Ophthal. Physiol. Opt. 2009 **29:** 127–137

A new desktop instrument for measuring macular pigment optical density based on a novel technique for setting flicker thresholds

Rob L. P. van der Veen[1], Tos T. J. M. Berendschot[1], Fred Hendrikse[1], David Carden[2], Maria Makridaki[2] and Ian J. Murray[2]

[1]University Eye Clinic Maastricht, Maastricht, The Netherlands, and [2]Faculty of Life Sciences, Moffat Building, University of Manchester, Manchester M60 1QD, UK

Abstract

A rapid portable technique for estimating macular pigment optical density (MPOD) in large populations is described. The new instrument utilises a novel method for setting flicker thresholds which is undemanding for naïve and elderly observers and easily operated by a non-technical person. The method has good repeatability ($r = 0.97$) and the data are comparable with an optical method based on retinal reflectometry ($r = 0.78$). MPOD spatial profiles are presented for seven normal observers and these are well described ($r = 0.99$) by a decaying exponential function consistent with previous reports. MPOD values are presented from 5581 (2435 females and 3146 males) individuals measured in 48 optometric practices. The mean MPOD of this population was 0.33 (S.D. ± 0.187) which is similar to previous large scale studies of MP.

Keywords: desktop instrument, flicker photometry, large data set, macular pigment, macular pigment optical density, naïve observers

Introduction

The role of the retinal carotenoids lutein (L) and zeaxanthin (Z) in slowing the effects of aging in the macular region of the eye continue to be of interest to the ophthalmic community (Seddon *et al*., 1994; Berendschot *et al*., 2000; Landrum and Bone, 2001; Beatty *et al*., 2004; Richer *et al*., 2004; Nolan *et al*., 2007; Schalch *et al*., 2007). These carotenoids form the macular pigment (MP). It is thought that MP protects the retina by shielding it from the damaging effects (Landrum *et al*., 1997) of high-energy blue light (Sharpe *et al*., 1998; Algvere *et al*., 2006; Wu *et al*., 2006) and by scavenging of free radicals formed by oxidative stress (Khachik *et al*., 1997; Beatty *et al*., 2000; Kim *et al*., 2006), thereby reducing the likelihood of degenerative macular disease.

Received: 8 June 2008
Revised form: 19 August 2008; 8 October 2008
Accepted: 12 October 2008

Correspondence and reprint requests to: Dr I. J. Murray.
E-mail address: ian.j.murray@manchester.ac.uk

To evaluate the role of MP and the extent of its protective properties, it would be helpful to measure it in a reliable and quantitative way in large populations. To this end a new instrument for measuring macular pigment optical density (MPOD) has been developed (MPS 9000 series: Tinsley Precision Instruments Ltd, Croydon, Essex, UK). It is compact and lightweight and designed to be easily operated in any clinical setting. The device uses the principle of heterochromatic flicker photometry (HFP). Like all HFP-based methods, observers are required to make flicker matches using two wavelengths of light, one of which is absorbed by the macular pigment (465 nm) and another (530 nm) which is not. Flicker matches are made at both a central and peripheral point in the retina. In the new method, flicker matches are obtained in a novel way which makes the determination of the minimum flicker point relatively quick and easy, even for naïve observers. Subjects are instructed to press a button as soon as flicker is detected, in contrast to the more conventional HFP approach where they are required to adjust a green-blue luminance ratio until flicker is minimised or eliminated (Bone and Landrum, 2004; Snodderly *et al*., 2004).

 doi: 10.1111/j.1475-1313.2008.00618.x

Estimating MP in large populations must be based on robust methodology. It must be reliable and reproducible when operated by non-professional staff and when applied to aging observers who may suffer from hazy ocular media, compromised physical skills and early degeneration of the retina. Apart from avoiding unnecessary demands on the observers, it is important that the technique is easy to implement and understand by those charged with obtaining the data. It should be reasonably light and mobile and electronically stable so that regular and costly calibration is not required. Perhaps most important of all, when both operator and observer are naïve, it should be obvious when the data are of poor quality. Simply producing a digital read-out of MPOD is insufficient. Some indication of the reliability, reproducibility and validity of the measurement as it is performed is helpful in busy clinics where operators may be required to make 10 or 20 measurements in a day. Furthermore, accuracy and repeatability are particularly important in clinical conditions where patients are supplementing their diets with L or Z. In these circumstances, practitioners will expect their MP apparatus to be capable of showing small but significant increments in MPOD during a phase of supplementation.

In this paper we describe the new technique in detail, concentrating in particular on the features which make it suitable for clinical conditions. We present data from a series of experiments designed to evaluate the new method in terms of: (1) repeatability; (2) how the data compare for a population of observers measured with a respected objective reflectometry method; (3) the spatial profile for a substantial number of observers; and (4) analysing data collected under clinical conditions for a large population of observers.

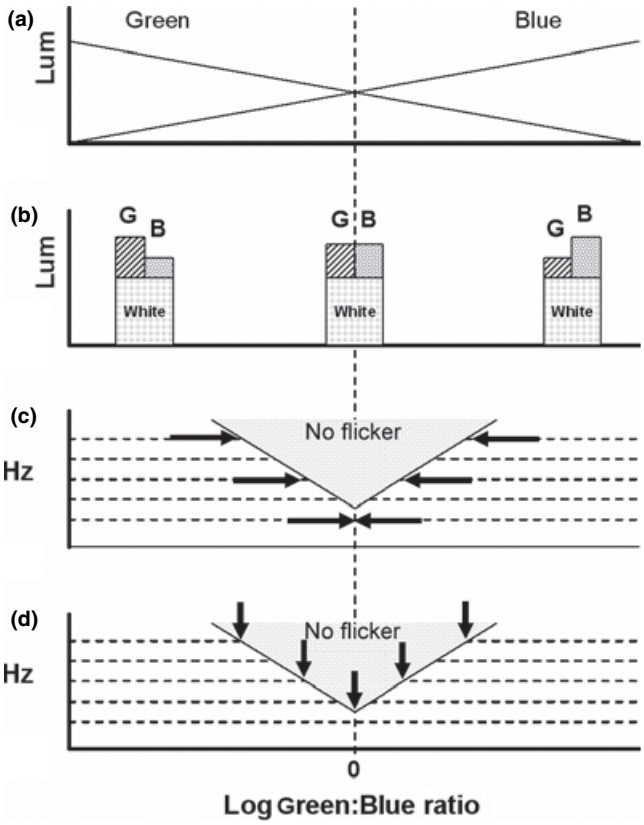

Figure 1. (a) The luminance of the blue and green light emitting diodes (LEDs) are yoked so that there is no change in mean luminance across the range of green-blue ratios; (b) Luminance profiles at different green-blue ratios, illustrating the white pedestal; (c) Conventional heterochromatic flicker photometry (HFP) where subjects adjust green-blue ratio for minimal flicker using a constant flicker rate; (d) method used in current study where flicker rate is gradually reduced and subject presses a button when they detect flicker.

Methods

Heterochromatic flicker photometry

Macular pigment optical density was determined using HFP, based on a new approach to obtaining the minimum flicker point. Instead of adjusting the green-blue ratio and detecting the *disappearance* or *minimisation* of flicker, the observer views the target whilst its flicker rate is gradually reduced from above the critical fusion frequency (CFF). They respond to the *appearance* of flicker by pressing a button. This procedure is repeated for a series of pre-set green-blue ratios. The intensity of the green and blue lights is reciprocally yoked so that there is no overall change in luminance for different green-blue ratios as illustrated in *Figure 1a*. The luminance profile at the different green-blue ratios is shown in *Figure 1b*. The main difference between the traditional approach and the method used in this study is highlighted in *Figures 1c and 1d*. Conventionally, observers view a target composed of two lights of different wavelength alternating at say, 15 Hz. They are given control of the intensity ratio of the two lights denoted by the horizontal arrows in *Figure 1c*. In this technique they see flicker for most of the time and this flicker is minimised, or disappears at a particular setting thus determining when the two lights are at equal luminance. Depending on their sensitivity to flicker, which varies with many factors, one of which is age, they will have either a wide or narrow range of ratios where flicker is absent, illustrated as horizontal arrows in *Figure 1c*.

This contrasts with the new method illustrated in *Figure 1d*. At the start of the test a particular green-blue ratio is selected and the flicker rate is automatically reduced from 60 Hz until the observer detects flicker when they press a button. Hence the arrows are vertical, indicating that the same function is plotted as in the conventional method, but by setting a series of flicker

rates corresponding to a series of pre-set green-blue ratios. Because the flicker starts above the CFF, the observer approaches the end point from the 'no flicker' direction as indicated in *Figure 1d*.

Stimulus and surround

The stimulus is composed of a cluster of blue (465 nm) and green (530 nm) light emitting diodes (LEDs). The blue and green LEDs are flickered in counter-phase. They are superimposed on a white light pedestal, which provides control of luminance contrast. The target consists of a 1° circular aperture in an integrating sphere. This is surrounded by a uniformly illuminated white area subtending approximately 30°. The peripheral measurement is achieved by fixating on a larger 1.75° red spot located at 8° horizontal eccentricity. The observer is instructed to maintain central fixation on the 1° target for central viewing. A more relaxed fixation strategy, allowing eye movements on or around the edge of the larger peripheral fixation target is recommended for the peripheral measurement. It is also suggested that the observer blinks frequently when obtaining the setting, particularly with peripheral viewing. The target is viewed from 200 mm through a +5D meniscus lens placed at 180 mm from the plane of the target. Subjects are requested to wear non-tinted normal distance correction spectacles if required.

Luminance control for all LEDs is by computer controlled fast pulse width modulation with accuracy better than 0.05 dB. The individual wavelength, bandwidth and luminance of the LEDs were as follows; green (530 nm), 30 nm, luminance variable up to 200 cd m^{-2}; blue (465 nm), 25 nm, luminance variable up to 200 cd m^{-2}. The surround (250 cd m^{-2}) was provided by three white LEDs of colour temperature 5500 K. Note that these generate a metameric white from two primary components with a broad peak around 550 nm and a narrow peak around 440 nm. A similar white LED was used for the target pedestal and could be varied up to 350 cd m^{-2}. The surround luminance was chosen to minimise the effect of age on pupil size resulting in a more stable retinal illuminance with age (Winn *et al*., 1994). On average, the luminance of the target area is close to that of the surround. Luminance measurements were carried out using a telespectroradiometer (PR 650, Photo Research Inc., Chatsworth, CA, USA) referenced to the V_λ function of Sharpe *et al.* (2005).

Procedure

The main procedure is shown in *Figure 2*. Prior to this, a short (30 s) pre-test flicker sensitivity routine is used to ensure observers are in the middle of their flicker sensitivity range when performing the main task. In this routine, the white light pedestal on which the blue and green lights are superimposed and the green-blue ratio remain constant at a preset value. The pedestal provides approximately 15% luminance contrast for the peripheral task and 10% contrast for the central task, with an overall pedestal luminance of 180 cd m^{-2}. Five settings are made for central, and five for peripheral viewing. For each, the temporal frequency is ramped down from 60 Hz, and the observer presses the response button when they see flicker. The mean of the five settings is used to set the luminance of the pedestal in the main test. Pre-setting the pedestal luminance in this way ensures the range of responses starts close to 30 Hz, with a minimum around 15 Hz. A similar approach was used by Snodderly *et al.* (2004) in that they determined optimal flicker frequency for each observer. Note that changing the luminance of the white pedestal does not alter the green-blue ratio. As has been shown previously (Anderson and Vingrys, 2000) increasing the luminance of the white pedestal raises flicker thresholds. Boundary conditions are such that the pedestal is always present.

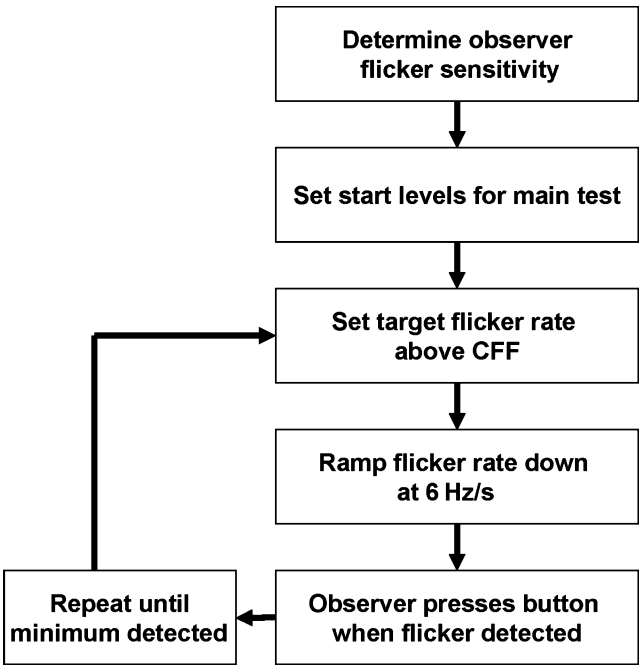

Figure 2. Algorithm of the new technique.

In the main part of the test, the initial value of Lr, the green-blue ratio, is set so that the luminance of the green light is higher than that of the blue, as illustrated in *Figure 1d*. The initial temporal frequency is set to 60 Hz, that is, above the normal critical flicker fusion frequency for these conditions. Again the flicker rate is gradually reduced at 6 Hz per sec. The subject fixates on the target and when flicker is detected, they respond by pressing the response button. The luminance ratio of blue and

green is then changed by 0.2 dB, incrementing blue and decrementing green, keeping mean luminance constant (see *Figure 1a*). The temporal frequency is re-set to 60 Hz and again ramped down at 6 Hz per sec, until the subject detects flicker for this new value of Lr and presses the response button. The algorithm is illustrated in *Figure 2*.

This sequence continues for a series of green-blue luminance ratios until a V-shaped function is obtained. The minimum corresponds to the equalization of the blue and green luminance. The process of detecting flicker for a series of green-blue luminance ratios is then repeated for peripheral viewing at a minimum of seven degrees eccentricity, and again a V-shaped curve is obtained. This then provides the minimum for peripheral viewing. The entire sequence usually takes around 2–3 min per eye. Example data for one observer (MM) are illustrated in *Figure 3*. The vertical axis is temporal frequency and the horizontal axis is the green component of the green-blue ratio, Lr in dBs, where Lr = 10−2 × L1 and L1 = luminance of the green LED in dB. The line through the data is a least squares best fit third order polynomial. Note that the zero MPOD value is equivalent to 4 dBs because of the preset calibration conditions.

Calculation of MPOD

In *Figure 3* the minima for central and peripheral viewing occur at different green-blue ratios (*x*-axis) because macular pigment selectively absorbs blue light, but is present only within the central 8–10° region of the retina. If macular pigment is absent, then the minima of the two curves would be superimposed under the assumption that flicker sensitivity is constant across this region. The optical density of the MP is calculated according to equation (1) below, assuming that all detected light is absorbed by the macular pigment and that MPOD is zero at the peripheral location.

$$\begin{aligned}\text{MPOD} &= k \log 10\left[\frac{\text{Lbc}}{\text{Lgc}}\right] - k \log 10\left[\frac{\text{Lbp}}{\text{Lgp}}\right] \\ &= k \log 10\left[\frac{\text{Lbc}}{\text{Lgc}}\right]\left[\frac{\text{Lgp}}{\text{Lbp}}\right],\end{aligned}$$

assume Lgc = Lgp,
then

$$= k \log 10\left[\frac{\text{Lbc}}{\text{Lbp}}\right], \quad (1)$$

where Lbc and Lgc are the luminance of the blue and green lights at minimum flicker for central viewing and Lbp and Lgp are the luminance of the blue and green lights at minimum flicker for peripheral viewing.

In the present device, a correction factor, $k = 1.2$ is applied to account for the following factors:

(1) overlap of the wavelength spectra of the green light with the MP absorbance spectrum
(2) overlap of the spectra of the blue and green LEDs
(3) λmax for the blue LED does not exactly correspond to λmax of the macular pigment as defined by Wyszecki and Stiles (1982). A similar compensation was made by Snodderly *et al.* (2004).

Under these conditions, with fast flicker (> 15 Hz) and high luminance (> 1000 td) then detection of flicker is predominantly mediated by the achromatic L/M cone magno-cellular pathway and any contribution from rods and S-cones is strongly suppressed (Werner *et al.*, 1987). The wavelengths of the LEDs were selected as a compromise to minimise chromatic contrast (λ_B–λ_G) between the two lights and thereby prevent incursion of chromatic detection mechanisms. The circular field of the stimulus is 1° and this integrates over the peak of the distribution of the MP. No correction has been introduced to account for this, although it is known that some observers have a multi-peaked spatial distribution of their macular pigment (Delori *et al.*, 2001; Delori, 2004; Berendschot and van Norren, 2006). Many previous studies have adopted a 1° stimulus but this is a compromise between many factors as discussed by Werner *et al.* (1987). One important point is that, unlike most techniques the present method does not rely on the assumption of equal ratio of sensitivities to the flickering blue/green target for the central and peripheral target. The reason for this is that observers set an isoluminant point (between blue and green) that may occur at slightly different temporal frequencies for centre and periphery. The minimum for the centre invariably occurs at a different temporal frequency to that for the

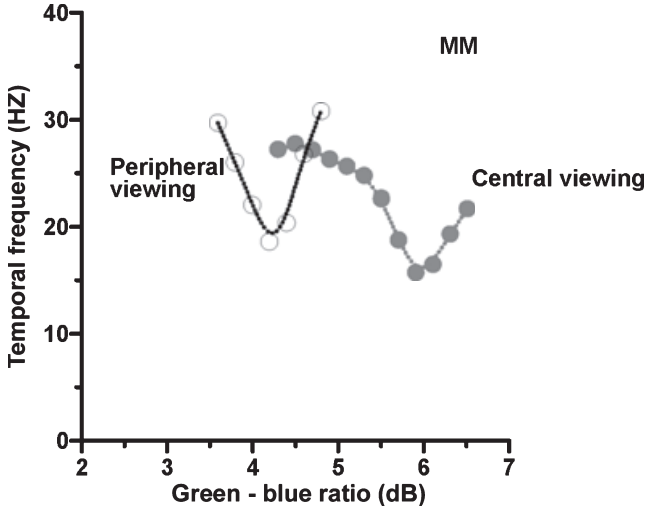

Figure 3. Example data from subject MM. The solid circles depict central viewing and the open circles, peripheral viewing. Note green-blue ratio is a logarithmic scale.

periphery. In *Figure 3* for instance, the minimum for peripheral viewing occurs at a higher temporal frequency than that for central viewing, indicating that for this subject sensitivity is higher in the periphery at isoluminance. Hence, although flicker sensitivity may vary between observers and for different stimulus size the blue/green ratio will remain independent of flicker sensitivity. However, increasing stimulus size beyond certain limits may result in decreased values of MPOD if sampling occurs over too large an area. Hence a 1° target is probably the best compromise taking into account our present knowledge on the spatial profile of most individuals.

The flicker frequency rate of decrease of 6 Hz per sec is a compromise between time taken for the test and variations in subject's reaction times. Over an age range of 60 years, reaction times vary by little more than 100 ms (Porciatti *et al*., 1999). Hence the flicker thresholds will vary by around 0.6 Hz but always less than 1 Hz. Reaction time remains approximately constant for a particular subject and we assume it has negligible effect on the measured MPOD. In pilot studies different rates were tested and 6 Hz per sec was regarded as the optimum. Any within-subject effects of reaction time would be negligible because they are the same for both the central and peripheral conditions.

In order to measure the spatial profile of the MP, the stimulus surround was modified to allow a series of fixation points at 0.5° intervals either side of the target. A profile of MPOD to one side of fixation takes around 25 min. All the instruments were calibrated by the manufacturer. They recommend a calibration check should be carried out every 6 months. The instruments used for the macular pigment reflectometer (MPR) comparison and the spatial profile investigation were checked regularly and remained stable throughout the study.

Macular pigment reflectometer

MPOD was also determined in a comparison study by spectral fundus reflectance with the MPR (van de Kraats *et al*., 2006) which has previously been validated against other techniques including SLO auto fluorescence, SLO reflectance and HFP (Berendschot and van Norren, 2005). The essentials of this setup are summarised as follows. The image of the filament of a 30 W halogen lamp is relayed to the pupil plane of the eye. The intensity of the light entering the eye is 1.04×10^7 Trolands. A 1° spot centred on the fovea is illuminated and the light that reflects from this spot is measured. An image of the retinal spot is focused on an optical fibre that has a mask on its tip to define a 1° diameter spot at the retinal plane. The fibre is the receiving part of a spectrometer with a range of 400–800 nm and an optical resolution of 5.8 nm full width half maximum (FWHM). To keep instrument stray-light to a minimum, the detection channel does not overlap with the illumination system. Operation with an undilated pupil is enabled by a mask in the pupil plane that limits the illumination and detection configuration (separation 0.8 mm) in the pupil to a 3 mm circle. Chin rest and temple pads were used to help maintain head position. MPOD was determined by a full spectral analysis of the reflected light. In short, the incoming light was assumed to reflect at the inner limiting membrane, the disks in the outer segments of photoreceptors and the sclera. Using known spectral characteristics of the different absorbers within the eye (lens, MP, melanin, blood), the densities of the pigments and % reflectance at the interfaces were optimized, to fit the measured data at all wavelengths (van De Kraats *et al*., 1996; For a detailed discussion of this analysis see Berendschot *et al*. (2003) and Berendschot and van Norren (2004).

Subjects

For the comparison study, 26 healthy volunteers were recruited at the University of Maastricht, The Netherlands. Their mean age was 39 ± 14 (range 22–64, men/women 16/10). Inclusion criteria were: aged 18 years or above; no known eye disease; clear ocular media; and corrected visual acuity of decimal 0.8 (Snellen 6/6.75) or higher.

For the spatial profile study, seven observers were recruited. All had normal vision, normal ocular fundi according to a fundus examination by a trained person. Their mean age was 36.2 ± 15.9, range 24–62.

This research followed the tenets of the Declaration of Helsinki and was approved by the Central Manchester Regional Ethics Committee (CMREC), UK and the ethics committee of the University Hospital of Maastricht, Netherlands. Before testing, all subjects gave written informed consent after the purpose and possible consequences of the study were explained.

Unselected monocular MPOD data from 5581 observers were collected from 48 optometric practices in the US between June 2006 and December 2007. The data were obtained as part of routine eye testing procedures. They have been de-identified in accordance with the Health Insurance Portability and Accountability Act (HIPAA). These regulations were introduced in 1996 to protect the privacy of individual's medical information whilst at the same time providing for the flow of information regarded as beneficial for research purposes. All the instruments used in this survey were carefully calibrated according to manufacturer's specification. The operators were trained by the distributor of the instruments who in turn received extensive training in the UK.

Data analysis

The SPSS statistical software package (Version 15.0.1: SPSS Inc., Chicago, IL, USA) was used for data analysis. Pearson correlation tests were used to quantify the agreement between different methods of determining MPOD. To determine age effects, Spearman's ρ was used. The large sample *z*-test was used to establish male/female differences in different age groups from the large data set.

Results

Repeatability

Mean MPOD for 26 subjects obtained with the new device was 0.40 ± 0.15. Each subject repeated measurements five times and the mean individual standard deviation (S.D.) was 0.067 ± 0.033.

Eleven subjects were measured a second time within 3–14 days after the initial measurement. *Figure 4* shows these test-retest data. The correlation coefficient was $r = 0.97$ ($p < 0.001$). Mean test-retest variability was 0.0195 ± 0.047. Another useful measure of repeatability of measurements is provided by the mean of differences divided by the mean values from the two estimates. In this case this was 11.7%.

Comparison with the Macular Pigment Reflectometer

The mean MPOD of all 26 subjects obtained with the MPR was 0.65 ± 0.20. Correlation between the two devices was $r = 0.78$ ($p < 0.001$) as seen in *Figure 5a*. The data show that correlation is high, but that there is a discrepancy between the two instruments of a factor of around 0.6 with the MPR giving consistently higher values. This is similar to other studies in which retinal reflection and HFP methods are compared (Delori *et al*., 2001; Berendschot and van Norren, 2005). It has been suggested that this discrepancy may be due to observers setting flicker thresholds using the edge of the stimulus. This would mean the measurement would give MPOD at 0.5° eccentricity. In *Figure 5b* we have corrected for this effect using the MPOD profile from Hammond *et al*. (1997). They recommend a factor of $10^{0.42x}$ where x is eccentricity. In our case $x = 0.5°$ because we have used a 1.0° stimulus. Accordingly the data in *Figure 5a* have been corrected by $10^{0.42x} = 1.62$. This does not affect the correlation coefficient but it changes the slope to close to 1.0, suggesting that the edge hypothesis may account for much of the discrepancy between optical and flicker-based methods of measuring MP (Delori *et al*., 2001; Berendschot and van Norren, 2005). An alternative approach is to use the MPOD profile data shown in *Figure 6* to make this correction. If we assume our data reflect the MPOD at 0.5 degrees the best fit exponential then indicates an underestimate of 42% i.e. a factor of 1.42. These issues are briefly reviewed in the Discussion.

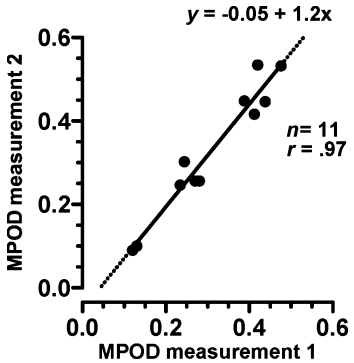

Figure 4. Comparison of macular pigment optical density (MPOD) with the new technique for two measurements obtained on separate occasions.

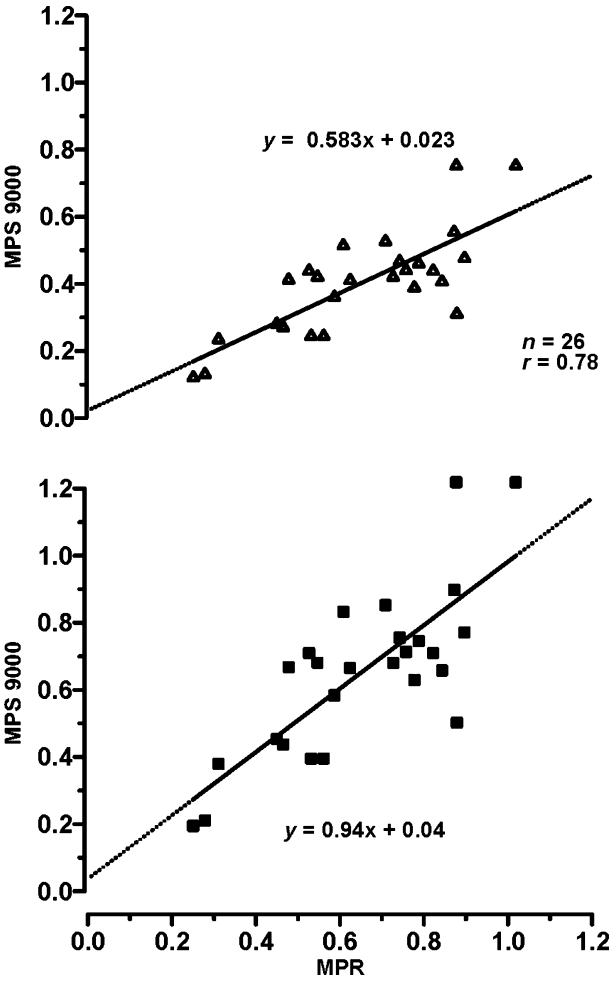

Figure 5. (a) Comparison of macular pigment optical density (MPOD) obtained with the new method (MPS 9000) and the Macular Pigment Reflectometer (MPR); (b) Data from (a) corrected assuming flicker settings were obtained at the edge of the 1.0° stimulus. Note that the correlation coefficient is the same in (a) and (b).

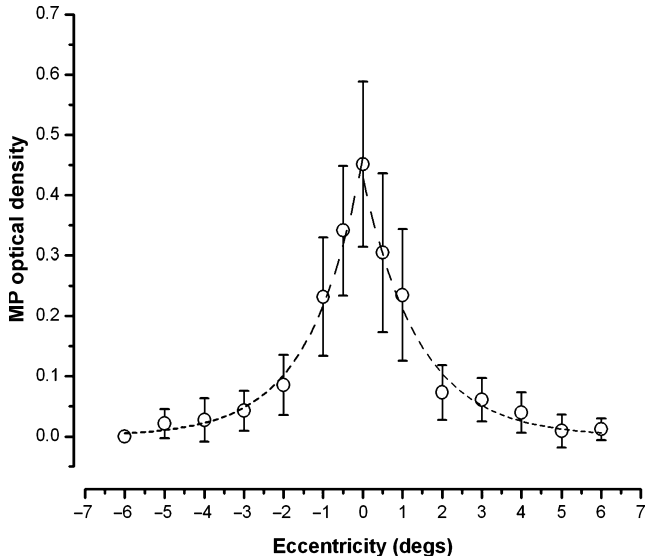

Figure 6. Spatial profile of the macular pigment (MP) using the new technique. Error bars are standard deviations (S.Ds.).

In *Figure 6*, the spatial profiles of the MPOD for seven normal observers obtained with the new apparatus are illustrated. As with previous reports (Hammond *et al*., 1997) the data show a sharp fall-off with eccentricity. There is considerable inter-subject variation in the profiles and some of this variation may be linked to foveal architecture (Nolan *et al*., 2008). We have used a decaying exponential of the form $y = \exp(-px)$, to fit the mean of all spatial profiles. The following functions were obtained: $y = 0.428 \times \exp(x/1.33816)$ with $r^2 = 0.992$ and $\chi^2 = 2.22 \times 10^{-4}$ for the nasal distribution and $y = 0.414 \times \exp(-x/1.30507)$ with $r^2 = 0.990$ and $\chi^2 = 2.4 \times 10^{-4}$ for the temporal distribution. It is evident from the figure and from the coefficients in these equations that there are only slight asymmetries between nasal and temporal distributions. The mean width of the distribution at half maximum in MPOD units was 2.2°.

Ideally, to investigate these spatial effects quantitatively, a smaller target should be used. Here we show the data using a 1° target to test the validity of the present technique compared with other similar approaches. As has been shown in other studies the standard deviation increases as eccentricity decreases because of the inherent inter-individual differences in MPOD. Non-monotonic MPOD spatial profiles, based on flicker photometric techniques (Hammond *et al*., 1997) and SLO reflectance maps showing a shoulder in the distribution at around 0.8° (Berendschot and van Norren, 2006), have been reported.

Unselected data from optometric practice

Figure 7a and 7b illustrate the frequency distribution of age, and MPOD, in 5581 males and females obtained from 48 optometric practices in the US. The details of the distribution are presented in *Table 1*. The data set includes year of birth (not date of birth) and gender. In this study, individuals under 20 and over 90 years old are not included. Their mean age is 52.37, median age is 53. The standard deviation is 13.02. The demography of this population is illustrated in *Figure 7a*. The overall mean and standard deviation of MPOD is 0.330 (±0.187). This is very similar to many other observations using flicker photometry (see Discussion). For males and females the means (S.D.) were 0.335 (±0.185) and 0.326 (±0.179) respectively, and this difference is not statistically significant. However, the male–female difference for the 20–30 year old group is statistically significant according to a z-test ($z = 2.187$, $p < .05$). None of the other age groups showed a significant male–female difference. As age increases, the male–female difference decreases and for the 80–90 year old age group females have slightly higher MPOD than males.

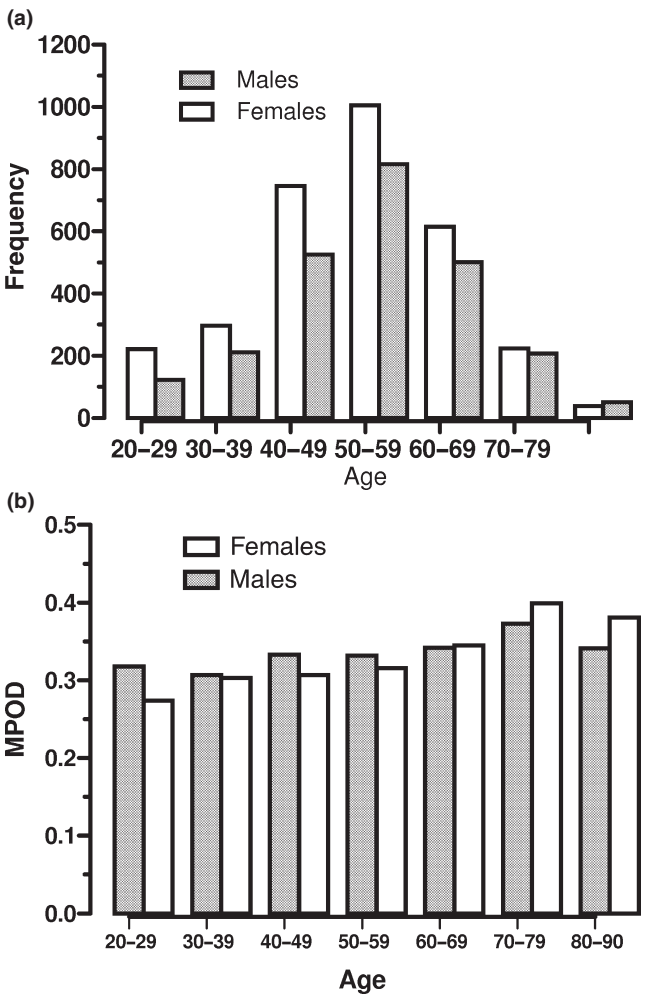

Figure 7. (a) Age profile of the data set; (b) macular pigment optical density (MPOD) of males and females.

Table 1. The means, standard deviations (S.Ds.) and medians for the macular pigment optical density (MPOD) for the different age groups in the large scale study

	MPOD	S.D.	Median	Number (*n*)
Males (age)				
20–30	0.318	0.188	0.276	123
30–40	0.307	0.169	0.312	212
40–50	0.333	0.171	0.312	525
50–60	0.332	0.179	0.312	816
60–70	0.342	0.197	0.312	501
70–80	0.373	0.202	0.360	207
80–90	0.341	0.247	0.312	51
Overall	0.335	0.184	0.312	2435
Females				
20–30	0.274	0.165	0.276	221
30–40	0.303	0.157	0.264	297
40–50	0.307	0.160	0.288	746
50–60	0.316	0.174	0.312	1005
60–70	0.345	0.186	0.360	615
70–80	0.399	0.223	0.408	223
80–90	0.381	0.265	0.360	39
Overall	0.332	0.178	0.312	3146

MPOD is directly correlated with age (Spearman's correlation coefficient = 0.083, $p < 0.1$). This effect is attributable mainly to the slightly low values for the 20–30 age group and higher values for the over 70s. Intermediate age groups, for example between 40 and 69 show only a small increase with age. The age effect may be due to a number of factors discussed below.

Discussion

Existing techniques for large-scale screening and measurement of MP in the general population have mostly used flicker-based psychophysical methods (Ciulla *et al.*, 2001; Ciulla and Hammond, 2004; Nolan *et al.*, 2004; Richer *et al.*, 2004; Snodderly *et al.*, 2004; Moeller *et al.*, 2006), although an SLO has been used for some large scale investigations (Berendschot *et al.*, 2002). The conventional flicker-based methods use the technique of allowing the subject to adjust the green-blue ratio of a flickering stimulus until flicker is either minimised or disappears. During the test, the subject continually perceives flicker which induces fading in many observers. Although techniques are used to minimise this effect, it creates problems for naïve observers which may mean many repeated measurements are required. A secondary problem is the flicker frequency chosen for the test. Snodderly *et al.* (2004) in their protocol for naïve observers used a preliminary flicker sensitivity test and modified the test flicker rate accordingly. The boundary conditions imposed in their study resulted in a flicker rate range of 11.5 ± 2.5 Hz in the fovea and 7.3 Hz ± 2.6 Hz in the periphery.

The main differences between the new technique and the more conventional methods of determining MPOD are illustrated in *Figures 1c and 1d*. In the conventional techniques observers see flicker for long periods of time as they adjust green-blue ratio (horizontal arrows *Figure 1c*), whereas in the new technique they approach the minimum flicker point from below flicker threshold (vertical arrows *Figure 1d*) and are not therefore exposed to flicker for more than 0.5 s when they press the button to indicate flicker has been detected. A series of these measurements is made, so that a V-shaped function as illustrated in *Figure 3* is obtained. An important advantage is that the data points appear on the screen each time the observer presses the button so that the operator can see the V-shape curve developing. This means that within a few seconds of the start, both operator and observer know that the technique is working as expected and time is not wasted collecting erroneous data. The operator can see the correct curve developing and the observer experiences the correct (predicted during instructions) sequence of the appearance of the stimuli. As in Snodderly *et al.* (2004) described above, a pre-test assessment is made of the flicker sensitivity of individual subjects and the initial test conditions are adjusted according to observer sensitivity. Effectively, this strategy ensures observers are making settings in the centre of their flicker sensitivity range.

Measurement error with the instrument was assessed by comparing two measurements a few days apart (see *Figure 4*). The data can be analysed either in terms of the within subjects standard deviation or by calculating the correlation co-efficient, r. The data show that there is a very high degree of association between the two measurements ($r = 0.97$, $p < 0.001$) despite the fact that the data set is limited to 11 observers. An *post hoc* power analysis shows power of 0.99, meaning with this number of subjects the statistical analysis had a 99% chance of detecting a correlation and greater number of observers was not necessary. It may be argued that as our device is designed for elderly observers that our sample of young subjects was unrepresentative of our target observer. Although this is true, we would argue that because the task is easy to perform, repeatability remains high even when the data are noisy as expected with older observers. For example with 15 observers correlation analysis would have power of >90% given a value of $r = 0.7$ with $\alpha = 0.05$. We have not conducted a repeatability study with older people but from *Figure 4* it seems likely that the correlation will reach 0.7.

Comparison with Macular Pigment Reflectometer

Correlations between MPOD values obtained with the new instrument and the reflectometry technique were

highly significant and similar to previous reports when flicker methods are compared with optical methods. Although the technique described here uses a novel method of determining minimum flicker it should be noted that the same principles as all flicker-based methods apply. Delori *et al.* (2001) used two estimates of MPOD derived from an SLO and compared these with a flicker-based technique. They found $r = 0.61$ between HFP and an SLO-based measure. van de Kraats *et al.* (2006) compared the MPR used in this study with another HFP method and obtained $r = 0.56$ based on 20 observers. In that study a ratio difference between the two measurements of around 0.6 was obtained, comparable with the uncorrected data illustrated in *Figure 5a*.

Correlation analysis reveals the strength of relation between techniques but does not necessarily reveal agreement in an absolute sense. In fact it is unlikely that different methods will agree quantitatively with each other, especially when they use different principles and rely on different assumptions, as in the case of the MPR and the new device described here. Statistically it may be argued, as with the repeatability measures, that the sample imposes a limitation on the interpretation of the results because it may not be representative in terms of age. This issue cannot be addressed in the present paper; here we place emphasis on describing the technique and accept that it must be subject to more rigorous testing involving observers of different ages.

The MPR measurements are based on averaged values of the MPOD obtained across a 1° sample area. Delori *et al.* (2001) made a correction for the differences in sample area between optical and flicker-based methods and this did not have a major impact on the comparisons. One source of any discrepancy between the flicker-based methods and the MPR is likely to be the fact that the latter does not rely on a peripheral null reference point. It is possible that in some individuals, especially those who are supplementing their diet with L and/or Z, the MP is spread beyond the normally-assumed zero point of eight degrees. Note that Hammond *et al.* (1997) suggest that higher MPOD is associated with a wider spatial distribution. This would artificially reduce the absolute measurement of MPOD when obtained with flicker-based methods. The MPR does not rely on a zero reference point for its determination of MPOD.

A further issue concerns the precise position on the flickering target on which observers make their judgment as to the absence/presence of flicker. It has been thought for some time that some observers use the edge of the flickering target to make their setting (Werner *et al.*, 1987; Hammond *et al.*, 1997; Bone *et al.*, 2004). It is claimed that when a 1° target is used, this may bias the measurement toward lower values because the luminance profile is highly peaked and reaches its maximum in the central 0.2°. Although this may be true for some observers who have high to moderate levels of MP, those with lower MPOD may be less likely to adopt this strategy because the luminance contrast between the edge of the target and its centre will be relatively low. It is possible however that, as discussed fully in Hammond *et al.* (1997), the value obtained for a 1° target actually represents the MPOD at 0.5°. We have tested this by correcting our data using their mean spatial profile characteristics as illustrated in *Figure 5b* above. Hammond *et al.* (1997) used a small (12′) target to obtain their spatial profiles and their data suggest that MPOD falls to 62% of its peak value at 0.5 eccentricity.

Importance of luminance

One factor which is important when measuring MPOD in older eyes is the luminance of the background. Flicker sensitivity is proportional to log luminance (up to approximately 1000 td) as described by the Ferry-Porter law. For HFP measurements at low luminance, pupil size as well as media aging effects may contribute to measurement errors, due for example to intrusion by rods and S-cones. As an example, at 44 cd m^{-2} the retinal illuminance will be reduced by approximately 4× for a 60-year-old eye compared with a young eye (Winn *et al.*, 1994). This compares with only a 2× reduction when the surround luminance is at 250 cd m^{-2}. This, added to the known reduction in flicker sensitivity with age, means it is important that the mean luminance of the background and the stimulus is sufficiently high to allow for these effects. Note that Snodderly *et al.* (2004) used relatively low background luminance, but they desensitized the rods and S-cones by using a blue (468 nm) background.

Data from clinical practice

We present MPOD data from 5581 individual eyes obtained using the new technique. The data set include year of birth (not date of birth) and gender. In this study, individuals under 20 and over 90 years old are not included. Their mean age is 52.37, median age is 53. The standard deviation is 13.02. The demography of this population is illustrated in *Figure 7a*.The means and standard deviations (overall 0.33 ± 0.17) of the data set are similar to previous findings. As discussed in Hammond *et al.* (2005), although the MPOD-age distribution is roughly normal in the general population, deviations from normality may easily be explained by unusual sample characteristics. For example Nolan *et al.* (2007) reported 0.306 ± 0.17 with 800 observers and Snodderly *et al.* (2004) 0.42 ± 0.22. As described above, these data are de-identified which means the details of the

individuals from whom the data were collected are not available. Nevertheless there are interesting trends which are worthy of mention. For example in the particular population reported in this study, there is a slight but significant increase in MPOD with age. However, generally there is unlikely to be a link between age and MPOD (Berendschot and van Norren, 2005) for individuals who are not supplementing their diet with L or Z. Observations drawn from more affluent communities can be expected to have a higher percentage of subjects with higher MPOD. Berendschot and van Norren (2005) showed that for a series of objective optical techniques there was no age effect in 134 observers with an age span of 18–70 years. They did notice a small decrease in MPOD with a flicker-based technique but considered this to be due largely to changes in the peripheral flicker setting at five degree eccentricity which may have caused an apparent reduction in MPOD in older observers. Delori *et al*. (2001) described between 15% and 17% higher MPOD in older observers (65–80 years) when testing with an autofluorescence and a reflectance method. In a smaller study, Beatty *et al* (2001) also found a slight reduction in MPOD with age and Nolan *et al*. (2007) have reported a more substantial reduction in MPOD in their older observers.

Notwithstanding these previous observations, we present in *Figure 7b* compelling evidence of higher levels of MPOD in older than younger observers. The most likely explanation for this is that the sample is biased by a particularly healthy group of elderly observers who may be supplementing their diet with either L and/or Z. Another interesting observation is that in the 20–30 age group, males have higher MPOD than females and this effect reaches statistical significance. The effect is not present as age increases however, and for the older age groups, females have higher MPOD than males. Again this almost certainly reflects the lifestyle of the subjects concerned. In the US it is widely thought that females are more susceptible to ARMD than males and these data would suggest that this message has reached older females who have been prompted to take L and Z supplements. A more detailed analysis of this large data set forms part of a separate study and for the most part the data are presented here mainly to substantiate the claim that the technique described can be easily incorporated into ophthalmic and optometric practice.

Conclusion

The new device is easy to operate. It provides fast, reliable and reproducible estimates of the MPOD. These are statistically repeatable, correlate well with an established objective method based on retinal reflectometry and reveal MP spatial profiles consistent with those previously reported. The new technique uses a novel strategy to obtain the minimum flicker point that is minimally demanding on both observer and operator and reduces the effects of flicker adaptation. Furthermore, it is small, portable and lightweight and can be readily operated by non-professional staff under office and/or clinical conditions. Therefore, the instrument is well suited to large-scale epidemiological studies of the MP.

Acknowledgements

This work was partly funded by ZeaVision L.L.C., St. Louis, MO, USA. We thank Tinsley Precision Instruments Limited, UK, for providing the Macular Pigment Screener. Jan van de Kraats and Dirk van Norren, from the University Medical Centre Utrecht, The Netherlands provided the Macular Pigment Reflectometer. Maria Makridaki is partly funded by Cognis GmbH and the UK MRC.

The instrument described in this paper is protected by US patent no 7390090 owned by Ian Murray and David Carden. A European patent is pending.

References

Algvere, P. V., Marshall, J. and Seregard, S. (2006) Age-related maculopathy and the impact of blue light hazard. *Acta Ophthalmol. Scand.* **84,** 4.

Anderson, A. J. and Vingrys, A. J. (2000) Interactions between flicker thresholds and luminance pedestals. *Vision Res.* **40,** 2579–2588.

Beatty, S., Koh, H., Phil, M., Henson, D. and Boulton, M. (2000) The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv.Ophthalmol.* **45,** 115.

Beatty, S., Murray, I. J., Henson, D. B., Carden, D., Koh, H. and Boulton, M. E. (2001) Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest. Ophthalmol. Vis. Sci.* **42,** 439–446.

Beatty, S., Nolan, J., Kavanagh, H. and O'Donovan, O. (2004) Macular pigment optical density and its relationship with serum and dietary levels of lutein and zeaxanthin. *Arch. Biochem. Biophys.* **430,** 70.

Berendschot, T. T. and van Norren, D. (2004) Objective determination of the macular pigment optical density using fundus reflectance spectroscopy. *Arch. Biochem. Biophys.* **430,** 149–155.

Berendschot, T. T. and van Norren, D. (2005) On the age dependency of the macular pigment optical density. *Exp. Eye Res.* **81,** 602–609.

Berendschot, T. T. and van Norren, D. (2006) Macular pigment shows ringlike structures. *Invest. Ophthalmol. Vis. Sci.* **47,** 709–714.

Berendschot, T. T., Goldbohm, R. A., Klopping, W. A., Van De Kraats, J., Van Norel, J. and Van Norren, D. (2000) Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest. Ophthalmol. Vis. Sci.* **41,** 3322–3326.

Berendschot, T. T., Willemse-Assink, J. J., Bastiaanse, M., De Jong, P. T. and Van Norren, D. (2002) Macular pigment and melanin in age-related maculopathy in a general population. *Invest. Ophthalmol. Vis. Sci.* **43,** 1928–1932.

Berendschot, T. T., Delint, P. J. and van Norren, D. (2003) Fundus reflectance – historical and present ideas. *Prog. Retin. Eye Res.* **22,** 171–200.

Bone, R. A. and Landrum, J. T. (2004) Heterochromatic flicker photometry. *Arch. Biochem. Biophys.* **430,** 137–142.

Bone, R. A., Landrum, J. T. and Gibert, J. C. (2004) Macular pigment and the edge hypothesis of flicker photometry. *Vision Res.* **44,** 3045–3051.

Ciulla, T. A. and Hammond, B. R. Jr. (2004) Macular pigment density and aging, assessed in the normal elderly and those with cataracts and age-related macular degeneration. *Am. J. Ophthalmol.* **138,** 582–587.

Ciulla, T. A., Curran-Celantano, J., Cooper, D. A., Hammond, B. R. Jr, Danis, R. P., Pratt, L. M., Riccardi, K. A. and Filloon, T. G. (2001) Macular pigment optical density in a midwestern sample. *Ophthalmology* **108,** 730–737.

Delori, F. C. (2004) Autofluorescence method to measure macular pigment optical densities fluorometry and autofluorescence imaging. *Arch. Biochem. Biophys.* **430,** 156–162.

Delori, F. C., Goger, D. G., Hammond, B. R., Snodderly, D. M. and Burns, S. A. (2001) Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. *J. Opt. Soc. Am.* **18,** 1212–1230.

Hammond, B. R. Jr, Johnson, E. J., Russell, R. M., Krinsky, N. I., Yeum, K. J., Edwards, R. B. and Snodderly, D. M. (1997) Dietary modification of human macular pigment density. *Invest. Ophthalmol. Vis. Sci.* **38,** 1795–1801.

Hammond, B. R. Jr, Wooten, B. R. and Smollon, B. (2005) Assessment of the validity of in vivo methods of measuring human macular pigment optical density. *Optom. Vis. Sci.* **82,** 387–404.

Khachik, F., Bernstein, P. S. and Garland, D. L. (1997) Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest. Ophthalmol. Vis. Sci.* **38,** 1802.

Kim, S. R., Nakanishi, K., Itagaki, Y. and Sparrow, J. R. (2006) Photooxidation of A2-PE, a photoreceptor outer segment fluorophore, and protection by lutein and zeaxanthin. *Exp. Eye Res.* **82,** 828–839.

van de Kraats, J., Berendschot, T. T. J. M. and van Norren, D. (1996) The pathways of light measured in fundus reflectometry. *Vision Res.* **36,** 2229.

van de Kraats, J., Berendschot, T. T. J. M., Valen, S. and van Norren, D. (2006) Fast assessment of the central macular pigment density with natural pupil using the macular pigment reflectometer. *J. Biomed. Opt.* **11,** 064031.

Landrum, J. T. and Bone, R. A. (2001) Lutein, zeaxanthin, and the macular pigment. *Arch. Biochem. Biophys.* **385,** 28.

Landrum, J. T., Bone, R. A. and Kilburn, M. D. (1997) The macular pigment: a possible role in protection from age-related macular degeneration. *Adv. Pharmacol.* **38,** 537.

Moeller, S. M., Parekh, N., Tinker, L., Ritenbaugh, C., Blodi, B., Wallace, R. B. and Mares, J. A. (2006) Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the carotenoids in age-related eye disease study (CAREDS): ancillary study of the women's health initiative. *Arch. Ophthalmol.* **124,** 1151–1162.

Nolan, J., O'Donovan, O., Kavanagh, H., Stack, J., Harrison, M., Muldoon, A., Mellerio, J. and Beatty, S. (2004) Macular pigment and percentage of body fat. *Invest. Ophthalmol. Vis. Sci.* **45,** 3940–3950.

Nolan, J. M., Stack, J., O' Donovan, O., Loane, E. and Beatty, S. (2007) Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp. Eye Res.* **84,** 61–74.

Nolan, J. M., Stringham, J. M., Beatty, S. and Snodderly, D. M. (2008) Spatial profile of macular pigment and its relationship to foveal architecture. *Invest. Ophthalmol. Vis. Sci.* **49,** 2134–2142.

Porciatti, V., Fiorentini, A., Morrone, M. C. and Burr, D. C. (1999) The effects of ageing on reaction times to motion onset. *Vision Res.* **39,** 2157–2164.

Richer, S., Stiles, W., Statkute, L., Pulido, J., Frankowski, J., Rudy, D., Pei, K., Tsipursky, M. and Nyland, J. (2004) Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry* **75,** 216–230.

Schalch, W., Cohn, W., Barker, F. M., Kopcke, W., Mellerio, J., Bird, A. C., Robson, A. G., Fitzke, F. W. and Van Kuijk, F. J. (2007) Xanthophyll accumulation in the human retina during supplementation with lutein or zeaxanthin - the LUXEA (LUtein Xanthophyll Eye Accumulation) study. *Arch. Biochem. Biophys.* **458,** 128–135.

Seddon, J. M., Ajani, U. A., Sperduto, R. D., Hiller, R., Blair, N., Burton, T. C., Farber, M. D., Gragoudas, E. S., Haller, J. and Miller, D. T. (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA* **272,** 1413.

Sharpe, L. T., Stockman, A., Knau, H. and Jagle, H. (1998) Macular pigment densities derived from central and peripheral spectral sensitivity differences. *Vision Res.* **38,** 3233–3239.

Sharpe, L. T., Stockman, A., Jagla, W. and Jagle, H. (2005) A luminous efficiency function, V*(lambda), for daylight adaptation. *J Vis* **5,** 948–968.

Snodderly, D. M., Mares, J. A., Wooten, B. R., Oxton, L., Gruber, M. and Ficek, T. (2004) Macular pigment measurement by heterochromatic flicker photometry in older subjects: the carotenoids and age-related eye disease study. *Invest. Ophthalmol. Vis. Sci.* **45,** 531–538.

Werner, J. S., Donnelly, S. K. and Kliegl, R. (1987) Aging and human macular pigment density. Appended with translations from the work of Max Schultze and Ewald Hering. *Vision Res.* **27,** 257–268.

Winn, B., Whitaker, D., Elliott, D. B. and Phillips, N. J. (1994) Factors affecting light-adapted pupil size in normal human subjects. *Invest. Ophthalmol. Vis. Sci.* **35,** 1132–1137.

Wu, J., Seregard, S. and Algvere, P. V. (2006) Photochemical Damage of the Retina. *Surv. Ophthalmol.* **51,** 461.

Wyszecki, G. and Stiles, W. S. (1982) *Color Science: Concepts and Methods, Quantitative Data and Formulae*, 2nd edn. John Willey & Sons, New York, NY.