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Macular pigment optical density in young Chinese adults

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Abstract

Purpose: The purpose of this study was to determine the macular pigment optical density (MPOD) in a group of Chinese subjects using a simple customized light emitting diode-based device.

Methods: Heterochromatic flicker photometry was used in this study. With a 1° diameter circular test stimulus, MPOD was estimated by comparing the relative sensitivities to a blue light, against a green reference, between foveal and parafoveal 4° temporal locations. Fixed alternating frequencies were used. Repeatability was determined on a small group of subjects. A further group of 67 young healthy subjects provided data for MPOD norms.

Results: All results were corrected to the common MPOD reference wavelength of 460 nm. The group-averaged MPOD was 0.48 (S.D. 0.23). We found no gender difference in MPOD. The coefficient of variability was 7.2–8.0% and the coefficient of repeatability was 0.12.

Conclusions: The MPOD of Chinese subjects did not differ greatly from the reported MPOD in white subjects.

Keywords: Chinese, macular pigment, optical density, heterochromatic photometry

Introduction

The human macular pigment is composed primarily of two hydroxycarotenoids, lutein and zeaxanthin (Bone *et al*., 1985; Landrum and Bone, 2001). The human pigment is concentrated in the central macular area and primarily localized in the region of the photoreceptor axons (fibres of Henle) and the inner plexiform layers (Snodderly *et al*., 1984a). Its density decreases exponentially with eccentricity, with only traces remaining beyond six degrees (Moreland and Bhatt, 1984; Snodderly *et al*., 1984b; Bone *et al*., 1988; Hammond *et al*., 1997a; Robson *et al*., 2003).

The macular pigment is believed to play an important role in the protection of the macular area from short-wavelength radiation induced damage. This is achieved by its ability to filter blue light (Snodderly *et al*., 1984a; Pease *et al*., 1987) and scavenge free radicals through the anti-oxidative nature of carotenoids (e.g. see Snodderly, 1995; Khachik *et al*., 1997; Landrum *et al*., 1997a; Schalch *et al*., 1999; Beatty *et al*., 2000; Landrum and Bone, 2001, for reviews). There is increasing evidence that macular pigment density can be modified by dietary means or taking lutein/zeaxanthin supplements (e.g. see Snodderly, 1995; Hammond *et al*., 1997b; Landrum *et al*., 1997b; Berendschot *et al*., 2000; Leung *et al*., 2001; Falsini *et al*., 2003, for reviews). Whether increasing macular pigment density will offer additional protection from degenerative changes of the retina remains to be proven.

Age-related macular degeneration (ARMD) is the most common form of retinal degeneration among the elderly (Klein *et al*., 1995). Macular pigment density is believed to be lower in patients with ARMD (Beatty *et al*., 2001; Bone *et al*., 2001) and it has been suggested that those with higher macular pigment density are better able to retain visual function (Hammond *et al*., 1998). However, in a study of a large group of non-ARMD and ARMD subjects, Berendschot *et al*. (2002), using spectral fundus reflectance, did not find any significant difference in the macular pigment density.

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Reflectance methods of quantifying macular pigment optical density (MPOD) are generally known to give smaller values because of intraocular scattering and reflections (Kilbride *et al*., 1989; Delori *et al*., 2001; Bour *et al*., 2002). These also reduce their sensitivity in detecting differences. ARMD prevalence in Chinese has been reported to be lower than in whites (Chang *et al*., 1999). It is of interest to see if ethnic Chinese have a lower macular pigment density.

Current information about the human macular pigment density is mainly derived from studies on non-Hispanic whites. Yet, ethnicity may play a role in macular pigment density. By comparing the foveal luminosity function of Egyptian subjects with those of other studies from the West, Ishak (1952) speculated that Egyptians might have a higher macular pigment density. Hammond *et al*. (1996a) and Hammond and Caruso-Avery (2000) reported higher macular pigment density in subjects with dark irises.

Macular pigment density can be measured by colour matching (Ruddock, 1963; Moreland and Bhatt, 1984; Davies and Morland, 2002), relative flicker sensitivities (Pease *et al*., 1987), motion photometry (Robson *et al*., 2003) and heterochromatic flicker photometry (HFP) (Werner *et al*., 1987; Snodderly and Hammond, 1999; Wooten *et al*., 1999; Bone *et al*., 2000; Beatty *et al*., 2001 and Mellerio *et al*., 2002). The latter is the most frequently used method and it is the method employed in the present study.

We report here the results of macular pigment density in Chinese subjects using a customized device, which uses HFP. The measuring device is portable, simple in design with easily administered testing procedures such that it can be developed into a clinical tool.

Methods

The HFP is a method to match visual luminous sensitivity to light of different colours. It has been extensively used to determine the luminous efficiency function (Le Grand, 1957; Wyszecki and Stiles, 1982a). Use of HFP to measure the macular pigment density is well established and has been described in detail by Wooten *et al*. (1999) and Snodderly and Hammond (1999).

Instrumentation

A HFP device was custom-built. The design of the system is shown in *Figure 1*. The system is portable with external dimensions of $23 \times 46 \times 44$ cm (width $\times$ height $\times$ depth). A notebook computer is connected to the device to control the radiance of the stimuli and flash frequencies. The computer also controls the measuring procedures, records and stores data for later

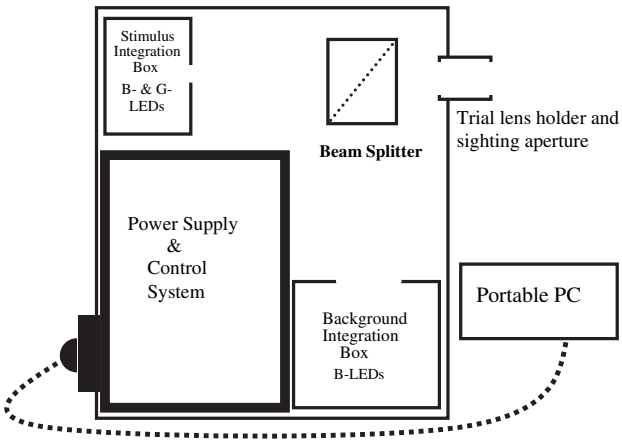

Figure 1. Design layout of the measuring device.

analysis. The system allows a test distance of 38 cm. The test field is composed of three elements: the background, fixation targets and a flashing stimulus. All light sources are light emitting diodes (LEDs). Multiple LEDs are used and uniform fields are achieved using integration boxes. Brightness and spectral composition of the background and test stimuli were measured with a Topcon SR3 radiospectrophotometer (Topcon, Tokyo, Japan).

The background field is an oval aperture in an integration box, major axes measuring 13° (horizontal) $\times$ 9° (vertical) at the viewing distance. The integration box contains uniformly distributed multiple LEDs. The LEDs have a maximum emission wavelength of 470 nm and a full-width at half maximum (FWHM) of 25 nm. The wavelength was selected to suppress the actions of the S-cones and the rods. Brightness of the background field is controlled by a constant voltage supply. This has a luminance of 40.4 cd m^{-2} or 2.90 log trolands when a 5 mm pupil diameter is assumed. Two tiny opaque fixation dots were incorporated in the centre of the oval opening, 4° apart at the viewing distance. They define the eccentricity of 4° for the parafoveal measurement.

The test stimulus flashes in repeated cycles, on for 1.5 s and off for 0.75 s. The test stimulus is composed of alternating blue and green lights. The same type of blue LEDs as the background are used for the blue portion of the stimulus. The green LED has the peak emission at 575 nm (FWHM = 15 nm). The blue light is near to the peak absorbance of macular pigment whereas the macular pigment absorption of the green light will be negligible (Ruddock, 1963; Wyszecki and Stiles, 1982b; Snodderly *et al*., 1984a; Pease *et al*., 1987; Werner *et al*., 1987). Green and blue LEDs are distributed evenly in a single integration box. This provides even distribution of light in the test stimulus. The blue and green stimuli flash alternately in a

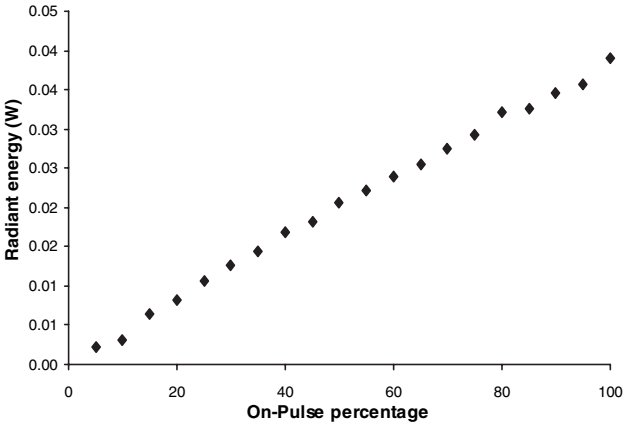

Figure 2. Variation of blue LED energy with percentages of activated on-pulses.

rectangular wave manner at a fixed temporal frequency. The stimulus alternation rates, 21 Hz at the fovea and 15 Hz in parafovea, are beyond the critical fusion frequency (CFF) of S-cones and rods (Mellerio *et al.*, 2002). This minimizes contributions from the S-cones and rod receptors in a brightness match. The integration box has an aperture of 6.5 mm. This provides the flashing stimulus of 1° at the working distance of 38 cm. The test stimulus is optically superimposed onto the left fixation spot in the background by a beam splitting cube. The green light is the reference and it has a constant luminance of 6.5 cd m^{-2} or 2.11 log trolands, assuming a 5 mm pupil diameter. The intensity of the blue light stimulus is controlled by regulating the number of evenly distributed brief on- and off-pulses within the blue semi-cycle of the blue-green alternating flashes. As we are matching the brightness of the blue light at foveal and parafoveal regions with a common reference, absolute values of blue light intensity are not needed. Setting the number of on-pulses as 100% when the intensity is at its maximum value, the instantaneous intensity of the blue stimulus is linearly related to the percentage of on-pulses activated (see *Figure 2*).

Subjects

Sixty-eight young ethnic Chinese subjects were studied, 36 females and 32 males, relatively homogeneous in age (range 18–23 years). There were eight non-optometry students and all others were optometry students. In addition, one of the authors (ethnic Chinese, male, age 45 years) participated in the reliability study. Only subjects free from any ocular pathology, having normal colour vision and visual acuities 6/6 or better were recruited. All subjects participating in this study were non-smokers. Dietary habits of subjects were not studied and were assumed to be typical to young Chinese in southern China. All measurements were done with natural pupils.

Procedures

Ethics approval was obtained from the Human Subjects Ethics Sub-Committee of the Hong Kong Polytechnic University. Signed consent was obtained from each subject after the purpose of the experiment, procedures and associated risks were explained. All subjects from the faculty had had a full eye examination within a year in the university's Optometry Clinic. Subjects were asked for information about their ocular health status. Ophthalmoscopy, visual acuity and colour vision (with Ishihara plates) were tested prior to the commencement of the experimental session. All ametropes were optically corrected. An additional +2.00 D trial lens was placed in the optical system to relax accommodation. This relieved possible accommodative fatigue during the course of the experiment. Irrespective of the ametropia (and including emmetropes), an equal number of trial lenses were inserted to maintain constant measuring conditions.

All measurements were taken in a room with an average illuminance level of about 50 lux (measured with DX-100, Digital Lux Meter, Tynset, INS). Each subject was given about 5 min to settle in, during which time experimental procedures were explained. He/she was then given some trial runs to ensure understanding of the measurement procedures. Subjects were instructed and reminded frequently to blink at regular intervals. Typically, the trial runs lasted about 8 min.

Only the right eye was studied and the other eye was occluded. The subject first looked at the left fixation point. The test stimulus, flashing at 21 Hz, was superimposed on the fixation point. The method of adjustment was used. The subject adjusted the blue stimulus luminance up and down to obtain the point of minimum/zero flicker. In cases when there was more than one point of minimum/zero flicker, the mid-point of the range was located. Five measurements were taken. For each measurement, the starting stimulus intensity of the blue portion was selected by means of a sub-routine in the controlling software utilizing a random function in the C++. No time limit was imposed on the subject for each measurement. On completion of the foveal measurements, the subject was then directed to the right fixation point. This brought the test stimulus to the temporal retina 4° from the fovea. All other measuring conditions and procedures remained unchanged except that the blue-green stimulus alternating rate of 15 Hz was used. On average, it took about 12 min to complete all measurements.

Table 1. Measurement precision

	Five measures in consequent days (MPOD)*	Three measures, each 7 days apart (MPOD)†					
Measure	**Experienced observer**	**Observer 1**	**Observer 2**	**Observer 3**	**Observer 4**	**Observer 5**	**Observer 6**
1	0.30	0.57	0.90	0.51	0.30	0.23	0.55
2	0.36	0.47	0.91	0.49	0.31	0.27	0.57
3	0.35	0.53	0.90	0.61	0.34	0.19	0.63
4	0.35						
5	0.36						

*Mean (S.D.) = 0.34 (0.02); S.D. of the differences against mean = 0.02.
†Mean = 0.52; Variance within subjects (s_w^2) = 0.00172.

Experiments

We conducted two experiments. The first experiment was a two-part reliability study. For part 1, the MPOD of one subject (male, 45 years) was measured everyday for 5 days. For part 2, the MPOD of six subjects (age range: 22–23 years) was measured every 7 days until three data sets were obtained. The second experiment was a study to determine the normative value of MPOD in young adults. Sixty-eight young normal adults were measured.

Data analysis

With considerations of the radiance distribution of the LED and its mismatched peak of emission, all measured results were corrected for the macular pigment (MP) transmission spectrum peaked at 460 nm (see Appendix). Of the five runs for each retinal location, the two extreme values were discarded and the three most clustered readings were averaged. Removing outliers in this unbiased way reduced data variability within a subject. A statistical software package (InStat3, GraphPad Software Inc., San Diego, California, USA) was used to analyse the results. (We also analysed our data with all five runs included. The final results of which, not shown here, differed from those after our treatment by not more than 0.02 log units.)

Reliability of the measurements was analysed by the coefficients of variability and the coefficient of repeatability (Bland and Altman, 1986; Chinn, 1990; Bland, 1995; Beatty *et al.*, 2001; Mellerio *et al.*, 2002; Snodderly *et al.*, 2004). Coefficient of variability is the ratio of the standard deviation against the mean of the measures. Coefficient of repeatability is defined as the 95% range for the difference between two repeated measurements. The variance within subjects (s_w^2) for repeated measurements of the six subjects was obtained in the intermediate calculation table of a one-way ANOVA test. For these subjects, coefficient of variability is: s_w/mean, and coefficient of repeatability is given by $\pm 2\sqrt{2s_w^2}$ (Bland and Altman, 1986; Chinn, 1990; Bland, 1995).

To test if there was difference in MPOD between males and females, the two-tailed Student's *t*-test was used.

Results

Reliability

Table 1 gives the results of the reliability study. Over a period of five consecutive days, the single observer gave a standard deviation of 0.02. The coefficient of variability for this subject is 7.2%. For the group of six subjects followed every 7 days, within subjects standard deviation (s_w) was 0.04 and a mean MPOD of 0.52, giving the coefficient of variability of 8.0% and the coefficient of repeatability of 0.12.

MPOD norms

The results of one female subject were excluded from the group analysis because of: (a) high variability from one run to another (over 0.8 log units); (b) negative resultant MOPD value (−0.19). A second trial was given and yielded no improvement. The summary of the results is shown in *Table 2* for the rest of the subjects. The mean MPOD of the whole sample was 0.48 (S.D. 0.23). Male subjects had a mean MPOD of 0.51 (S.D. 0.25) and females, a mean MPOD of 0.45 (S.D. 0.22). The MPOD distribution of the subjects is shown in *Figure 3*. There was no significant difference in MPOD between the male and female subjects ($t = -1.028$, d.f. = 65, two-tailed, $p = 0.308$).

Table 2. Result summary

	All subjects	**Male**	**Female**
Mean	0.48	0.51	0.45
Number of subjects	67	32	35
S.D.	0.23	0.25	0.22
Minimum	0.02	0.12	0.02
Maximum	1.11	1.11	1.02
Median	0.47	0.52	0.44

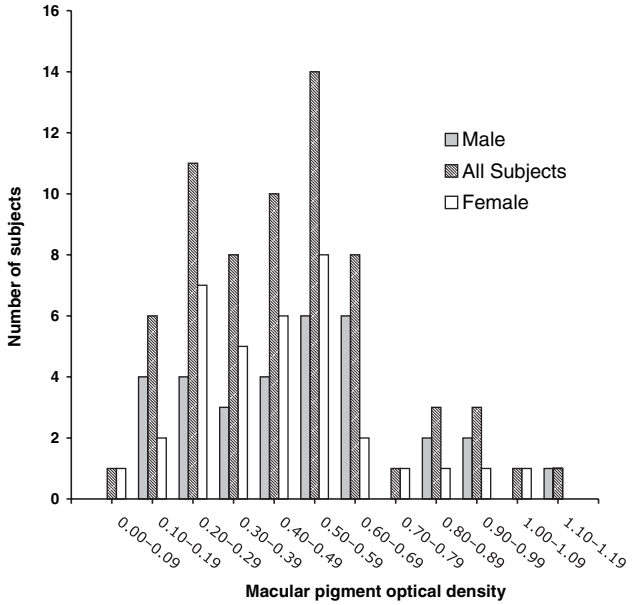

Figure 3. Distribution of MPOD.

Discussion

Our results on a sample of young healthy Chinese subjects present some normative values of macular pigment density, measured using a psychophysical method, in the Chinese population.

The HFP is known to give reliable results. We obtained a coefficient of variability of about 8% and a coefficient of repeatability of 0.12. These values are comparable with previous MPOD studies by HFP (Beatty *et al*., 2001; Delori *et al*., 2001; Mellerio *et al*., 2002; Snodderly *et al*., 2004). These coefficients have been slightly amplified because of the peak wavelength and bandwidth corrections. One of the important factors contributing to the variability of HFP measurements is the alternating frequency. We used fixed alternating rates and they were set to high levels to ensure that the rods and S-cones were not contributing to the end-points. The disadvantage of using a fixed flicker frequency is that the null range for minimum or no flicker may vary, depending on individual sensitivity. Snodderly *et al*. (2004) emphasized the importance of testing the CFF for individual subjects to establish the alternating rate. A higher flicker frequency gives a wider null zone and determination of the mid-point of the range can be more difficult. Our subjects were university students and they are generally able to perform better in psychophysical tests.

The MPOD is known to vary with diet and iris colour. Given these factors, it is reasonable to speculate that MPOD might vary with ethnicity. Woo and Lee (2002) suggested a possible ethnic difference of macular pigment density in a study of colour discrimination by the Farnsworth Munsell 100 hue test. It is however difficult to compare MPOD across studies because of the different methodologies, experimental setup and procedures used. Psychophysical methods tend to yield higher MPOD compared with methods utilizing reflection of the retina. We note that our group-averaged MPOD was higher than those reported in studies using the retinal reflection method. For instance, Chang *et al*. (2002) found a MPOD of 0.23 (S.D. = 0.07) in a group of Chinese subjects by reflectometry whereas our mean MPOD was 0.48(S.D. = 0.23).

The average MPOD from previous studies in normal subjects using heterochromatic photometry yielded results varying from about 0.2 to 0.6 (e.g. see Werner *et al*., 1987; Hammond *et al*., 1996b,c, 1997a, 1998; Wooten *et al*., 1999; Hammond and Caruso-Avery, 2000; Beatty *et al*., 2001; Delori *et al*., 2001; Aleman *et al*., 2001, for reviews). This wide range of MPOD may not necessarily reflect a true macular pigment density difference among subjects, but may reflect the difference in methodologies and/or diversity in subject samples. Given that our group-averaged MPOD is within the range of MPOD values reported elsewhere in predominantly non-Hispanic white samples, it is reasonable to suggest that there is no substantial difference in MPOD between Chinese and non-Hispanic whites. However, to confirm this, we need to measure MPOD in white subjects using our experimental setup. Consistent with most previous studies (e.g. Werner *et al*., 1987; Hammond and Caruso-Avery, 2000; Beatty *et al*., 2001), our results show inter-subject variability of MPOD, amounting to 1.1 units.

The mean MPOD was 12% higher in our male subjects but this was not statistically significant. A gender difference in macular pigment density was reported in a number of studies, for instance, Hammond *et al*. (1996b), Broekmans *et al*. (2002) and Mellerio *et al*. (2002). Although Hammond and Caruso-Avery (2000) found macular pigment density lower in females, by 13%, they also commented that when 5% of men with the highest density were excluded, the gender difference was minimal. Beatty *et al*. (2001) did not find an association of MPOD with gender.

In common with some previous studies using HFP (Wooten *et al*., 1999; Ciulla *et al*., 2001a,b; Hammond *et al*., 2002), we used an eccentricity of 4° as the zero MPOD reference. This assumes the ratio of M- and L-cone concentrations in this retinal location is comparable with that at the fovea (Nerger and Cicerone, 1992). There is evidence that the L/M ratio shows little change within the central 40° of the central fundus (Knau *et al*., 2001). Equal L/M ratios in the two testing locations ensure equal relative sensitivities towards the blue and green light of the stimulus. A smaller eccentricity also allows for easier subjective responses. The

obvious disadvantage of a smaller eccentricity is the possible residual macular pigment in this reference retinal location (Bone *et al*., 1988; Hammond *et al*., 1997a; Robson *et al*., 2003). It is estimated that macular pigment measured at 4° eccentricity could have induced an under-estimation by as much as 25% (Bour *et al*., 2002).

Conclusion

In this study, we report MPOD normative data in young adult Chinese subjects. The group-averaged MPOD was 0.48 (S.D. 0.23), referencing to a wavelength of 460 nm. This is well within the range of MPOD reported for non-Hispanic white subjects. We did not find a significant gender difference in MPOD. The system of measurement was proven to be efficient and can be developed into a clinical device.

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Appendix

Correction of MPOD for LED spectral radiance distribution and peak emission

Because of radiant energy dispersion and mismatch of the MPOD peak absorption at 460 nm to the blue LEDs (470 nm) used in our instrument, our measured results require correction. Results given in the manuscript have been adjusted to macular pigment density specified by the MP absorption peak at 460 nm.

The principle of correction is to find the extra amount of light needed for the blue LEDs to compensate for the filtering effect of the macular pigment. We adopted the luminosity function of Stockman and Sharpe (2000) $V_2^*(\lambda)$ and its associated MPOD function of standard observers. The luminosity function was first corrected for the MPOD spectrum to give a macular pigment free sensitivity function V_λ^P. This function was assumed for both the foveal and parafoveal matches. Brightness stimulus (B) of the blue LEDs at a peripheral match (deprived of macular pigment) is proportional to the sum of the multiples of the modified luminosity coefficient and the blue LED spectral radiant energy (E_λ), i.e. $B = k\Sigma V_\lambda^P E_\lambda$, where k is a scaling constant. Under foveal viewing, a subject looks at the blue light through the layer of macular pigment of spectral transmittance T_λ and the new brightness stimulus $(B') : B' = k\Sigma T_\lambda V_\lambda^P E_\lambda$. The overall transmittance (T_{MP}) is then given by:

$$T_{MP} = \frac{\sum T_\lambda V_\lambda^P E_\lambda}{\sum V_\lambda^P E_\lambda} \tag{1}$$

We want to establish a relation between the MPOD of a subject as described by the peak OD at 460 nm (OD_{460}) and the matched optical density with the blue LED spectrum (OD_{LED}). For a given peak MPOD value at 460 nm (OD_{460}), the standard MPOD function was modified according to the Beer-Lambert law (Wyszecki and Stiles, 1982c and Bridgeman, 1987). Iteratively, intensity of the blue LEDs was changed to give spectral radiant energy (E'_λ) until it reached a factor, say α (i.e. $E'_\lambda = \alpha E_\lambda$), that gave the matched brightness through the layer of macular pigment. Hence,

$$B' = k\sum T_\lambda V_\lambda^P E'_\lambda = \alpha k \sum T_\lambda V_\lambda^P E_\lambda$$

On a matched condition, $B' = B$, this gives $\alpha\Sigma T_\lambda V_\lambda^P E_\lambda = \Sigma V_\lambda^P E_\lambda$. Rearrangement gives:

$$\alpha = \frac{\sum V_\lambda^P E_\lambda}{\sum T_\lambda V_\lambda^P E_\lambda} \tag{2}$$

Comparing Equations (1) and (2) gives $T_{MP} = \frac{1}{\alpha}$. Hence, the logarithmic value of α gives the expected MPOD measure (OD_{LED}).

By assuming a range of OD_{460} in 0.2 steps, appropriate α or OD_{LED} values were located. A curve fitting procedure gave a relation ($R^2 = 1$) that:

$$OD_{460} = 0.2794OD_{LED}^2 + 1.1786OD_{LED} + 0.0128$$

This relation was used to correct all measured OD values to give the corrected macular pigment optical density as specified at the peak value of 460 nm.