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Macular Pigment: its Associations with Color Discrimination and Matching

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1 table, 5 figures

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

2 **[Davison et al.]**

3

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

18

19 Hue discrimination and color vision in general are most acute at the fovea ¹³
20 corresponding to increased cone density, specialized anatomic relationships and
21 minimal spatial summation in this region (although with appropriate stimulus size
22 scaling, surprisingly good color vision is possible beyond the fovea¹⁴). It is
23 plausible that color discrimination at a small angular subtense would be

24 influenced by the optical density of MP at the fovea. Indeed it has long been
25 speculated that inter-observer differences in color matching by color-normal
26 observers are at least partially due to differences in macular pigmentation^{15, 16}.
27 Also it is known that even subjects with ophthalmoscopically-normal fundi exhibit
28 substantial variations in MP optical density (MPOD), contributing to a range of
29 prereceptor light absorption at 460 nm from 3% to almost 100%¹⁷. Dietary
30 supplementation with the macular carotenoids has been shown to increase
31 MPOD¹⁸ and may retard development of age-related macular degeneration
32 (AMD) because of its antioxidant and short wavelength light filtering properties.
33 Such hypotheses are currently the subject of a major randomized controlled
34 clinical study (AREDS 2)¹⁹ and follows potentially significant results from the
35 LAST2 study²⁰.

36

37 Since the MP absorption spectrum ranges from about 400 to 520 nm and peaks
38 at 460 nm²¹, it would seem likely that these pigments influence color vision
39 through selective absorption of short wavelengths, thereby influencing the short-
40 wave sensitive (SWS) cones and the blue-yellow opponent-color channel.

41 Moreland and Dain²² (1995) reported that hue discrimination, measured using
42 the Farnsworth-Munsell 100-Hue test (FM100), is indeed adversely affected
43 primarily for short wavelengths by simulation of high MPOD using liquid filters
44 containing carotene in a benzene solution. Comparing the results with those
45 obtained with a neutral filter, they concluded that this effect was not simply the
46 result of reduced retinal illuminance. However, to our knowledge there are no

47 published studies on the effects of actual (rather than simulated) MPOD on
48 conventional measurements of hue discrimination thresholds. Further evidence
49 supporting an effect of MPOD on short wavelength vision has been obtained
50 from studies of SWS cone sensitivity^{8, 23}. Finally, it has been shown that color
51 discrimination measured by a color matching technique is influenced by MPOD
52^{24, 25}.

53

54 However, two recent studies using alternative methods, produced conclusions
55 differing from those of the above mentioned studies. Firstly, a study of the effects
56 of dietary supplementation with macular carotenoids on MP found no correlation
57 between the level of MP (measured by heterochromatic flicker photometry) and
58 red-green (RG) or yellow-blue (YB) color discrimination thresholds, though it was
59 reported that RG vision tends to improve with augmentation of MP²⁶. Secondly,
60 RG cancellation profiles have been reported to be highly correlated with MPOD,
61 while profiles for YB were independent of both eccentricity and MPOD¹⁷.

62 However, changes in spectral sensitivity across the fovea, macula and
63 paramacula are accompanied by relatively little change in color appearance,
64 depending on whether corrections are made for macular pigment absorption^{27,28}.

65

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

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

75

76 The objective of the present study was to evaluate, in a cross sectional manner,
77 the associations between color variables and MPOD, using a much larger
78 sample of subjects than in most previous studies and a battery of color
79 assessments rather than relying on a single method of quantification. This study
80 was part of a larger study of the association between MPOD and a wide range of
81 vision parameters¹¹.

82

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

88

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

91

92

93 **METHODS**

94

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

97

98 **Subjects**

99

100 102 healthy subjects aged 18 to 40 years and resident in either Dublin or
101 Waterford, Ireland, were recruited to participate in this dual-center study, which
102 was approved by Research Ethics Committees of Waterford Institute of
103 Technology and of Dublin Institute of Technology. Informed consent was
104 obtained from each volunteer, and the experimental procedures adhered to the
105 tenets of the Declaration of Helsinki.

106

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

113

114 **Color Threshold/Sensitivity Techniques**

115

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

123

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

129

130 (b) Anomaloscope

131 This test was administered using the Moreland match on an HMC MR
132 anomaloscope (type 7700: Oculus, Wetzlar, Germany). This provides a 2 degree
133 field within which 436 and 490 nm sources are matched to a mixture of 480 and
134 589 nm, the latter mixture providing a brightness match. Control of stimuli and
135 calculation of blue/green mixture were achieved with the anomaloscope under
136 computer control using the manufacturer's software. Neutral pre-adaption was
137 not used as this was found to produce transient adaptation effects on stimulus
138 saturation. Stimuli were presented under continuous viewing mode. Following

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

142

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

144 Foveal and parafoveal increment sensitivities were measured using an
145 adaptation of the standard SWAP routine on a Humphrey Field Analyzer 2i (Carl
146 Zeiss Medetec, Jena, Germany). Yellow (530nm) background luminance was
147 100 cd.m^2 . Size V targets of 440 nm and 200msec duration subtending 1.7
148 degrees at the eye were presented at 0, 1, 2, 3, 4 and 5 degrees eccentricity
149 from a fixation target. The number of targets at each eccentricity beyond the
150 foveal center varied from 4 to 20. On each presentation, a single target was
151 presented. Increment thresholds were obtained using the SWAP adaptive
152 staircase full thresholding technique. Subjects were given 3 minutes to adapt to
153 the background before testing began. Sensitivity for each eccentricity was the
154 mean of values for all targets in the group at that eccentricity.

155

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

157

158 MPOD was measured by customized heterochromatic flicker photometry (cHFP)
159 using a densitometer (Macular Metrics Corp., Providence, RI) which alternates
160 460 and 550 nm stimuli, the former being maximally absorbed by MP while the
161 latter is not absorbed by MP. A spatial profile of MPOD was obtained by

162 performing 5 measurements at each eccentricity (0.25, 0.5, 1, 1.75 and 3
163 degrees), and at 7 degrees, to provide a reference point at which MP is optically
164 undetectable. Further details have been published elsewhere³⁰. This instrument
165 and technique have been shown to be valid and have high reproducibility³¹.

166

167 **Statistical Methods**

168

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

175

176 **RESULTS**

177

178 Figure 1 shows the MPOD spatial profile. These data compare well with
179 previously published data using the same cHFP method³². Mean (\pm SD) MPOD
180 for the 0.25 degree stimulus was 0.45 (\pm 0.18), range 0.16 to 0.93.

181

182 Mean (\pm SD) hue discrimination TES for our subjects was 55 (\pm 23), comparable
183 to Kinnear and Sahraie's data for the 30-39 age group³³. TES was found not to
184 correlate significantly ($p > .001$ after Bonferroni correction). Possible

185 associations between MPOD and (1) short wavelength hue discrimination in the
186 region of peak absorption by MP and (2) discrimination at the short wavelength
187 end of the expected axis of a type III acquired color vision defect were
188 investigated by calculating %PES for color samples 50-68 and 36-54
189 respectively, i.e. $\%(\text{PES}/\text{TES})$. An example of this analysis is provided in Figure
190 2, which is a scattergram of % partial error scores (%PES) for FM100 samples
191 36-54 against macular pigment optical density (MPOD) at 1.75° eccentricity.
192 Despite an apparent trend of increased %PES with higher MPOD, both (1) and
193 (2) were found to be non-significantly correlated ($p > .001$ with Bonferroni
194 correction) to MPOD at all eccentricities.

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

202

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

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

212

213 **DISCUSSION**

214

215 Our hue discrimination data do not support the findings of Moreland and Dain
216 (1995)²², who found a significant increase in both TES and PES in the blue-
217 green region with their MP1 carotene filter of 1.0 maximum absorbance. We
218 found no statistically significant association between MPOD at any retinal
219 eccentricity and TES or PES after application of Bonferroni correction. This
220 discrepancy may be a reflection of the nature of Moreland and Dain's filter, which
221 was considerably denser than typical MPOD values; it exceeded the MPOD of all
222 of our subjects at and between 1.75 degrees and the foveola) and did not provide
223 an exact fit to the spectral absorbance of MP. It may also reflect a difference
224 between a physiological filter, to which the visual system has adapted, and a filter
225 placed before the eye.

226

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

231 over a 3 month period for cataract patients post-surgery³⁴, 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 mechanisms³⁶.
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 eccentricities. No directly comparable data exist in the literature,
243 though Stringham and Hammond¹⁷ 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 wavelength-absorbing intra-ocular lenses (IOLs) and those with clear
248 IOLs³⁷.

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

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

263

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

271

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

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

283

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

300 mean age \pm SD = 29 \pm 6 years). However, the age factor may be important in
301 older subjects.

302

303 Stimulus size and location are known to affect both color vision⁴⁶ and measures
304 of MPOD³. In the present study MPOD was measured using targets subtending
305 between 30 minutes and 3.5 degrees at eccentricities between 0 and 3 degrees.
306 Color thresholds were measured using centrally fixated targets subtending
307 approximately 1.5 degrees (FM100), 2 degrees (anomaloscope), and 1.7
308 degrees at between 0 and 5⁰ eccentricity (cSWAP). A clear pattern is evident
309 from our data: MPOD correlated consistently across size and eccentricity
310 parameters with cSWAP and Moreland midpoint. MPOD values were reported in
311 this study at a range of eccentricities in order to assess the consistency of
312 correlations, and because retinal images extend beyond their geometric optical
313 limits as a result of aberrations, diffraction and scatter. Furthermore eye
314 movements produce translational shift of retinal images in a natural viewing
315 environment.

316

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

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

324

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330

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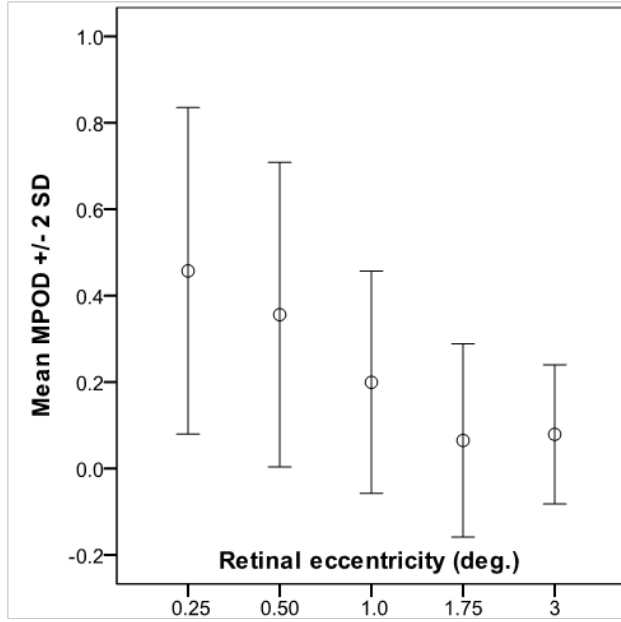
421 **FIGURE 1**
422 **Spatial profile of macular pigment optical density (MPOD).** Abscissa:
423 eccentricity in degrees. Ordinate: mean MPOD across subjects +/- 2 standard
424 deviations.

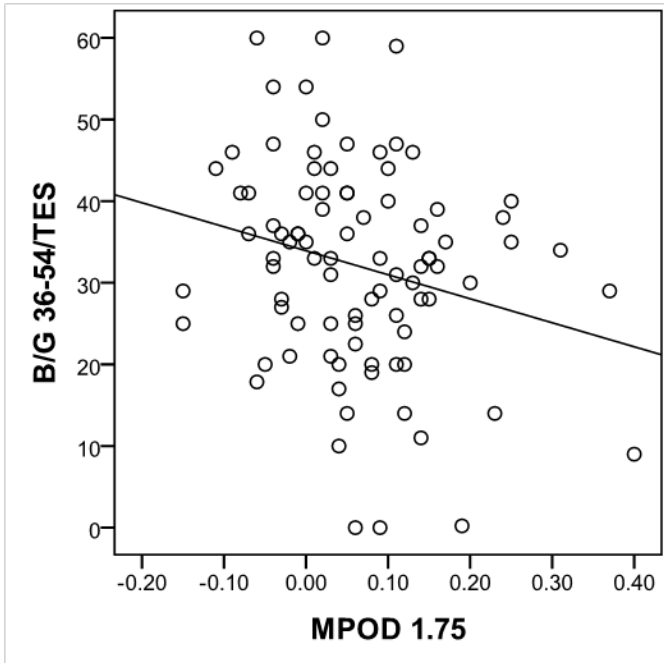
425
426 **FIGURE 2**
427 **Scattergram of % partial error scores (%PES) for FM100 caps 36-54 against**
428 **macular pigment optical density (MPOD) at 1.75⁰ eccentricity.** Solid line =
429 linear model least-squares regression ($\%PES = -0.239 * MPOD + 33.92$)
430

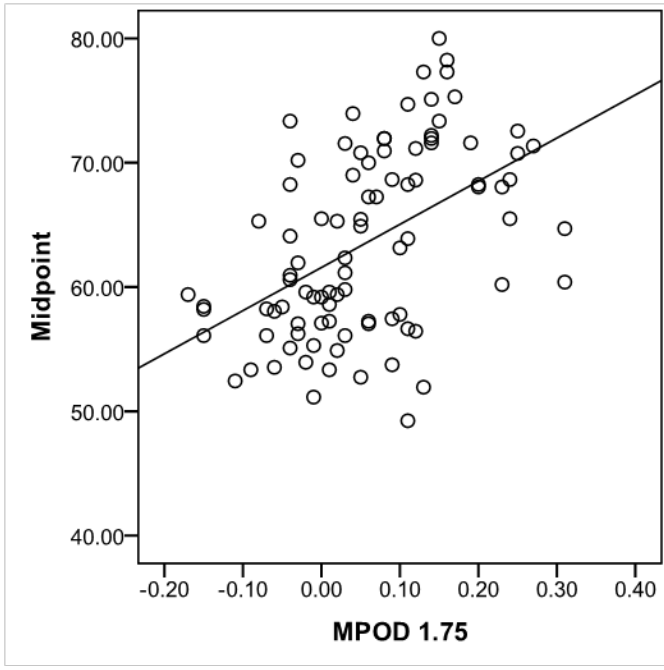
431 **FIGURE 3**
432 **Scattergram of anomaloscope Moreland match midpoints against macular**
433 **pigment optical density (MPOD) at 1.75⁰ eccentricity.** Solid line = linear model
434 least-squares regression. (Midpoint = $35.91 * MPOD + 61.46$)
435

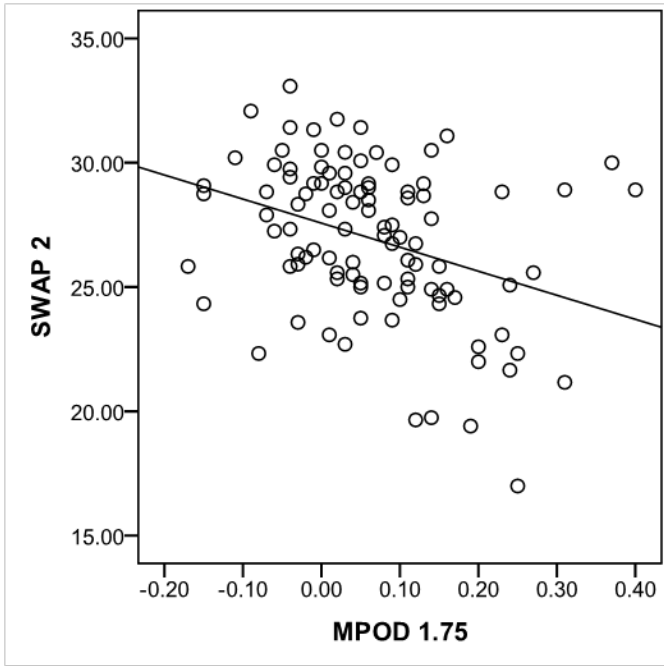
436 **FIGURE 4**
437 **Scattergram of sensitivity data on customized shortwave automated**
438 **perimetry (cSWAP) at 2⁰ eccentricity against macular pigment optical**
439 **density (MPOD) at 1.75⁰ eccentricity.** Solid line = linear model least-squares
440 regression ($cSWAP = -9.67 * MPOD + 27.57$)
441

442 **FIGURE 5**
443 **cSWAP spatial profile.** Abscissa: eccentricity in degrees. Ordinate: mean
444 cSWAP sensitivity in decibels across subjects +/- 2 standard deviations.









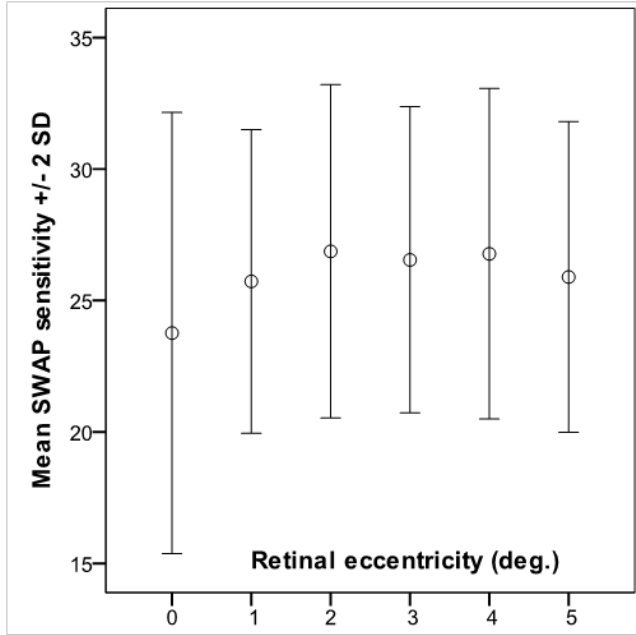


TABLE 1.

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 °	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 °	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 °	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 °	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 °	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

r₀ = Pearson correlation coefficient, r₁ = 1st-order partial correlation coefficient controlling for age

p₀ = 2-tailed significance for r₀, df₀ = degrees of freedom for r₀, * 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°, %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°.