A portable instrument for measuring macular pigment with central fixation.

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A PORTABLE INSTRUMENT FOR MEASURING MACULAR PIGMENT WITH CENTRAL FIXATION

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Running title: **PORTABLE INSTRUMENT FOR MEASURING MACULAR PIGMENT**

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Abstract

Purpose: To evaluate the reliability and validity of a portable instrument for measuring macular pigment optical density.

Methods: The instrument is small, uses light emitting diodes as light sources and the principles of heterochromatic flicker photometry of comparing foveal and extra-foveal minimum flicker matches. It uses central fixation for the extra-foveal matches, which subjects found easier than eccentric fixation. Subjects with healthy eyes used the instrument to measure their pigment density in a number of eye clinics.

Results: The mean pigment density in 124 eyes in 124 individuals was 0.41 ± 0.16 (mean ± sd), there was no significant change with age but the density was less in females, those with light irides, smokers, subjects on diets low in precursor carotenoids and in those exposed to several hours of daylight every day or who used sun beds.

Conclusions: The portable instrument gave valid and reliable data that confirmed published values for macular pigment. It was convenient to use in the clinic and has potential as a screening tool.

Introduction

Recently there has been considerable interest in macular pigment (MP) and its possible role of protecting the central retina from degenerative processes associated with age or chronic exposure to light (e.g, Landrum *et al.* [1], but see Werner *et al.* [2]). If the anti-oxidant and free radical scavenging properties of MP, demonstrated *in vitro* [3, 4], are also operative *in vivo* then the presence of high quantities of the pigment might be a useful prognostic for protection against the incidence of age-related macular degeneration (AMD). Consequently, it would be useful to be able to measure MP levels in patients, or even the general population, and perhaps advise life style or dietary changes to increase MP and thus preserve retinal function in old age.

MP may be measured *in vivo* with objective methods such as TV densitometry [5], reflection densitometry [6], using the autofluorescence of the retina [7], with a scanning laser ophthalmoscope [8] or Raman spectroscopy [9]. These methods have employed sophisticated equipment that is more suited in size, complexity and expense to the research clinic or laboratory. Careful immobilisation of the subject's head, sometimes with a dental bite, may be required, and pupillary dilation and careful fixation by the subject are necessary.

Several subjective psychophysical methods have been developed such as measurement of spectral sensitivity [10] and motion photometry with the Moreland anomaloscope [11], but recently most workers have employed some version of heterochromatic flicker photometry (HCFP). This was first applied to measuring MP by Werner and Wooten[12] in 1979 who used a dual monochromator and dental bite. HCFP has appeared in a number of formats since 1979 and several authors, e.g. Hammond *et al.* [13, 14, 15, 16], have pioneered many interesting applications of monochromator-based psychophysical measurement to questions of MP concentration. In this form, the typical apparatus has been laboratory based and has not been suitable for routine use in the clinic.

Mellerio, Palmer and Rayner [17] described the first portable instrument (which they called a maculometer) for measuring MP that used light emitting diodes (LED) as the (near) monochromatic light sources for the HCFP task. The instrument was small, light and portable, the size of a shoe box. It employed free viewing with the subject unrestrained and had promise as a screening tool. Since that time, Wooten *et al.* [18] have also described an instrument using LED's and Beatty *et al.* [19] have made another portable instrument based on LED's. This paper describes the current version of the maculometer which features easy to use central fixation for foveal and parafoveal measurements, describes its use in a busy eye clinic and compares the data from 124 subjects with those of the literature.

Materials and Methods

Maculometer

The principles of HCFP were well described by Werner and Wooten [12] and these have recently been re-evaluated by Werner *et al.* [2]. In summary, these are that a test field flickers between a monochromatic blue light that is highly absorbed by the MP and monochromatic light of longer wavelength, e.g. green, that is not absorbed by the MP. A minimum flicker match is made by adjusting the intensity of the blue light when the retinal image of the test field lies on the fovea and another match is made when the image lies several degrees away from the macula in an area of the retina where there is less MP[2, 20]. The logarithm of the ratio of the blue luminosity for the foveal match to that for the extra-macular match gives the optical density of the MP. The first necessity is to choose a monochromatic blue light source with a spectral power distribution (SPD) that matches the peak of the MP spectral absorption curve.

Light Sources

LED's are good light sources for portable instruments because they are small, inexpensive and are easily driven from simple power supplies. They also emit near monochromatic light. The LED's used were type 235-9916 for the blue and type 228-1879 for the green sources (RS Components, Corby, UK) and figure 1 shows their normalised SPD's together with the normalised absorption spectrum of MP. The peak wavelength of each LED of a batch of each type was measured (modified Zeiss monochromator type M4 QIII) and those with the λ_{max} closest to that of the MP spectrum were used.

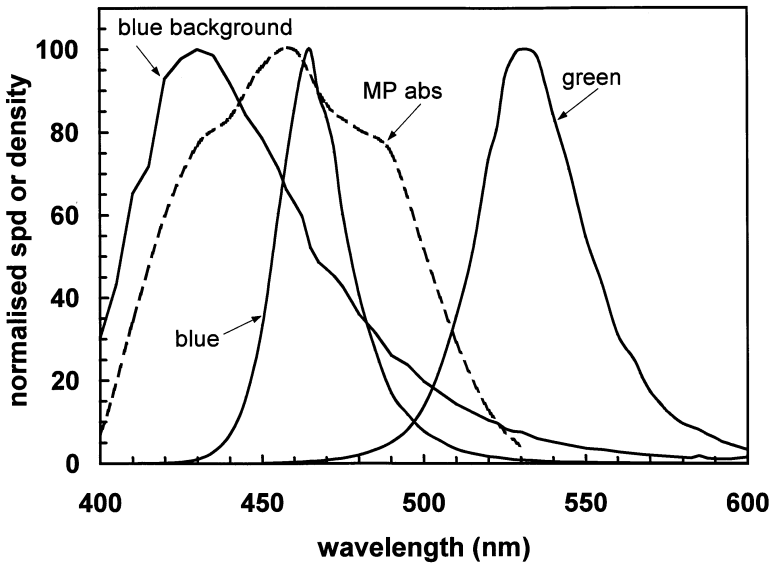

FIGURE 1 Normalised spectral power distributions for the three types of light emitting diodes (LED's) in the maculometer plus the normalised absorption of the macular pigment.

Test Fields

The maculometer described in 1998 (Mellerio, Palmer & Rayner [17]) had only one test field and required eccentric fixation for the parafoveal measurement. This field was imaged on the fovea by direct fixation by the subject or on a patch of retina 5 degrees from the fovea by getting the subject to fixate on a small red light placed to one side of the single test field. Many subjects found this eccentric fixation was not easy to maintain. Consequently, the maculometer was modified [21] to provide central fixation for both the foveal and parafoveal condition. For this, a central test field (fovea) was surrounded by an annular test field (parafovea) as described in detail below.

The field that was imaged on the fovea was viewed at a distance of 330 mm and subtended a diameter of 1 degree at the eye. For the minimum flicker match that was made away from the fovea, and where it is assumed there was no MP, the test field was an annulus of 10 degrees inner diameter and of 1 degree width. This was centred on the foveal test field that, whilst making matches in the parafovea, was switched from the flickering blue and green LED light to a dim red to provide a fixation target for these matches. Thus, the subject always fixated the central 1 deg. field, first for the foveal match when it flickered blue/green and the annulus was extinguished, and second as a red fixation target when the annulus was flickering blue/green. The test fields were formed from apertures in a matte white screen and each aperture opened into a small integrating chamber either cylindrical (foveal) or annular

(parafoveal) in shape - see figure 2. In each chamber the appropriate type and number of LED's were mounted, as shown in the figure.

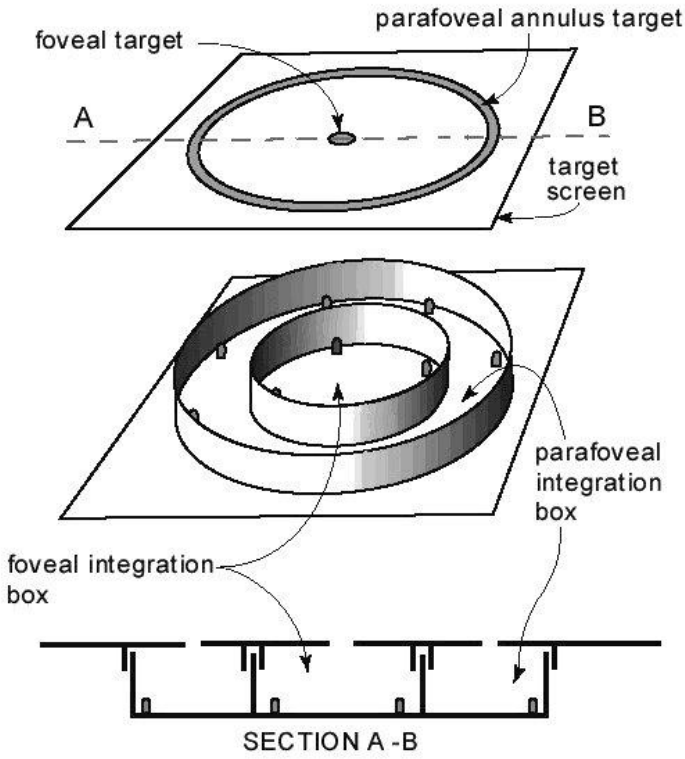

FIGURE 2 Top: exploded diagram showing the arrangement of the LED's and concentric integrating chambers behind the target screen that defines the central foveal and the annular parafoveal target fields. Bottom: cross section of the integrating chambers on the axis A-B shown above.

The electronics that drove the LED's was arranged so that each set of LED's in each field could be switched on individually without flicker to allow luminosity calibration. The green luminosity of both test fields could be varied over a large range (up to 250 $cd.m^{-2}$) so that the instrument could be set up for use in either brightly or dimly lit rooms. Eventually, settings were chosen that, from experience, were found to work well in the normal range of lighting found in offices and clinics. These were 120 $cd.m^{-2}$ for the fovea and 95 $cd.m^{-2}$ for the parafoveal annulus. That the drive circuits delivered the same currents whether continuously delivering current in calibration mode or pulsed in flicker mode, was verified by measuring the currents directly and indirectly by measuring the output of a calibrated photodiode and op-amp on the oscilloscope. Measurement of the test field luminances was done with a field lens and a calibrated photodiode (UDT, Orlando, Fa, USA, type S371 with photometric filter) and calibrated op-amp and digital voltmeter. Calibrations were traceable to NIST (National Institute for Standards and Technology) and NPL (National Physical Laboratory) and luminance accuracy was specified to be ±10%.

Background Adapting Field

The blue/green minimum flicker matches should ideally be made using only the L and the M cones. To ensure the S cones play no part in the match they are saturated by flooding the matte white screen with blue light. This background adapting field was provided by eight blue LED's (Type 247-1628, RS Components) with λ_{max} of about 428 nm, a wavelength that

corresponds well with the peak of the S cone sensitivity curve. Figure 1 shows the normalised SPD of a typical blue background LED. To ensure that the adaptation of the S cones was equal across the field of view, blue LED's of the same type as those used for the adapting background were also included in the integrating chambers. Their luminance was adjusted to match that of the background LED's illuminating the front of the white screen. The luminance was 5 $cd.m^{-2}$.

Frequency of Flicker

Just as it is important to ensure that the matches were made without the S cones taking part, neither must the rods be involved. This was achieved by arranging the frequency of switching from blue to green to be above the critical fusion frequency for rods [22]. In the parafoveal annulus, this frequency was set to 13 Hz and in the foveal field to 18 Hz. The frequency was higher in the foveal field so that it was also above the critical frequency of the S cones, should any that might be present not be adapted by the blue background. The blue and green LED's were driven with 50% mark-space ratio square wave current pulses in exact counter phase.

Luminance Measurements

In operation, the luminance of the blue LED's in the test fields was varied by altering the drive current from constant current sources by rotating a control on a small unit that was conveniently situated on the bench beside the subject (figure 3). The luminance of each integrating chamber was measured by a photodiode permanently embedded in the chamber. The diode's output was amplified by a calibrated op-amp and displayed via a sample-and-hold circuit on a digital voltmeter (DVM). The sample was taken when the subject, satisfied that the match was at a minimum flicker, pushed a button adjacent to the luminance control.

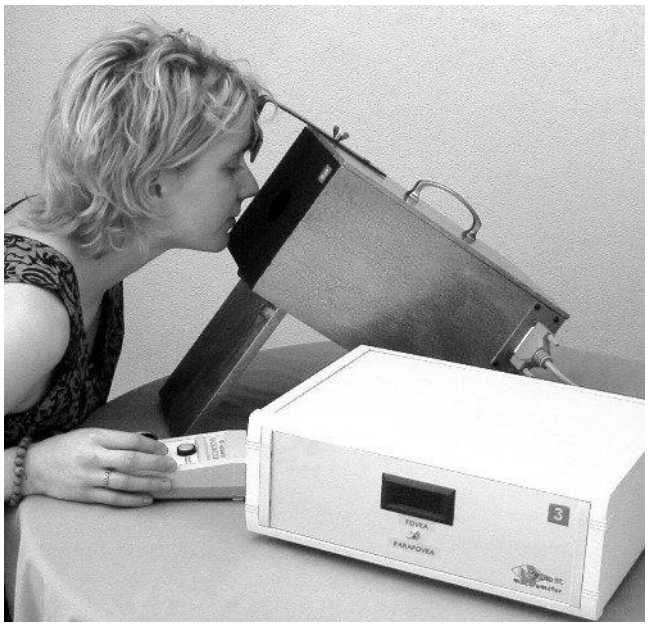

FIGURE 3 Photograph of a subject using the maculometer. She is viewing the test fields in the unit at the rear and is making an adjustment for a minimal flicker match with the control unit under her right hand. The display and electronic unit is at the bottom right.

The response law of the variable luminance control and the LED current sources was carefully designed so that control rotation was highly linear with respect to the luminance of

the test fields. Failure to achieve such linearity made the measurement of MP very variable and subjects reported that the task was difficult.

The DVM readings for the foveal and the parafoveal conditions were entered into a spreadsheet on a lap top PC. The spreadsheet contained the appropriate calibration relationships to change DVM readings into luminance values and to calculate the MP optical density.

Measuring MP Optical Density

The maculometer was set up on a table top at an angle of about 35 degrees as shown in figure 3. The room lighting varied from clinic to clinic and was typical of a modern office - dim lighting was not required as the test fields were of sufficient luminance. Subjects' visual acuity was checked to be better than 6/6 with correction. They were shown pictures of the two different test fields and had the principles of making a minimum flicker match explained. They viewed the test fields through the viewing aperture of the maculometer: a guide rested against the forehead to keep the viewing distance at 330 mm. Subjects were allowed to make two or three trial minimum flicker matches before recording of the measurements started. They were encouraged to make the matches quickly and pernickety and perfectionist adjustment of the control was discouraged as there is never a no-flicker setting. The end point was found by the method of adjustment and when satisfied with the match, the subject pressed the sample-and-hold button. After a match was recorded, the experimenter set the luminance control to some new arbitrary position so that the subject did not learn how far to turn the control to obtain a match. As it was easier to obtain minimum flicker matches with the parafoveal annulus, these matches were made first. Usually four settings were recorded, further values being taken if the coefficient of variation of the DVM readings was greater than 20%. If the coefficient still exceeded 20% by the time eight values had been obtained, the four worst outliers were removed so only four values were used by the spreadsheet. If the coefficient still remained greater than 20% the subject was rejected. In this study only 5 subjects were rejected for poor coefficient values or for being too perfectionist in attempting to set the control for minimum match.

For comparison, four of the subjects measured their MP density with the Moreland anomaloscope [11], and the values were compared with those obtained with the maculometer. In addition, 5 subjects were measured with and without neutral density filters and tinted lenses, and the MPOD values compared.

Subjects

The local research ethics committee approved the study. Volunteers were recruited - some were patients in ophthalmic clinics whose good eyes were used and some were accompanying relatives or members of staff or students. Subjects were in good general health except three who had type II diabetes who were excluded, as also were two who reported they had ocular problems. Five were excluded because their coefficients of variation for either the foveal or the parafoveal condition exceeded the 20% limit. The remaining group of 124 consisted of 64 women and 60 men. The MP was measured in only one eye in 117 subjects and in both eyes in the remaining seven. Subjects wore their reading correction, untinted, if necessary.

Each subject completed a simple questionnaire before using the maculometer to record personal characteristics and life style factors that the literature had previously reported as being associated with variations in MP. Besides ascertaining each subject’s age, gender and iris colour, the questionnaire asked subjects about their diet, tobacco smoking habit and exposure to sun light so that each subject could be allocated to the groups specified in table 1. These factors were simply self-reported and were not verified for accuracy.

Factor	Score	Criterion
Score for diet	Score 1	16 or more servings of fruit, vegetables and eggs per week
	Score 2	15 or less servings of fruit, vegetables and eggs per week
Score for smoking	Score 1	non-smoker
	Score 2	cigarette smoker or recent ex-smoker
score for exposure to	score 1	less than 3 hours per day
	score 2	more than 3 hours per day
	score 1	regular sunbathing or use of sun beds or

TABLE 1 Showing the criteria for scoring the diet, smoking and light exposure as self-reported by subjects. A subject who sunbathed or used sunbeds or tanning salons and who also had more than three hours exposure per day to outside day light was scored as 3 whilst a subject who had less than 3 hours outside but used a sunbed or tanning salon was scored 2.

Results

Convenience of use

The maculometer was easy to transport from clinic to clinic and to set up. Most subjects, after instruction that there was never a no-flicker condition and that they should not take too long to reach a setting, found the task of setting minimum flicker not difficult. Subjects reported that making the settings for the parafoveal annulus was easier than for the foveal test field. The whole procedure typically took ten minutes and did not disrupt the usual procedures of the clinic.

Repeatability and Validity

To see if the MP measures made with the maculometer were repeatable, three males measured their MP on four successive days. Table 2 shows coefficients of variation well below 10%.

	Subject		
	One	Two	Three
1	0.421	0.341	0.326
2	0.371	0.340	0.278
3	0.349	0.375	0.316
4	0.408	0.338	0.309
mean	**0.387**	**0.349**	**0.307**
sd	0.033	0.018	0.021
cv (%)	**8.6**	**5.1**	**6.7**

TABLE 2 Repeated measures of MP density on three subjects on four consecutive days

Validity of the measures of MP density was assessed by a number of subjects measuring their MP densities by a second technique. Correlation was always good and four subjects were investigated in detail. Table 3 shows their densities measured on the maculometer and the Moreland anomaloscope. For the latter technique, the densities at an eccentricity of 0.5 degree were taken because the foveal test field in the maculometer is one degree in diameter and because Werner *et al.* [23] showed that for HCFP the density that is measured is that corresponding to the edge of the foveal field. The time intervals between the measures were only a matter of a few days.

subject	alpha	beta	gamma	delta
maculometer	0.77	0.46	0.23	0.32
Moreland anomaloscope	0.79	0.45	0.12	0.30

TABLE 3 The MP density measured in four subjects with the maculometer and with the Moreland anomaloscope [11].

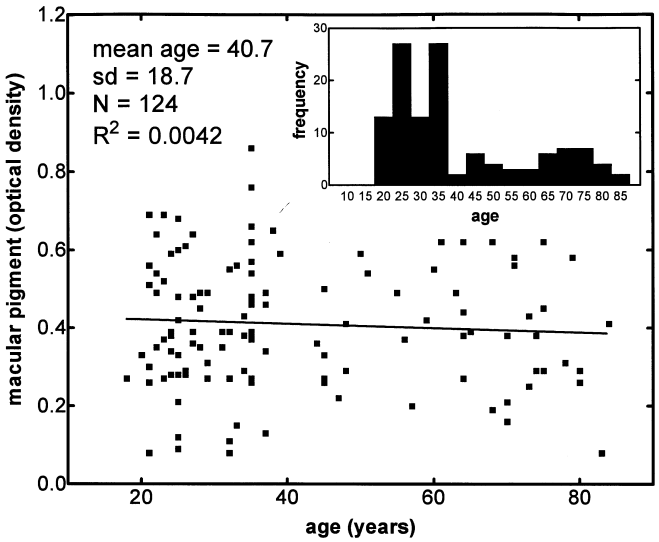

FIGURE 4 Plot of macular pigment optical density (MPOD) versus age. The regression line shows no significant correlation between MPOD and age (R^2 = 0.0042). Inset are relevant parameters and a frequency plot which shows the bimodal nature of the distribution.

Age

The subjects ranged from 18 to 84 years old. The optical density of the MP in 124 healthy subjects had a mean value of 0.41 ± 0.16 (mean ± sd) with a range of 0.08-0.86. Figure 4 shows MP density plotted against age. There is no significant correlation between MP density and age: R^2 = 0.0042.

Gender

The mean MP density for the 60 males was 0.48 ± 0.16 (mean ± sd) and for the 64 females it was 0.36 ± 0.15 and the difference between the means was significant, $p < 0.001$ (two tailed *t*-test, equal variance).

Iris Colour

67 subjects had light irides, defined as blue, grey or light brown, and 57 had dark irides that were mid-brown or darker. The MP density was 0.35 ± 0.14 (mean ± sd) for light and 0.48 ± 0.16 for dark irides. The difference between the two means was significant, $p < 0.001$ (two tailed *t*-test, equal variance).

Diet

The mean MP density for the 62 subjects on the fruit, vegetable and egg rich diet (score 1) was 0.48 ± 0.16 (mean ± sd) and for the 62 on the poorer diet (score 2) was 0.34 ± 0.14 and the difference was significant, $p < 0.001$ (two tailed *t*-test, equal variance).

Smoking

There were 93 non-smokers and 31 current cigarette or recent ex-smokers and their MP densities were 0.43 ± 0.16 (mean ± sd) and 0.35 ± 0.16 respectively. The difference between the two means was significant, $p = 0.014$ (two tailed *t*-test, equal variance).

Light History

Table 4 shows the MP density, and the relevant light exposure score. One-way ANOVA and Bonferroni multiple comparison post-tests were used to test the significance of the difference between the means of the three light groups.

	MP density	number of	
1 - minimal	0.47 ± 0.15	71	1 vs 2 $p < 0.05$ sig 1 vs 3 $p < 0.001$ sig 2 vs 3 $p > 0.05$ not sig
2 - moderate	0.38 ± 0.16	34	
3 - heavy	0.30 ± 0.14	19	

TABLE 4 Showing the MP density (mean ± sd) for the three light exposure groups, and the significance of the comparison of the groups' means using one-way ANOVA and Bonferroni post-tests.

Discussion

Correction for Non-monochromatic Light Sources

HCFP is preferably carried out with monochromatic blue and green light sources, but as figure 1 shows, the LED's were not truly monochromatic. Thus, when there is a minimum flicker match and the blue and green luminances are equal, the width of the LED's SPD's have to be considered and a correction can be calculated. In the current instrument, the correction factor depends crucially on which photopic sensitivity curve is selected. Taking the recently published curve by Stockman and Sharpe [24] which shows greater sensitivity in the short wavelengths, especially between 400 and 500 nm, than the V_λ CIE curve of 1924, the MP density is under-estimated and would be corrected by multiplying by 1.09. This correction is not large and has not been applied to the MP values given in this paper.

Correction for Lens Pigment

In effect, lens pigmentation applies a yellow filter across the pupil and its density increases with age. It is well known that the changes with age are variable [25] and older individuals of the same age may show a difference in lenticular absorption at 400 nm of a log unit or more. However, because HCFP compares a luminance match in one portion of the retina with that in another, a filter interposed in the light entering the eye will have no effect

on the comparison, provided the filter is not too dense. Measurements of MP density were made on the authors with interposed neutral density filters up to 0.6 log units and a range of tinted filters similar to those employed in category 1 and 2 sunglare filters (CEN: 1995 [26]) without any appreciable or systematic changes in MPOD density. This confirms the expectation and moderately tinted spectacles do not influence the values for MPOD obtained with the maculometer.

There is the possibility that the lens will fluoresce green under the influence of the blue light in the test fields and this would cause the MP density to be under-estimated. Lens fluorescence increases with age, so any errors would be worse in older subjects. However, to a first approximation, the fluorescence should be the same for the foveal and parafoveal condition and thus cancel out but Weale [27] has shown that for very old subjects with large amounts of macular pigment, the under-estimate might reach 20% or more. However, Ciulla *et al.* [28] reported that variable opacification of the crystalline lens does not significantly influence MPOD values and this supports the idea that lenticular fluorescence and lens pigmentation effects do not cause significant interference with HCFP methods.

Calibration, Repeatability & Validity

There is no way an instrument that uses HCFP can be calibrated absolutely in terms of MP optical density because of the subjective nature of its operation and its underlying psychophysical principles. It is nevertheless possible to demonstrate consistency with repeat measurements and with measurements made on or by the subjects in other ways. The maculometer shows good repeatability (Table 2) with coefficients of variation well below 10%. This figure is better than that shown by Beatty *et al.* [19] but similar to the day to day scatter shown in the study by Landrum *et al.* [29] where two subjects made daily MP density measures during a dietary supplementation experiment. Other studies sometimes show repeat measures of MP density [23], and the variation is similar to that found here. For the four subjects who had their MP density determined by a second method (Table 3), the agreement is good except for subject *gamma*. This subject had a low value of MP density that he has maintained over the years (he has been measured in several laboratories) and it is our experience that subjects with small MP densities yield results that are more variable. However, the above observations and the demonstration that the mean MP density values, and the way they change with parameters like diet, iris colour and so on, are similar to the changes found in the literature, is evidence that the maculometer makes satisfactory measurements, subject, of course, to the limitations that underlie all psychophysical measurements. The more important limitations are discussed below.

The HCFP method of measuring MP optical density makes some assumptions about the distribution of MP across the retina and about the contributions of the rods and all three cone types to the minimum match condition. The first point is that HCFP is based, in effect, upon comparing sensitivity in the fovea where there is macular pigment with an extrafoveal region where there is assumed to be none. It is established [20, 30] that carotenoids are distributed throughout the eye but the major concentration is in the fovea and is before the photoreceptor outer segments [31, 32, 33]. We have to rely, therefore, on those authors who have plotted the spatial distribution of pre-outer segment pigment to choose a retinal location where the pigment is sensibly absent and which can act as a baseline or zero-point for the foveal/parafoveal comparison. If the baseline area has pre-receptoral pigment then most methods of measuring MPOD *in vivo*, both physical and psychophysical, will under-estimate the amount of pigment present. Moreland and Bhatt [34] considered variations in individual MP densities and spatial distributions across the retina. They showed that the pigment has a symmetrical spread that approximates an exponential distribution rotated about the foveal centre with a value of zero considered by the average of MP at 5-7 degrees eccentricity.[34]

Hammond *et al.* [35] demonstrated similar distributions and showed that an exponential function fitted the distributions best. The residuals of their exponential function fitted through their data (their figure 3) are about 0.005 at 5 degrees eccentricity. Other authors have reported spatial distributions determined by psychophysics but the amount of pigment at 5 degrees was always small [8] except in one or two cases, e.g. Werner *et al* [2] where one subject might have an MPOD approaching 0.1 log units. Indeed, Werner *et al* [2] chose an eccentricity of eight degrees for their baseline retinal area, but five degrees is the best compromise between instrumental necessity and retinal limitations. So, provided fixation is sound, the minimum flicker match set with the parafoveal target is made in a retinal region with, at most, insignificant macular pigment and for most subjects, because the MP density is effectively zero much closer to the fovea, there is room for a margin for fixation error.

For the second consideration, it is important that the minimum flicker matches are made only with inputs from the M and L cones, and that the ratio of their sensitivities does not vary from the fovea to the parafovea. The maculometer follows the established practice of HCFP by working with flicker frequencies above the rod critical fusion frequency and using a blue adapting background light to swamp the S cones. However, it is assumed that the relative number of M and L cones, their content of visual pigment and the lengths of their outer segments (and thus the ratio of their sensitivities) is the same in the fovea and the parafovea. An analysis of the problem was made by Sharpe *et al.* [36] who showed that for wavelengths below about 450 nm it was not safe to assume that the kind of comparisons made in the HCFP MP measurements would yield correct results. As the maculometer, and many other of the HCFP instruments reported in the literature, are tuned to measure MP absorption only at its maximum of 463 nm, the measures of optical density may be taken as valid. Knau *et al* [37] reviewed the evidence for constancy of the L/M cone ratio from the fovea to more peripheral sites. They support the view from the literature by their own measurements using HCFP that show that the ratio of L/M cones is approximately constant at 0 , 25 and 40^{O}. Elsner *et al.* [8] investigated, with a reflectometric technique, small scale irregularities in the distribution of cones which could, of course, vitiate the assumption of equal sensitivity ratios between the M and L cones in the fovea and the parafovea. They found irregularities that could upset MP density measures and these were marked in older subjects. In our study, the nature of the target fields and the matching task will not reveal small spatial changes in receptor sensitivity but such changes may affect MP density measurements although there is no evidence that this is so.

Measurements of Macular Pigment

The results of our study (MP density = 0.41 ± 0.16) are consistent with previous studies such as that by Beatty *et al* [19] who measured MP densities between 0.08 and 0.84 and Werner *et al* [2] who showed an overall mean MPOD of 0.47, range 0.07 to 1.07. As reported elsewhere, large inter-subject differences in MP density have been documented, for example, by Bone and Sparrock [38] who recorded a wide range of optical densities, from 0 to more than 1.0, and standard deviations for individual subjects between 0.15 and 0.2. Pease *et al.* [10] tabulated the results of 14 papers that used four different techniques to measure MP and showed that most reported variations of a similar magnitude. More recent studies, such as those by Hammond and colleagues [13, 14, 15, 16], show broadly similar values for MP density whilst some of the latest reports show mean MP densities that are lower, in the region of 0.2 to 0.3 log units [28, 39, 40]. However, Hammond *et al* [40] and Ciuall et al[28] used a parafoveal baseline area of 4 degrees eccentricity and there is a real possibility that the concentration of pre-receptor macular pigment is higher there than at five degrees, and this would reduce the measured MPOD. Delori *et al* [7] compared three different techniques of measuring MPOD but their HCFP mean is 0.37, not very different from our value. The reasons for the different

mean values in HCFP studies is probably due partly to the subtly different ways the stimuli are generated and to sampling and population differences.

The large sample size in this study allows examination of several factors that influence MP density and which may thus be associated with AMD onset. The main findings have been highly significant differences in MP between males and females, dark and light irises, smokers and non-smokers, dietary intake of fruit, vegetables and eggs and exposure to light. Even though the analysis was simple, no allowance being made for the confounding effects of each factor upon the others, the results show good agreement with the data reported in the literature.

Unlike some previous findings, e.g. refs 2 and 40, we failed to demonstrate any significant age-related changes in MP density. Beatty *et al.* [39] showed a significant inverse relation between age and MP density, but Hammond and Caruso-Avery [40] found only a small decline of MP with age, whilst Werner *et al* [2] found a significant increase, as did Delori *et al*. [7] Thus there is at present no consistency in the reports of MPOD changes with age. It should be noted that in every study the data are scattered and the regression coefficients are small and may be greatly influenced by outliers. For example, removal of three outliers in the data of Werner *et al*[2] make the MPOD regression with age non-significant. This lack of agreement between reports may again arise from sampling problems: for example, the age distribution in our study is bimodal with more subjects younger than 50 years than older so simple regression statistics do not apply.

Highly significant differences in MP densities were observed between males and females. The MP density was approximately 37% higher in men. This pattern is similar to that seen in past studies [13, 40]. However, although several epidemiological studies have shown that women are at greater risk of developing AMD, there is a lack of consensus on whether or not female sex is a risk factor for AMD [41] and the basis for sex-differences in AMD susceptibility remain unresolved (for a recent review, see Evans, [42]). Indeed, not all studies have found lower levels of macular pigment in females. For instance, Bone and Sparrock [38], who used HCFP with 49 subjects, did not find any sex differences in MP density. However, any differences that may have been present might have been obscured because, as these authors suggested, the contribution of S cones was not eliminated thus making the MP measurements less accurate. In the current study, the S cone effect is minimised and the sample size is large enough to give weight to the gender difference.

The relationship that was found between MPOD and iris colour is also similar to past studies [15, 40]. There is a striking difference of 27% between dark (MP = 0.48) and light irises (MP = 0.35). Evidence indicates that differences in MP density between individuals are not completely genetically determined [43] although iris colour is. Thus, it is not clear to what extent MP differences may be due to genetic and/or environmental factors. For instance, it has been suggested that iris colour and MP density may be traits that are inherited together. Furthermore, MP depletion may occur as a result of increased oxidative stress in eyes with light coloured irises due to increased transmission of light and it is this that may be partly responsible for the genesis of AMD [44]. Despite the inverse relation observed here between iris colour and MP density, not every study has shown such a relationship (e.g. Bone & Sparrock[38]) and factors such as ethnic origin, which may also play a role, require further evaluation.

A significant difference in MP density was found between the two dietary groups. The division into the two groups was based upon the fact that lutein and zeaxanthin, the carotenoid constituents of MP, are not synthesised in the body [4]. Hence, people who eat a diet rich in the food stuffs that contain zeaxanthin and lutein [45] might be expected to have more MP. We relied on a simple questionnaire where respondents could self-report their "average"

input of fruit, vegetables and eggs. This is a very crude measure, subject to many criticisms, but the differences in MP showed clearly. Subjects who consumed 16 or more servings of fruits, vegetables and eggs per week had MP density values approximately 41% higher than those who reported lower intakes of these foods. This finding agrees with previous studies that have suggested that MP density can be increased by dietary modifications to include carotenoid rich foods [45, 46]. Werner *et al.* [2] used a sophisticated dietary reporting questionnaire and showed a significant increase of MP with increasing intake of lutein in the diet in their study of 50 subjects. Hammond *et al.* [46] supplemented subjects' diets with spinach and maize and showed in those subjects who they classed as "retinal responders", increases of mean MP optical density from around 0.37 before the diet change to about 0.49 after 12 to 14 weeks of enhanced diet. Bone *et al.* [47] found a weaker relation between MP and diet. They examined dietary intake of L and Z using food diaries and compared the intake with MP density. They concluded that about 17% of the variation of MP density could be explained by dietary intake of L and Z. In our study, diet diaries and plasma measures of carotenoids would have been more accurate, but a carefully designed yet simple questionnaire can produce responses that seem to be valid.

A significantly lower MP density was observed in smokers (MP = 0.35) as compared to non-smokers (MP = 0.43). This replicates the findings of previous studies. For example, Hammond *et al.* [13] reported an MP density of 0.16 for a group of 34 smokers and 0.34 for 34 non-smokers. This difference is larger than found in our study but Hammond *et al.* used more stringent methods to record smoking, and established a significant inverse relationship between smoking frequency and MP density. There is mounting evidence that smoking increases oxidative stress [48] and lowers antioxidant protection throughout the body, including the retina. Of course, the increased prevalence of AMD amongst smokers [42] may merely be a result of increased oxidative stress that has led to choroidal neovascularisation [49] and not be related to the effects of reduced macular pigment. However, this is unlikely because the carotenoids, which are internal to Bruch's membrane, must have a protective role [3] and their reduction may be due to removal by oxidative processes, or to some other cause that allows oxidative stress to harm the neural retina. Nevertheless, the present study supports the concept that it is not unreasonable to suggest that smoking contributes to macular pigment depletion, thus making it a candidate risk factor for AMD onset.

As with the determination of dietary habits discussed above, the questionnaire on light exposure, and the scoring system derived from it, is very crude and cannot be verified. However, it is again interesting to see that simple questions can apparently yield consistent results because the light exposure score was inversely associated with MP density. The implication of this finding is that MP is reduced by light exposure, as was suggested by Hammond and Caruso-Avery [40] when comparing northern and southern populations in the USA. They found the southern group had a reduction of mean MP density of 40% compared to the northern group: this is nearly the same as the difference we found (36%) between the groups with light scores of 1 and 3. This view that MPOD is negatively correlated with light exposure is not supported by data from Ciulla *et al* [50] that show low pigment levels, similar to those from the south, at a more northerly latitude, and no significant correlation with sun exposure, sunglass or hat use. This study [50] also fails to find other significant associations reported in other papers – perhaps this is a true reflection of differences between sample populations. More detailed and larger surveys may help to settle the status of the differences in MPOD found in the literature.

Conclusion

An instrument that is designed for routine use in a clinic must be judged on the results it gives and how easily and efficiently these are obtained. The measurements of MP density

on the group of 124 subjects described above are sufficient to allow judgments to be made on both these heads. First, the measures of macular pigment complement, and even extend, those reported in the literature and have yielded considerable experience of measuring MP density in busy ophthalmic clinics. This experience, together with that derived from duplicate instruments in clinics outside the UK, show that the maculometer is an entirely practical instrument well suited to measuring macular pigment in volunteers and patients alike.

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