Counterphase modulation photometry: comparison of two instruments

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The ratio of long-wavelength to medium-wavelength sensitive cones varies significantly among 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 been utilized previously for this purpose, but it is no longer available commercially. Having access to one of the few remaining OSCAR instruments, we compared the observers' mean settings to those obtained with the Medmont C100, a newer but apparently similar device. We also obtained Rayleigh matches for each participant. One hundred volunteers took part in the study. Settings on the OSCAR test were highly correlated with those on the Medmont C100. Both tests appeared to be influenced not only by L:M cone ratios but also by the spectral positions of the cone photopigments, since anomaloscope midmatch 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 among 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 by using psychophysics, ERG, and retinal densitometry [3]. The psychophysical methods included cone modulation thresholds, minimal flicker perception, and heterochromatic flicker photometry. Though individual cone ratios were not given, all of the measures used indicated that there was 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 *et al.* [4] explored the variation in cone ratios using ERG in 62 males with normal color vision and estimated the subjects' L-cone spectral absorbance curves from their respective L-opsin gene sequences. The corrected estimates of the 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 (from 1:1 to 4:1).

To explain the substantial variability in the L:M cone ratio, two factors can be considered. First, 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]. Second, 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, Estévez *et al.* 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 *et al.* showed that their test reliably

distinguishes protans and deutans, and this has subsequently been confirmed in a number of studies [12–14]; however, the test proved to be unsuitable as a general screening test for color deficiencies, since many deutan subjects were 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 OSCAR's ability to estimate cone ratios lies in the fact that the strength of the signal from either the L or the M cone depends on the total number of each cone type. For example, a participant with a lower than average L:M ratio 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 details). 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 identify carriers of protan deficiencies reliably [18]. Despite the similarity in design and appearance, the two tests have never been directly compared, and the potential of the Medmont C100 to estimate 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

There were originally 114 participants recruited for this study. Out of these, 101 (44 male, 59 female) completed all of the measures. One female participant was removed from the analysis, owing to her lack of comprehension of one of the tasks. A total of 100 participants were included in the analyses.

The age range was 7–65 years, with a mean age of 31 years. There was no difference in the mean age between males and females (mean female age was 31 years, mean male age was 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

1. 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 16 Hz. 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.

2. 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 16 Hz. As in the case of the OSCAR test, the 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 among those already diagnosed with color deficiency.

3. Oculus Anomaloscope

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 deg 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 midpoint and range were found for each participant on the anomaloscope using his or her dominant eye.

The OSCAR and Medmont C100 tests were completed under fluorescent room light (standard daylight ceiling source, Philips TL514W/840 HE). The CIE 1931 chromaticity coordinates were x: 0.388, y: 0.391. 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 [Fig. 1(b)] and the OSCAR test [Fig. 1(c)] are also shown. Kolmogorov–Smirnov tests of normality show that when participants with color vision deficiencies are excluded, neither the OSCAR test settings nor the Medmont C100 test settings are normally distributed [$D(95) = 0.095$, $p < 0.05$, $D(95) = 0.133$, $p < 0.001$, respectively]. The settings range from −8 to +2.9 on the OSCAR test (mean setting = −0.82) and from −4.4 to +2 on the Medmont C100 test (mean setting = −0.59). 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

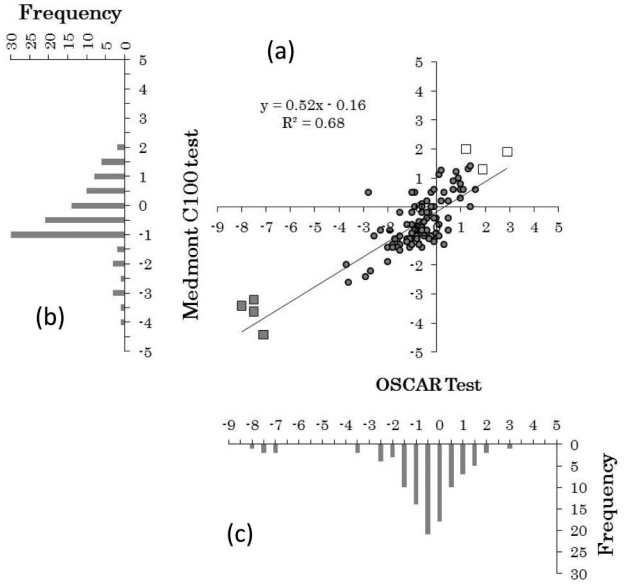

Fig. 1. Average settings on OSCAR and Medmont C100 for 102 participants. (a) Correlation between mean OSCAR test settings and mean Medmont C100 test settings. Color-deficient observers are represented by squares and are solid for a protan deficiency and open for a deutan deficiency. Color-normal observers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

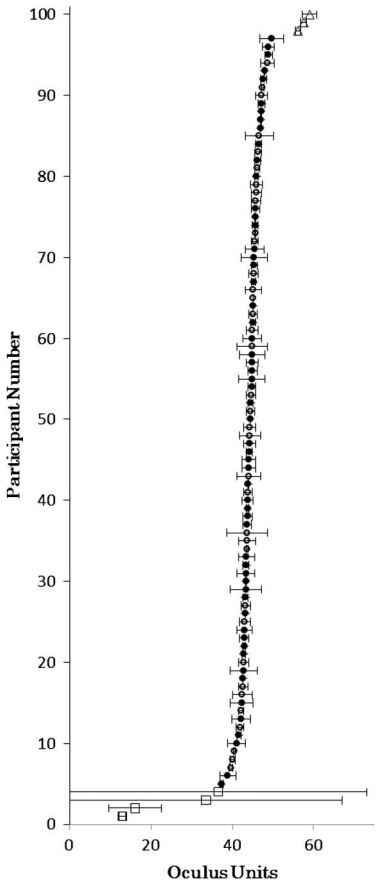

Fig. 2. Rayleigh match midpoints and matching ranges (horizontal bars) for 102 participants. Male and female observers are represented by open and closed symbols, respectively. Observers with protan deficiencies are represented by triangles, those with deutan deficiencies by squares, and those with normal color 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 Tests

	OSCAR		Medmont	
Contributor	R^2	p-value	R^2	p-value
Age	0.005	0.484	0.029	0.081
Experimenter	0.007	0.546	0.009	0.149
Rayleigh match	0.217	0.0001[a]	0.212	0.0001[a]

[a]Significant p-values.

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 ($t = -0.299$, $p = 0.766$ and $t = -0.469$, $p = 0.640$, respectively).

Figure 2 shows the Rayleigh match midpoints and ranges for each participant, sorted according to midpoints. The closed circles indicate female participants, and the open circles indicate male participants. The figure demonstrates the typical range of Rayleigh match midpoints and matching ranges among those with normal color vision. The mean midpoint excluding color-deficient observers was 44.5 (s.d. = 2.16) ranging from 37.35 to 49.7. As expected, anomalous trichromats were 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 correlated significantly with the Rayleigh match midpoints ($r = -0.468$, $p < 0.001$ and $r = -0.464$, $p < 0.001$, respectively). This correlation dropped once the individuals with color deficiency were taken out of the analysis ($r = -0.247$, $p < 0.001$ and $r = -0.223$, $p = 0.002$, respectively).

A hierarchical multiple regression analysis was carried out to calculate the variance of factors other than those of interest. Age, experimenter, and Rayleigh match midpoint 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 insignificant 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 the observers' Rayleigh match midpoints.

4. 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 *et al.* [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 midpoints account for a significant fraction of the variance in the OSCAR and Medmont C100 settings, it is likely that the 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 interindividual differences in our perception of color? de Vries originally suggested that fewer cones of either type would lead to degradation in color vision [1].

Subsequently, it has been suggested that this variation may lead to interindividual 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 ratios (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.

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