2011

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## Recommended Citation

Davison, P., Makunda, A., Loughman, J., Scanlon, G., Nolan,J., Beatty, S.," Macular Pigment: Its Associations with Color Discrimination and Matching", Optometry & Vision Science, 88 (7), pp. 816-822. doi:10.1097/OPX.0b013e31821798ec



Funder: Bausch & Lomb

# Macular Pigment: its Associations with Color Discrimination and Matching

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1 table, 5 figures

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Submitted May 18th, 2010. Re-submitted November 4th, 2010.

**Macular Pigment: its Associations with Color Discrimination and Matching**

**[Davison et al.]**

Macular pigment (MP), consisting of the carotenoids lutein, zeaxanthin and meso-zeaxanthin, is concentrated at the macula and is not detectable optically beyond about 7 degrees from the foveal center [1]. Of these carotenoids, the zeaxanthins predominate at the fovea whereas lutein dominates beyond the fovea [2]. The extent of macular pigmentation has recently been found to be related to the width of the foveal cup, as assessed by optical coherence tomography [3]. Since these pigments are located in the fibers of Henle at the foveola and in the inner nuclear layer beyond the foveola [4], they act as a pre-receptoral filter and are believed to contribute a variety of potentially beneficial properties for vision, including reduction of the effects of chromatic aberration [5] (though not supported by Engles et al.[6],), improvement of spatial vision and contrast enhancement [7], increased photopic increment sensitivity [8], reduced glare sensitivity in some studies [9,10] but not others [11], and increased critical flicker frequency [12].

Hue discrimination and color vision in general are most acute at the fovea [13] corresponding to increased cone density, specialized anatomic relationships and minimal spatial summation in this region (although with appropriate stimulus size scaling, surprisingly good color vision is possible beyond the fovea[14]). It is plausible that color discrimination at a small angular subtense would be

influenced by the optical density of MP at the fovea. Indeed it has long been speculated that inter-observer differences in color matching by color-normal observers are at least partially due to differences in macular pigmentation [15, 16]. Also it is known that even subjects with ophthalmoscopically-normal fundi exhibit substantial variations in MP optical density (MPOD), contributing to a range of prereceptoral light absorption at 460 nm from 3% to almost 100%[17]. Dietary supplementation with the macular carotenoids has been shown to increase MPOD [18] and may retard development of age-related macular degeneration (AMD) because of its antioxidant and short wavelength light filtering properties. Such hypotheses are currently the subject of a major randomized controlled clinical study (AREDS 2)[19] and follows potentially significant results from the LAST2 study [20].

Since the MP absorption spectrum ranges from about 400 to 520 nm and peaks at 460 nm [21], it would seem likely that these pigments influence color vision through selective absorption of short wavelengths, thereby influencing the short-wave sensitive (SWS) cones and the blue-yellow opponent-color channel. Moreland and Dain [22] (1995) reported that hue discrimination, measured using the Farnsworth-Munsell 100-Hue test (FM100), is indeed adversely affected primarily for short wavelengths by simulation of high MPOD using liquid filters containing carotene in a benzene solution. Comparing the results with those obtained with a neutral filter, they concluded that this effect was not simply the result of reduced retinal illuminance. However, to our knowledge there are no

published studies on the effects of actual (rather than simulated) MPOD on conventional measurements of hue discrimination thresholds. Further evidence supporting an effect of MPOD on short wavelength vision has been obtained from studies of SWS cone sensitivity [8, 23]. Finally, it has been shown that color discrimination measured by a color matching technique is influenced by MPOD [24, 25].

However, two recent studies using alternative methods, produced conclusions differing from those of the above mentioned studies. Firstly, a study of the effects of dietary supplementation with macular carotenoids on MP found no correlation between the level of MP (measured by heterochromatic flicker photometry) and red-green (RG) or yellow-blue (YB) color discrimination thresholds, though it was reported that RG vision tends to improve with augmentation of MP [26]. Secondly, RG cancellation profiles have been reported to be highly correlated with MPOD, while profiles for YB were independent of both eccentricity and MPOD [17]. However, changes in spectral sensitivity across the fovea, macula and paramacula are accompanied by relatively little change in color appearance, depending on whether corrections are made for macular pigment absorption [27,28].

Thus there is no consensus in the literature on the relationships, if any, between MPOD and color vision parameters on the one hand, and mechanisms on the other hand. This may or may not simply reflect the innate differences between, for example, spectral sensitivity measurements of the isolated SWS cone

mechanism and the overarching hue discrimination function at short wavelengths. It is also necessary to distinguish between the effects on color vision (mechanisms, sensitivity or appearance) of (1) distribution of macular pigment across the retina, and (2) variation of MPOD between subjects at a given retinal locus.

The objective of the present study was to evaluate, in a cross sectional manner, the associations between color variables and MPOD, using a much larger sample of subjects than in most previous studies and a battery of color assessments rather than relying on a single method of quantification. This study was part of a larger study of the association between MPOD and a wide range of vision parameters[11].

The color vision tests used in the present study were (a) hue discrimination using the FM100 test, (b) hue matching using the Moreland match on an anomaloscope, and (c) short wavelength automated perimetry (SWAP) increment thresholds using a customized procedure (cSWAP) to provide optimal foveal and para-foveal stimuli.

The present study has clinical implications for the visual effects of dietary supplementation of patients with AMD and at-risk patients.

## METHODS

Identical instrumentation and test protocols were used in the Macular Pigment Research Group laboratories in Dublin and Waterford, Ireland.

### Subjects

102 healthy subjects aged 18 to 40 years and resident in either Dublin or Waterford, Ireland, were recruited to participate in this dual-center study, which was approved by Research Ethics Committees of Waterford Institute of Technology and of Dublin Institute of Technology. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

Potential subjects underwent a full eye examination. The exclusion criteria comprised: any ocular pathology (including abnormal macula appearance or cataract); corrected visual acuity less than 6/9 in the better eye; refractive error outside -6 to +6 diopters; defective color vision. One eye only of each subject was tested, that with better corrected acuity. Full color vision data were available for 84 subjects.

### Color Threshold/Sensitivity Techniques

(a) The FM100 test (X-Rite UK, Poynton) was administered under color-corrected fluorescent lighting supplied by a pair of 15W 46 cm lamps (The Daylight Co., London, UK) providing minimum luminance of 94 $cd.m^{-2}$ reflected from each color sample as measured with a spot telephotometer. Maximum background luminance reflected from the supplied black sample trays was 12 $cd.m^{-2}$. Color temperature is rated at 6400 $^{o}$ K. Subjects were allowed to review the arrangement in each tray if they so requested.

Individual error scores and total error scores (TES), summed across the visible spectrum and purple hues, were determined using the software supplied by the manufacturer. Partial error scores (PES) were used to assess hue discrimination specifically among blue and cyan hues using samples 50 to 68 and 36 to 54 respectively and were divided by TES to obtain percentage values (%PES).

(b) Anomaloscope

This test was administered using the Moreland match on an HMC MR anomaloscope (type 7700: Oculus, Wetzlar, Germany). This provides a 2 degree field within which 436 and 490 nm sources are matched to a mixture of 480 and 589 nm, the latter mixture providing a brightness match. Control of stimuli and calculation of blue/green mixture were achieved with the anomaloscope under computer control using the manufacturer's software. Neutral pre-adaption was not used as this was found to produce transient adaptation effects on stimulus saturation. Stimuli were presented under continuous viewing mode. Following

practice, subjects toggled the mixture to obtain 4 matches, 2 each with the mixture preset to blue bias and green bias. The mean of 6 blue/green matches was calculated for each subject to obtain the midpoint.

(c) Customized short-wavelength automated perimetry (cSWAP)

Foveal and parafoveal increment sensitivities were measured using an adaptation of the standard SWAP routine on a Humphrey Field Analyzer 2i (Carl Zeiss Medetec, Jena, Germany). Yellow (530nm) background luminance was 100 $cd.m^2$ . Size V targets of 440 nm and 200msec duration subtending 1.7 degrees at the eye were presented at 0, 1, 2, 3, 4 and 5 degrees eccentricity from a fixation target. The number of targets at each eccentricity beyond the foveal center varied from 4 to 20. On each presentation, a single target was presented. Increment thresholds were obtained using the SWAP adaptive staircase full thresholding technique. Subjects were given 3 minutes to adapt to the background before testing began. Sensitivity for each eccentricity was the mean of values for all targets in the group at that eccentricity.

**Macular pigment optical density** (**MPOD)**

MPOD was measured by customized heterochromatic flicker photometry (cHFP) using a densitometer (Macular Metrics Corp., Providence, RI) which alternates 460 and 550 nm stimuli, the former being maximally absorbed by MP while the latter is not absorbed by MP. A spatial profile of MPOD was obtained by

performing 5 measurements at each eccentricity (0.25, 0.5, 1, 1.75 and 3 degrees), and at 7 degrees, to provide a reference point at which MP is optically undetectable. Further details have been published elsewhere [30]. This instrument and technique have been shown to be valid and have high reproducibility [31].

## Statistical Methods

Data were analyzed using PASW Statistics 17 (SPSS Inc, Chicago, Illinois). Correlation coefficients and first-order partial correlation coefficients were calculated using the Pearson product-moment method since scatter-plots showed no evidence of non-linearity. Statistical analysis was based on two-tailed tests and interpreted with reference to 0.05 significance levels and Bonferroni correction.

## RESULTS

Figure 1 shows the MPOD spatial profile. These data compare well with previously published data using the same cHFP method [32]. Mean (± SD) MPOD for the 0.25 degree stimulus was 0.45 (+/-0.18), range 0.16 to 0.93.

Mean (± SD) hue discrimination TES for our subjects was 55 (± 23), comparable to Kinnear and Sahraie's data for the 30-39 age group [33]. TES was found not to correlate significantly ($p > .001$ after Bonferroni correction). Possible

associations between MPOD and (1) short wavelength hue discrimination in the region of peak absorption by MP and (2) discrimination at the short wavelength end of the expected axis of a type III acquired color vision defect were investigated by calculating %PES for color samples 50-68 and 36-54 respectively, i.e. %(PES/TES). An example of this analysis is provided in Figure 2, which is a scattergram of % partial error scores (%PES) for FM100 samples 36-54 against macular pigment optical density (MPOD) at $1.75^0$ eccentricity. Despite an apparent trend of increased %PES with higher MPOD, both (1) and (2) were found to be non-significantly correlated ($p>.001$ with Bonferroni correction) to MPOD at all eccentricities.

The anomaloscope Moreland match midpoints were found to be negatively correlated to MPOD at all eccentricities (see Table 1 and Figure 3), indicating a shift towards green mixtures to match cyan. The coefficient was maximal for MPOD at $1.75^0$, corresponding to the anomaloscope stimulus diameter of $2^0$. MPOD at $1.75^0$ accounted for 23.9% of variability ($r^2$) in Moreland match data. Coefficients were still significant after Bonferroni correction at all eccentricities except at 0.5 degrees.

cSWAP data (sensitivity in dB) at all eccentricities measured were negatively correlated at high significance levels, with MPOD at both 1.75 and 3 degrees of retinal eccentricity: see Table 1. Figure 4 is a scattergram of the data for cSWAP at $2^0$ and MPOD at $1.75^0$. Furthermore, cSWAP at the fovea correlated negatively and significantly with MPOD at all eccentricities. Thus high cSWAP

sensitivities were associated with low MPOD.However, after Bonferroni correction, only foveal cSWAP correlated significantly with MPOD at 1.75 and 3 degrees. The maximal proportion of variability in cSWAP attributable to MPOD ($r^2$) is 21.2% (for foveolar cSWAP and MPOD at $1.75^0$).

## DISCUSSION

Our hue discrimination data do not support the findings of Moreland and Dain (1995) [22], who found a significant increase in both TES and PES in the blue-green region with their MP1 carotene filter of 1.0 maximum absorbance. We found no statistically significant association between MPOD at any retinal eccentricity and TES or PES after application of Bonferroni correction. This discrepancy may be a reflection of the nature of Moreland and Dain's filter, which was considerably denser than typical MPOD values; it exceeded the MPOD of all of our subjects at and between 1.75 degrees and the foveola) and did not provide an exact fit to the spectral absorbance of MP. It may also reflect a difference between a physiological filter, to which the visual system has adapted, and a filter placed before the eye.

It is possible that an artificial filter creates short-term changes in color vision and that an autoregulatory process adjusts retinal and/or cortical color mechanisms on a long-term basis in response to their naturally occurring MPOD. This hypothesis is supported by data showing a consistent shift in achromatic locus

231 over a 3 month period for cataract patients post-surgery [34] , by color constancy
232 effects for blue and green targets despite crystalline lens brunescence (Hardy et
233 al. 2005), and by evidence of plasticity of adult neural color mechanisms [36].
234 Rodriguez-Carmona et al. [26] found no correlation between yellow-blue thresholds
235 and MPOD using a technique in which threshold color differences were
236 measured for detection of movement of a stimulus within a checkered array.
237
238 We did not assess the association, if any, of MPOD across subjects with color
239 appearance other than by using the HMC anomaloscope Moreland match. Using
240 this technique, we found that midpoint data were surprising in that subjects with
241 high MPOD required less blue to match cyan; this finding was consistent for
242 MPOD at all eccentrities. No directly comparable data exist in the literature,
243 though Stringham and Hammond [17] found that yellow-blue cancellation
244 thresholds were constant across the retina despite significant MPOD variability
245 across the retinal region tested. It is of interest that in one study of Moreland
246 match midpoint data, no difference was reported between post-cataract patients
247 with short wavelengthe-absorbing intra-ocular lenses (IOLs) and those with clear
248 IOLs [37].
249
250 The cSWAP data show relatively constant sensitivity across the retina beyond
251 the foveola (Figure 5) despite substantial differences in MPOD across the retina
252 (Figure 1). This finding is consistent with that of Stringham et al. [29] who used
253 Maxwellian-view multi-channel optics except that they found slightly lower

sensitivity at the foveola compared to parafovea using 16 subjects of similar age to those in the present study. This suggests that parafoveal (but not foveolar) cSWAP may provide a valid clinical test of SWS cone function. The fact that we found statistically significant inverse correlations between short-wave sensitivity for the foveal stimulus and MPOD at two eccentricities does not in fact contradict Stringham et al.'s conclusions; our correlations relate to differences between subjects rather than to averaged measures across the retina which would not take into account the effects of inter-subject variance in both SWS cone sensitivity and MPOD at any single retinal locus.

We hypothesize that the fact that SWS cone *sensitivity* exhibited significant inverse associations with MPOD, while hue discrimination *thresholds* showed no significant associations with MPOD, may be related to temporal differences between the 2 measures. It is possible that, by using short stimulus presentations, the cSWAP technique (200 msec) produces transient effects quite different to those found with much longer presentations such as those of the FM100 test.

Confounding variables which might influence the relationship between MPOD and color vision include: iris and choroidal pigmentation, age, stimulus size, and pupil diameter. The effect of iris pigment density has been studied by Woo and Lee (2002) [38], who found that Asians have poorer PES in the blue quadrant, and by Hammond and Caruso-Avery (2000) [39], who reported that subjects with darker

irides had higher MPOD. Since all subjects in the present study were Caucasian, the density range of both iris pigment and choroidal pigment was limited, and yet MPOD was found to correlate significantly with color sensitivity across a variety of measures. We suggest that our findings are independent of iris pigmentation, though such pigmentation is a factor in a less racially homogenous group of subjects [40].

The effect of age on hue discrimination, in the blue-green spectral region in particular, is well known [41] and is partly due to wavelength-selective loss of light transmission by the aging crystalline lens [42]. An age effect on MPOD has also been reported, some studies having shown a statistically significant age related decline in MPOD [39,43]. It is therefore possible that age is a confounding factor influencing our findings on MPOD and hue discrimination in the blue-green spectral region. A similar age effect is possible in relation to SWS cone function as measured by cSWAP [44,45]. Although our subjects were restricted to the age range 18 to 40 years, and our exclusion criteria included any evidence of cataract, potentially confounding contributions attributable to age cannot be dismissed. However, inspection of Table 1 shows that first-order partial correlation coefficients with age as the control variable are very similar to zero-order coefficients. In no case did a significance level change from significant to non-significant by controlling for age. We therefore suggest that our observed associations between MPOD and both Moreland midpoint and cSWAP are independent of age within the age range of the present study (18 to 40 years,

mean age ± SD = 29 ± 6 years). However, the age factor may be important in older subjects.

Stimulus size and location are known to affect both color vision [46] and measures of MPOD [3]. In the present study MPOD was measured using targets subtending between 30 minutes and 3.5 degrees at eccentricities between 0 and 3 degrees. Color thresholds were measured using centrally fixated targets subtending approximately 1.5 degrees (FM100), 2 degrees (anomaloscope), and 1.7 degrees at between 0 and $5^0$ eccentricity (cSWAP). A clear pattern is evident from our data: MPOD correlated consistently across size and eccentricity parameters with cSWAP and Moreland midpoint. MPOD values were reported in this study at a range of eccentricities in order to assess the consistency of correlations, and because retinal images extend beyond their geometric optical limits as a result of aberrations, diffraction and scatter. Furthermore eye movements produce translational shift of retinal images in a natural viewing environment.

The practical implications of the present study are two-fold. Firstly, dietary supplementation to increase MPOD is not likely to adversely affect hue discrimination. However, a longitudinal study of the effects of supplementation on color vision is needed to support this. Secondly, we have shown that appropriate customization of a standard clinical automated perimetry test (cSWAP) provides

a potential clinical test for foveal SWS-cone sensitivity, though this awaits confirmation by a concordance study using Maxwellian view instrumentation.

## ACKNOWLEDGEMENTS

We acknowledge Enterprise Ireland and the Innovation Partnership of Bausch & Lomb Inc. who provided research grants. The authors have no financial interest in any of the instruments mentioned in the manuscript.

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420

**FIGURE 1**

**Spatial profile of macular pigment optical density (MPOD).** Abscissa: eccentricity in degrees. Ordinate: mean MPOD across subjects +/- 2 standard deviations.

**FIGURE 2**

**Scattergram of % partial error scores (%PES) for FM100 caps 36-54 against macular pigment optical density (MPOD) at $1.75^0$ eccentricity**. Solid line = linear model least-squares regression (%PES = -0.239*MPOD + 33.92)

**FIGURE 3**

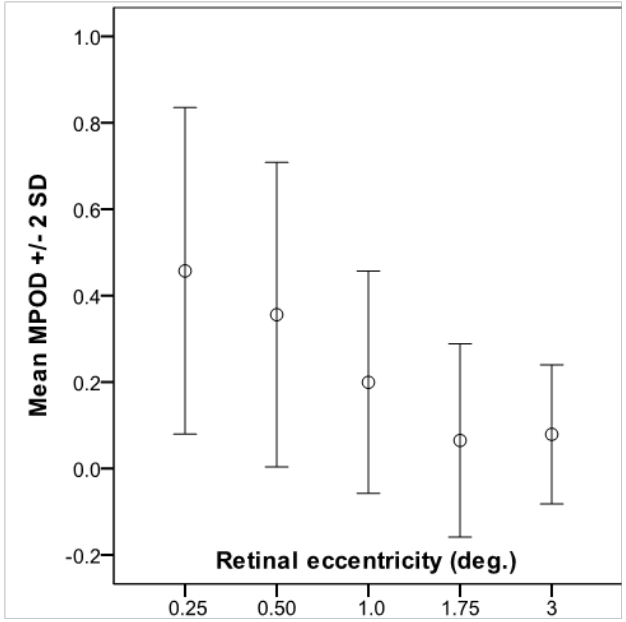
**Scattergram of anomaloscope Moreland match midpoints against macular pigment optical density (MPOD) at $1.75^0$ eccentricity**. Solid line = linear model least-squares regression. (Midpoint = 35.91*MPOD + 61.46)

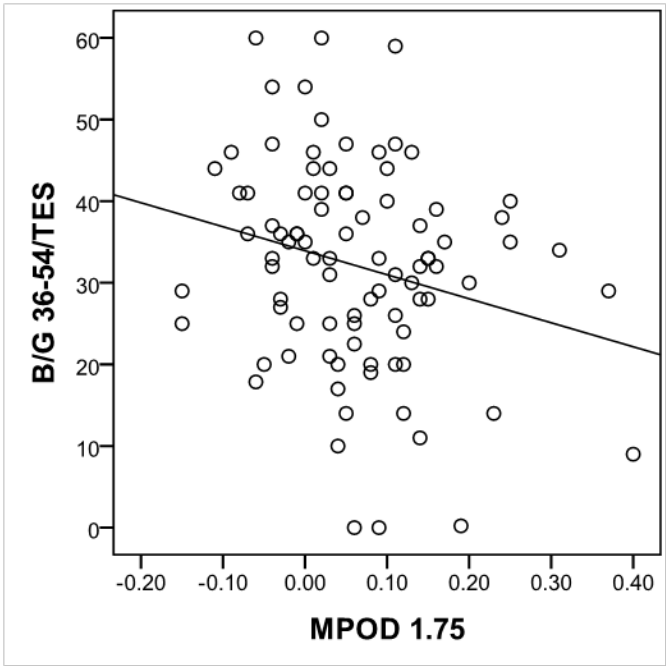
**FIGURE 4**

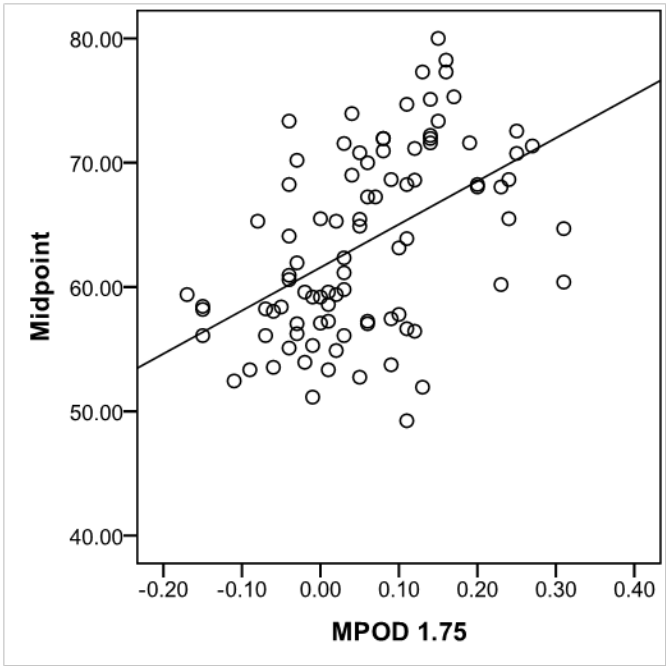
**Scattergram of sensitivity data on customized shortwave automated perimetry (cSWAP) at $2^0$ eccentricity against macular pigment optical density (MPOD) at $1.75^0$ eccentricity**. Solid line = linear model least-squares regression (cSWAP = -9.67*MPOD + 27.57)

**FIGURE 5**

**cSWAP spatial profile**. Abscissa: eccentricity in degrees. Ordinate: mean cSWAP sensitivity in decibels across subjects +/- 2 standard deviations.


Mean MPOD +/- 2 SD
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Retinal eccentricity (deg.)
0.25
0.50
1.0
1.75
3


B/G 36-54/TES
60
50
40
30
20
10
0
-0.20
-0.10
0.00
0.10
0.20
0.30
0.40
MPOD 1.75


80.00
70.00
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Midpoint
-0.20
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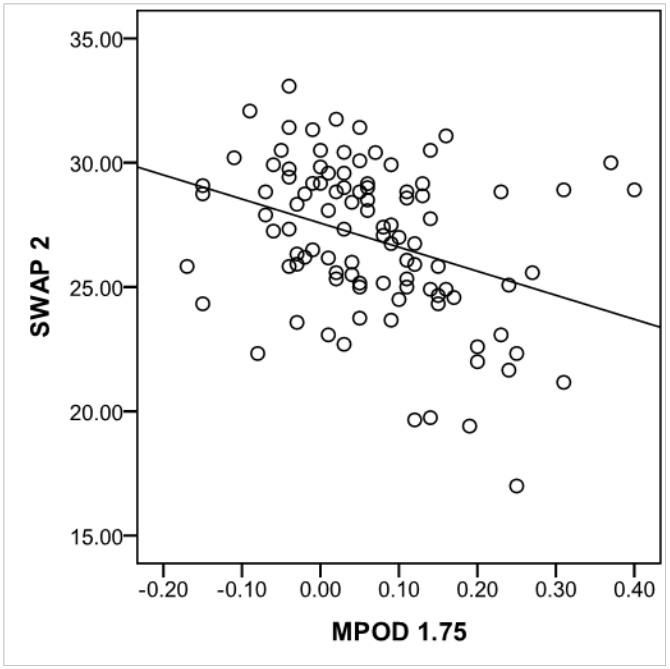
0.30
0.40
MPOD 1.75


35.00
30.00
25.00
20.00
15.00
SWAP 2
-0.20
-0.10
0.00
0.10
0.20
0.30
0.40
MPOD 1.75

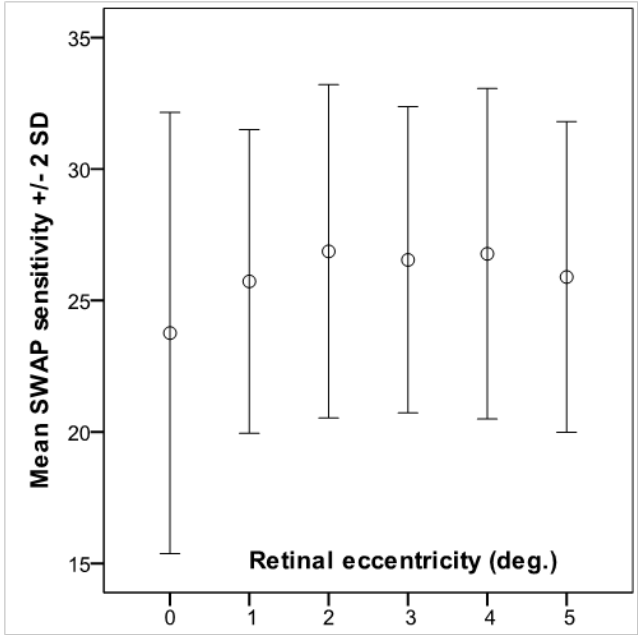

Mean SWAP sensitivity +/- 2 SD
35
30
25
20
15
Retinal eccentricity (deg.)
0
1
2
3
4
5

**TABLE 1.**

Correlations between Color Vision Variables and MPOD

| MPOD | | %PES | | Moreland midpoint | cSWAP | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | B/G 36-54 | B 50-68 | | Fovea | 1 | 2 | 3 | 4 | 5 |
| 0.25 $^{o}$ | $r_0$ | -.188 | .114 | .343 | -.331 | -.189 | -.110 | -.003 | -.097 | -.032 |
| | $r_1$ | -.183 | .121 | .343 | -.328 | -.186 | -.106 | .005 | -.089 | -.025 |
| | $p_0$ | .084 | .301 | .001** | .002* | .083 | .314 | .982 | .378 | .769 |
| | $df_0$ | 83 | 83 | 91 | 83 | 83 | 83 | 83 | 83 | 83 |
| 0.5 $^{o}$ | $r_0$ | -.142 | .094 | .298 | -.267 | -.191 | -.116 | -.047 | -.134 | -.063 |
| | $r_1$ | -.138 | .099 | .295 | -.264 | -.189 | -.112 | -.042 | -.128 | -.057 |
| | $p_0$ | .195 | .393 | .004* | .014* | .079 | .292 | .667 | .223 | .567 |
| | $df_0$ | 83 | 83 | 91 | 83 | 83 | 83 | 83 | 83 | 83 |
| 1 $^{o}$ | $r_0$ | -.219 | .026 | .329 | -.285 | -.180 | -.200 | -.132 | -.165 | -.125 |
| | $r_1$ | -.218 | .028 | .331 | -.285 | -.178 | -.198 | -.130 | -.163 | -.123 |
| | $p_0$ | .044* | .816 | .001** | .008* | .100 | .067 | .229 | .132 | .256 |
| | $df_0$ | 83 | 83 | 90 | 83 | 83 | 83 | 83 | 83 | 83 |
| 1.75 $^{o}$ | $r_0$ | -.224 | .113 | .489 | -.461 | -.288 | -.295 | -.215 | -.267 | -.203 |
| | $r_1$ | -.217 | .121 | .484 | -.458 | -.284 | -.291 | -.209 | -.261 | -.196 |
| | $p_0$ | .040* | .304 | .000** | .000** | .008* | .006* | .048* | .013* | .063 |
| | $df_0$ | 83 | 83 | 90 | 83 | 83 | 83 | 83 | 83 | 83 |
| 3 $^{o}$ | $r_0$ | -.177 | .230 | .387 | -.393 | -.288 | -.317 | -.249 | -.307 | -.283 |
| | $r_1$ | -.154 | .258 | .371 | -.386 | -.278 | -.306 | -.229 | -.284 | -.263 |
| | $p_0$ | .105 | .034* | .000** | .000** | .008* | .003* | .021* | .004* | .009* |
| | $df_0$ | 83 | 83 | 90 | 83 | 83 | 83 | 83 | 83 | 83 |

$r_0$ = Pearson correlation coefficient, $r_1$ = 1$^{st}$-order partial correlation coefficient controlling for age

$p_0$ = 2-tailed significance for $r_0$, $df_0$ = degrees of freedom for $r_0$ ,* indicates p<= .05 without Bonferroni correction, ** indicates significant with correction for a 5 by 9 correlation matrix.

MPOD=macular pigment optical density at eccentricities 0.25 to 3$^0$, %PES=FM100 percentage partial error scores, B/G 36-54=blue/green caps (36-54), B 50-68=blue caps (50-68), cSWAP= sensitivity values on customized shortwave automated perimetry at fovea and eccentricities from 1 to 5$^0$.