deficiency.

Oculus Anomalsocope:

The anomaloscope measures the Rayleigh equation, i.e. the ratio of red (666 nm) and green (549 nm) primaries needed to match a monochromatic yellow (589 nm). The participant views a 2-degree bipartite circular field and adjusts the ratio of red to green light in the top half to match the monochromatic yellow light in the lower half. The brightness of the yellow light is also adjustable. The range of red/green ratios accepted as a perfect match to the yellow standard light is taken as the Rayleigh matching range and is indicative of an observer's color discrimination.

C. Procedure

On arrival each participant was first asked to make five settings on the OSCAR test and on the Medmont C100 respectively. The order of the tests was randomized. Finally, the Rayleigh match mid–point and range were found for each participant on the anomaloscope using his or her dominant eye.

The OSCAR and Medmont C100 test were completed under fluorescent room light (standard daylight ceiling source, Philips TL514W/840 HE). The CIE 1931 chromaticity coordinates are x: 0.388, y: 0.391 with a luminance of . The measurements were taken on a barium sulphate reference standard. The Rayleigh matches were measured in a dark room.

3.Results

Figure 1(a) shows the relationship between the average settings on the OSCAR test and on the Medmont C100 for each participant. The frequency distributions for the Medmont C100 (b) and the OSCAR test (c) are also shown. Settings range from -8 to +2.9 on the OSCAR test (mean setting = -0.89) and from -4.4 to 3.7 on the Medmont C100 test (mean setting = -0.57). Normal observers are represented by filled circles whereas color-deficient observers are shown as closed and open squares for protan and deutan observers respectively. These groups commonly make settings at the extremes of the range. There was a highly significant correlation between the OSCAR test settings and the Medmont C100 test settings ($r = 0.82$, $p < 0.001$). No significant difference was found between males and females in either their OSCAR settings or their Medmont C100 settings once those with color deficiency had been taken out of the analysis ($t = -0.877$, $p = 0.911$; $t = -0.439$, $p = 0.383$ respectively).

Figure 2 shows the Rayleigh match mid-points and ranges for each participant sorted according to mid-points. Closed circles indicate female participants and open circles indicate male participants. The figure demonstrates the typical range of Rayleigh match mid-points and ranges amongst those with normal color vision. The mean mid-point excluding color-deficient observers was 44.5 (s.d. = 2.16) ranging from 37.35 to 49.7. As expected, anomalous trichromats are found at either end of the distribution. In our sample, there were four protan and three deutan observers represented by triangles and squares respectively.

Both the Oscar and the Medmont average scores correlate significantly with the Rayleigh match mid-points ($r = -0.464$, $p < 0.001$; $r = -0.461$, $p < 0.001$ respectively). This correlation dropped once individuals with color deficiency were taken out of the analysis ($r = -0.247$, $p < 0.001$; $r = -0.223$, $p = 0.002$ respectively).

[Figure 1 about here]

A hierarchical multiple regression analysis was carried out to calculate the variance of factors other than those of interest. Age, experimenter, and Rayleigh match mid-point were entered as separate blocks in this order. There were two experimenters (authors 1 and 3) who each tested approximately 50% of the cohort. The analysis shows that a small but non-significant proportion of the variance in both OSCAR and Medmont C100 settings could be explained by age (Table 1). However, a significant proportion of the variance in both OSCAR and Medmont C100 settings can be explained by observers' Rayleigh match mid-points.

[Table 1 about here]

5. Discussion

The main goal of the study was to find out whether the Medmont C100 test is a suitable replacement for the OSCAR test. A highly significant correlation was indeed found between the mean settings of the two tests and we conclude that the Medmont C100 test is appropriate for a first, quick estimate of the ratio of L:M cones in a participant's retina.

The correlation between the OSCAR and the Medmont instrument is impressive, given that the LEDs differ in their peak wavelengths and given that the scales differ in the two devices. The scale on the OSCAR test is continuous and ranges from -9 to +5 whereas the scale on the Medmont C100 test is split into discrete portions ranging from -5 to +5. Some resolution is therefore lost in the Medmont C100 test. We note that the correlation between the two instruments is comparable to the test-retest reliability of the OSCAR test in 104 participants tested by Lawrance-Owen and colleagues [16].

Rayleigh matches are determined by the spectral sensitivities of the L and M cones and not affected by the relative numbers of the two types of cone [19]. Since Rayleigh match mid-points account for a significant fraction of the variance in the OSCAR and Medmont C100 settings, it is likely that settings on the two instruments reflect not only variations in cone ratios but also variations in the spectral position of the photopigments. This is theoretically expected: An observer whose L pigment is shifted to shorter wavelengths will need a greater depth of modulation in the red LED to balance the modulation of the green LED. Thus neither the OSCAR nor the Medmont C100 test offers a pure estimate of the L:M cone ratio.

Could the variation in L:M cone ratios lead to inter-individual differences in our perception of color? De Vries originally suggested that fewer cones of either type would lead to a degradation in color vision [1].

[Figure 2 about here]

Subsequently, it has been suggested that this variation may lead to inter-individual differences in, for example, unique hues [20] or chromatic contrast sensitivity [21]. However, there is continuing disagreement on this matter and several researchers suggest that the differences in cone ratio have no effect at all on color vision. For example, Miyahara et al. studied two carriers of protanopia with extreme L:M cone ratios. They found that although their estimated cone ratios were 0.09:1 and 0.03:1, their Rayleigh matches, FM 100 Hue test scores and equilibrium yellow were all in the range of normal trichromats who had ratios ranging from 0.6:1 to 10:1 [22]. Similarly, Jordan and Mollon did not find any correlation between settings of unique yellow and estimates of L:M cones ratios using the OSCAR test in carriers for deutan or protan deficiencies [23]. Finally, two observers investigated by Brainard et al. [24] were also shown to vary only slightly in their settings of unique yellow, despite differences in their cone ratio (1.15:1 and 3.79:1) This research has led to the suggestion that although the sensitivity of the luminance channel has a direct relationship with the L:M cone ratio, the red-green chromatic channel may compensate for those differences.

In order to facilitate further investigations of a possible influence of cone ratios on other mechanisms of color perception, the Medmont C100 test could indeed be used to give a quick estimate of L:M cone ratios. We confirm that the test also has clear value in distinguishing protans and deutans once a diagnosis of color deficiency has been made with another screening test.

6. Acknowledgements

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7. References

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Figure 1. Average settings on OSCAR and Medmont C100 for 102 participants. (a) Correlation between mean OSCAR test settings and mean Medmont C100 test settings. Color-deficient observers are represented by squares and are solid for a protan deficiency and open for a deutan deficiency. Color-normal observers are represented by closed circles. (b) Frequency distribution of average Medmont C100 setting. (c) Frequency distribution of average OSCAR test setting.

Figure 2. Rayleigh match mid-points and matching ranges (horizontal bars) for 102 participants. Male and female observers are represented by open and closed symbols respectively. Observers with protan deficiencies are represented by triangles, those with deutan deficiencies by squares and those with normal colour vision by circles.

Table 1. Hierarchical multiple regression analyses showing the proportion of variance attributable to age, experimenter and Rayleigh match for each of the OSCAR and Medmont test. Significant p-values are denoted by a *.

	OSCAR	Medmont		
Contributor	**R^2**	**P value**	**R^2**	**P value**
Age	0.005	0.464	0.003	0.091
Experimenter	0.003	0.686	0.001	0.231
Rayleigh Match	0.245	0.0001*	0.206	0.0001*