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Macular pigment measurement in clinics: controlling the effect of the ageing media

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Abstract

We report a series of experiments designed to ensure that Macular Pigment Optical Density (MPOD) measurements obtained with a clinical instrument are not influenced by lens yellowing and ocular media optical density. These effects were determined in six subjects using seven Lee Colour Temperature Correcting filters to simulate changes in the transmittance of the ocular media with age. Calculated simulated age matched the data linking age and optical density reported in the literature, and the MPOD was independent of simulated age. The instrument allows an estimation of MPOD to be made which is based only on a foveal (centre-only) measurement rather than, as is conventional, making a comparison between foveal and peripheral measurements. We assessed the performance of this facility by comparing the centre-only estimate of MPOD with that obtained from both central and peripheral measurements in 5616 eyes. The 95% limits of agreement for the two estimates was 0.13 OD units.

Keywords: flicker, large sample, macular pigment, media yellowing, photometry

Introduction

Macular pigment (MP) is composed of the hydroxycarotenoids lutein (L) and zeaxanthin (Z). It is located in the fibres of Henle of the photoreceptors in the outer retina and is thought to be mainly confined to the central 12 degrees (Seddon *et al*., 1994). It has been claimed that the macular pigment (MP) protects against ageing processes in the eye. Epidemiologically it is accepted that low MP is a risk factor for macular disease as described by Beatty *et al*. (1999, 2000, 2001) and Hammond *et al*. (1998). There can be no disputing the purely biological benefits of L and Z: they act as a passive filter, attenuating damaging short-wavelength light and they have strong anti-oxidant properties (Reading and Weale, 1974; Beatty *et al*., 2000; Kvansakul *et al*., 2006). It is difficult to establish whether these biological properties translate into real clinical benefits. Some reports find no link between MP and macular disease (Berendschot *et al*., 2002; Kanis *et al*., 2006), whilst other do (Seddon *et al*., 1994; Snodderly, 1995; Beatty *et al*., 2001; Richer *et al*., 2004; Delcourt *et al*., 2006; Nolan *et al*., 2007).

Approximately 30% of those over 70 years in the developed countries have early stage macular disease and recent statistics indicate that this is increasing, due mainly to demographical factors, but it is thought certain features of life-style in developed countries also play a part (Evans, 2001; Klein *et al*., 2004; Hogg and Chakravarthy, 2006). The therapeutic options for AMD are limited, but improving. Recently there has been substantial progress in the management of the wet form of the disease using intravitreal injections of anti-VEGF medications, (Avery *et al*., 2006). However, there is at present no systematic approach to the management of the more common dry or atrophic form of the condition. The main reason for this is the lack of understanding of its aetiology. Statistically, the biggest risk factor is age, but other factors, such as heritance, smoking, general lifestyle, diet and obesity have also been identified, see ((Ambati *et al*., 2003) for a review).

In the absence of management strategies for atrophic macular disease, interest has been focused on prevention by controlling the known environmental factors. Patients who are at risk, perhaps for familial reasons, are therefore advised to maintain a healthy lifestyle. Among the factors which can be manipulated is the level of retinal carotenoids (macular pigment; MP) in the eye.

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There are many studies describing how MP may be enhanced, either by following a diet rich in one of the foods which are high in lutein (e.g. spinach) or by supplementing the diet with capsules containing either lutein or zeaxanthin or both (Hammond *et al*., 1997; Landrum *et al*., 1997; Koh *et al*., 2004). Note that there are some reports of subjects not responding to diet supplementation. Many of these observations are based on flicker-based techniques and it has been suggested this method systematically underestimates MPOD compared with reflectometry (van der Veen *et al*., 2009). Another possible reason for so called non-responders is that supplementation may result in a spreading of the spatial distribution of MP and any enhancement may then be missed unless a spatial profile is obtained.

The putative link between MP and macular disease, and the fact that many people are supplementing their diet in order to increase MP, have heightened awareness of macular disease in the population. This in turn has meant that there is a demand for a clinical technique for measuring MP. There is also a strong case for conducting large-scale studies of the distribution of MP in the population in order to advance our understanding of its link with macular disease and its possible beneficial effects. Conducting large-scale studies of MP and obtaining measurements under clinical conditions is difficult with traditional techniques, which are largely designed for use in the laboratory. There are however some methods developed specifically for measuring large populations (Mellerio *et al*., 2002; van der Veen *et al*., 2009) and guidelines have been published for how such studies should be conducted (Hammond *et al*., 2005).

As with all flicker-based procedures, the calculation of MP is based on the luminance ratio of a blue light presented in the peripheral retina to that presented in the central retina. Therefore it might be thought that ageing of the ocular media should not affect the measurements, because any changes in the crystalline lens are assumed to be common to both the central and peripheral measurements. However, it is well known that with advancing age, the changes in the ocular media, particularly across the crystalline lens, are not necessarily uniform. Also, the lens undergoes a two-stage increase in optical density throughout life and, as might be expected, individual variation in lens inhomogeneities increase with age. At 400 nm, OD increases slowly (0.12 density units per decade) up to age 60, but much more rapidly (0.4 density units per decade) beyond 60 (Pokorny *et al*., 1987).

Although the likelihood of media changes affecting MPOD is low, the instrument described here [and in more detail by van der Veen *et al*. (2009)] was developed specifically to measure MPOD in large numbers of elderly subjects. It is therefore important to confirm empirically that media yellowing and other factors, such as reduced retinal illumination associated with the aging/cataractous eye, do not affect the value of MPOD obtained. Hence the main aims of the experiments reported here are as follows: first to show the effects of simulated ocular lens yellowing and optical density changes; second, to test observers of different ages having the same levels of MPOD; and third, to establish the validity of a unique technique of obtaining estimates of MP based only on the central measurement.

Materials and methods

MPOD measurement

A desktop device designed for assessing MPOD in large populations was used (van der Veen *et al*., 2009). The instrument is called M|POD (Macular Pigment Optical Densitometer) in the UK and used in the US under the name QuantifEye. The manufacture's name for the device is the MPS 9000 (Tinsley Ophthalmic Instruments, Redhill, Surrey). The device uses the principles of Heterochromatic Flicker Photometry (HFP) and is described in detail in van der Veen *et al*. (2009). The traditional HFP method for determining MPOD presents a target of two superimposed lights, one of which is blue and is absorbed by the MP, the other green and absorbed negligibly by the MP. The lights are flickered at a pre-set rate, around 12–15 Hz, and the observer is asked to modify the relative intensity of two lights (the green-blue luminance ratio) until the flicker is minimised, thus identifying the equal luminance point for the two lights. The setting is made for central and peripheral viewing. In the presence of MP, which selectively absorbs blue light, the luminance ratio is different for the two settings. The MPOD is then the log ratio of the perceived intensity of blue light in the centre, to that for the periphery.

The MPS 9000 uses the same principle, but a different method for obtaining the equal luminance point. The subject fixates a 1 degree central target composed of two superimposed flickering LEDs (465 nm peak wavelength, 25 nm bandwidth and 530 nm peak wavelength 30 nm bandwidth, luminance up to 200 cd m^{-2} for both LEDs) on a white light pedestal (luminance up to 350 cd m^{-2}). The flicker rate is initially set to 60 Hz which is above the critical flicker fusion rate for these stimulus conditions and therefore is perceived as static. It is then gradually reduced at a rate of 6 Hz s^{-1}. The subject is required to press a response button as soon as they detect flicker. This process is repeated for a series of green-blue luminance ratios. A V-shaped curve is obtained when temporal frequency is plotted against log green-blue ratio. The minimum of the curve indicates the equal luminance point of the blue and the green lights. The observer then fixates a 2° diameter

red target placed 8° eccentrically and a second set of data are recorded for peripheral viewing. A second V-shaped curve is recorded. It is assumed that the sensitivity of the L- and M- photoreceptors, responsible for flicker detection, in the two wavelengths remains the same for foveal and extra-foveal retinal locations (Bone and Landrum, 2004). MPOD is calculated by subtracting the *x*-axis values of the two minima points of the curves. In order to compensate for the overlap of the spectra of the two LEDs and the slight overlapping of the green LED spectrum with that of the MP, a correction factor of 1.2 is applied. More details on this calculation and a detailed description of the *x*-axis are available in van der Veen *et al*. (2009). Briefly, the *x*-axis is a measure, in dBs, of the attenuation of the luminance of the green LED. In order to maintain equal luminance of the target across all intensity ratios, the intensities of the two LEDs are yoked so that as one is increased the other is equally decreased.

The 1 degree central target is surrounded by a 30° white area (colour temperature 5500 K, $x = 0.33$, $y = 0.34$). The luminance of the background is set to 250 cd m^{-2} in order to maintain adaptation in the photopic range, thus ensuring the validity of the principles of flicker photometry in cases where media opacities cause reduction in retinal illumination (this point is amplified in the Discussion). Exemplar data for a 25-year-old subject (MM) and an 82-year-old subject (TG) are illustrated in *Figure 1*. These subjects have the same level of MP. Filled symbols represent central viewing and open symbols peripheral viewing. The arrows indicate the minima obtained for TG. The graph demonstrates the effect of the media yellowing on the data, in that the V-shaped curves for the older subject are shifted rightward along the *x*-axis. Any effect of retinal sensitivity changes will be common to both curves. Note however that the difference between the central and peripheral minima is the same in both subjects. The rightward shift along the *x*-axis indicates that more blue light is absorbed by the media of the older subject for both central and peripheral measurements.

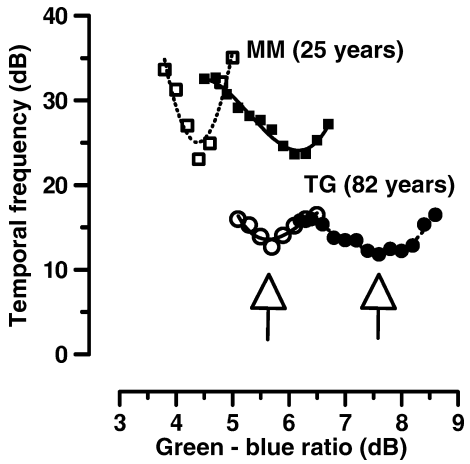

Figure 1. MPOD data from a 25 year old (MM, squares) subject and a 82 year old subject (TG, circles). The filled symbols represent central viewing and the open symbols peripheral viewing. Arrow heads indicate minima for subject TG. Both subjects have an MPOD of 0.41.

Subjects

For the age-simulating experiments, six young healthy subjects were recruited. They were selected to be in the same age group (age range 24–27 year, three males and three females). In all cases visual acuity was 6/5 or better. Only right eyes were tested. Data were also collected from nine healthy individuals of different ages recruited through advertisements in the University of Manchester. Their age range varied from 11 to 68 years of age. The participants in both experiments had clear media and no ocular pathology according to an examination by a qualified person. The study was approved by the South Manchester Research Ethics Committee.

Further MPOD data are presented from a survey conducted in the US, of MP measurements made as part of routine eye testing procedures. The data were collected in 48 optometric practices using the MPS 9000 between June 2006 and December 2007. The age range of these individuals was 20–90. In accordance with the Health Insurance Portability and Accountability Act (HIPAA) these data had been de-identified so that only their gender and year of birth are known. HIPAA was introduced in 1996 to protect the privacy of medical information but at the same time allow the release of information that can be regarded as beneficial for research purposes. Since no information on the ocular pathology of the subjects was available, the MPOD data were filtered prior to the analysis. Data with unusually high or poor pre-test flicker sensitivity were removed, as well as data from subjects with peripheral minima suggesting a very much younger eye than was possible from their known age (e.g. an 80 year old with a media-aged eye of a 20 year old).

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.

Filters for simulating the aging media

Media changes for different ages were simulated by viewing the flickering targets through seven colour temperature (CT) modifying filters (Lee Filter numbers; 204, 285, 205, 206 223, 218, 203; Andover, Hampshire, UK). No account was taken of retinal sensitivity loss

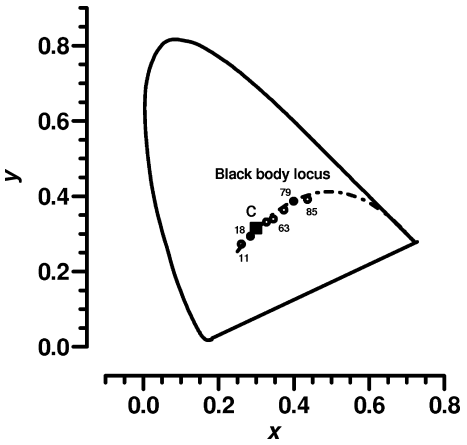

Figure 2. The locations in CIE 1931 colour space of the filters used to simulate ageing. The square indicates the location of illuminant C. Numbers indicate simulated age.

due to ageing. In total, eight MPOD measurements were obtained for every subject: one without a filter, five with orange CT filters which were selectively absorbing blue, reducing colour temperature and simulating elderly eyes, and two blue filters selectively absorbing red, increasing colour temperature, and thus simulating younger eyes. They follow the Planckian locus as described in Werner and Schefrin (1993) and illustrated in the 1931 CIE chromaticity diagram in *Figure 2*. Their absorption spectra are similar to the changes in the lens optical density spectrum with age (Pokorny *et al*., 1987). A similar approach was used in Hogg *et al.* (2007). The age values for each transmittance were calculated by adding transmittance at 465 and 530 nm for the filters to those of a normal 27 year old observer obtained from Pokorny *et al.* (1987). The equivalent age values for each filter were then obtained from Pokorny *et al.* (1987). Optical densities at 465 nm for each calculated age were as follows: 0.154 at 11 years, 0.195 at 18 years, 0.240 at 27 years, 0.371 at 51 years, 0.462 at 63 years, 0.541 at 67 years, 0.763 at 79 years, 0.860 at 85 years.

Results

Simulated ocular media density data collected from six subjects are presented in *Figure 3*. The filled symbols are the minima obtained for central viewing and the empty symbols are the minima for peripheral viewing (note that examples of minima are indicated by arrows for one of the observers in *Figure 1*). The horizontal axis is simulated age and the vertical axis is the minimum green-blue ratio in dBs. Note that this ordinate axis has a log scale and as green-blue ratio is increased the blue component is increasing. It is for this reason that it is referred to as green-blue ratio. The scale is attenuation of green in dBs. Each panel illustrates data from an

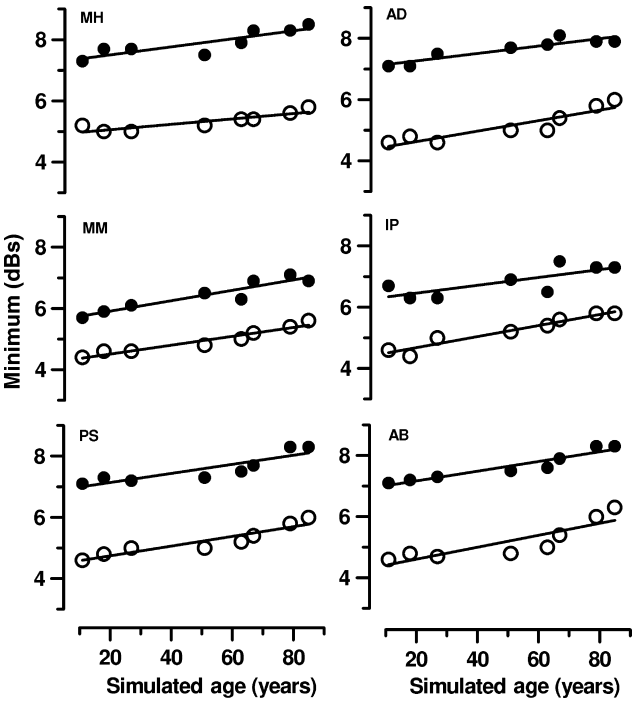

Figure 3. Simulated aging effects. Minima obtained for central (closed circles) and peripheral (open circles) viewing for six subjects and for each measurement.

individual subject. MPOD is calculated by subtracting the minimum green-blue ratio for central viewing from that obtained for peripheral viewing. The solid lines are linear regressions, with r^2 values varying between 0.68 and 0.99. The fact that for all subjects the two lines are approximately parallel indicates that MP measurements remain stable during the simulation experiments. Minor differences in slope may be present but are not significant and the small variations in the MP levels observed during the experiments are within expected experimental error for the instrument (van der Veen *et al*., 2009).

Note that, as expected, green-blue ratio increases with simulated age, indicating that both minima are shifting to the right (towards increasing levels of blue luminance) in *Figure 1*. This agrees with the idea that media yellowing should affect the central and peripheral minimum equally and thus not influence the estimation of MPOD.

To test this idea further, MPOD measurements were conducted with nine subjects who had the same level of MP (MPOD = 0.31) and whose age ranged from 11 to 65 years. The data are presented in *Figure 4*. Again, approximately parallel lines are obtained when regression lines are drawn through the data (solid lines). r^2 values are indicated in the figure.

MPOD based on central flicker setting only

Observers with abnormalities such as cataract and macular degeneration may encounter difficulty setting

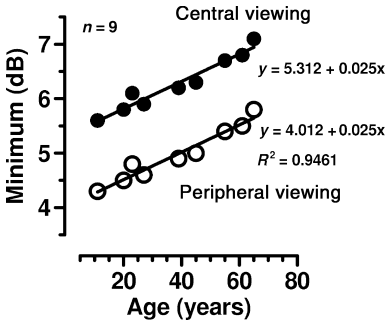

Figure 4. Minima obtained for central and peripheral viewing for subjects of different ages but having the same MPOD. Note both functions have the same regression coefficient.

flicker detection thresholds when viewing peripherally. The instrument used in these experiments incorporates a facility for estimating the position of the minimum for the peripheral setting from the extent of ocular media yellowing which can be derived from the patient's age.

The data for this estimate of MPOD and how the estimate is related to the conventional centre-periphery measurement are illustrated in *Figure 5* for 5616 eyes. The overall mean of the MPOD based on central and peripheral measurements is 0.34 with a standard deviation of ± 0.17. It is clear from the correlation analysis that, although there is a wide spread, the MPOD can be estimated reasonably well, suggesting that 84% of the variance in the estimated MP can be accounted for in terms of actual MP i.e. $r^2 = 0.84$. Despite the high correlation between the measurements, it should be recognised that some error may occur in individuals, depending on whether their crystalline lenses have aged as predicted by the chronological age. The best estimate of this error is calculated as 1.96 × the standard deviation of the differences between measurements, referred to as the 95% limits of agreement (Bland and Altman, 1999). For these data this is ±0.13 OD units. Note that this assumes the distribution of differences is normal and this assumption is valid for these data.

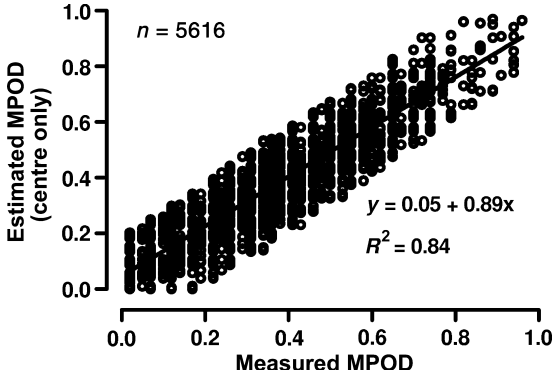

Figure 5. Actual vs age-based estimate of MPOD for 5616 eyes. The vertical axis is the estimate of the MPOD obtained from a central measurement only which is derived from the extent of lens yellowing according to the literature.

There are some important points to note here. The regression line does not intercept the *y*-axis at zero, showing that, for observers with low MP, the instrument slightly over-estimates MP when only central measurements are obtained. For those with higher levels of MP, the true MP is more likely to be a little lower than indicated by the central-only measurement. For example, when the centre and periphery measurement is 0.2, the estimate from the centre only will be 0.228 and when the centre and periphery measurement is 0.75, the central-only estimate is 0.72.

Discussion

It seems obvious that flicker-based MPOD measurements should not be affected by media changes; the technique relies on a comparison between central and peripheral viewing and any media yellowing is common to both. However the method described here was designed specifically to determine MPOD in older eyes and it was thought important to establish empirically that the measurements are immune to the shifts in wavelength-selective absorption and reduction in overall luminance that are commonplace in the older eye. As outlined in van de Kraats and van Norren (2007), other less well documented factors such as light scatter (Zlatkova *et al.*, 2006) and corneal abnormalities also influence the image-forming properties of the aging media. In order to specifically study the effects of media yellowing with age on the MPOD measurements we have used filters to simulate the effects of lens yellowing and tested observers of different ages but who have the same MPOD. In all cases we find the effects of the aging media to be negligible when compared with the noise inherent in the measurements.

The instrument has a facility to estimate MPOD from only a central measurement by correcting for the well-documented increased absorption in the blue region of the visible spectrum with age. We have evaluated this by comparing the MPOD obtained from central-only measurements with that obtained for both central and peripheral viewing for 5616 eyes. There is a close association between the two measurements, indicating that to a first approximation, the age-correcting equation used in the instrument is valid. The central-only facility is further discussed below.

As a result of media absorption properties and senile miosis, retinal illumination in the elderly eye is severely reduced. This is taken into account in the choice of background luminance of 250 cd m^{-2} in the instrument described here. According to the Ferry-Porter law, critical flicker frequency is proportional to perceived log luminance but this relationship holds only for

photopic conditions when rods do not mediate the response. Using a high luminance is therefore essential when testing the elderly eye. It ensures that rod intrusion does not affect the flicker settings despite age-related media and pupil-size changes.

The effect of the media yellowing in MP measurements was tested using filters having spectral absorptions similar to those of crystalline lenses of different ages. Effectively this procedure simply validates the choice of LED peak wavelength. Central and peripheral measurements should show a linear shift of the green-blue isoluminant point along the *x*-axis in *Figure 1* and this is what is seen in *Figure 3*. Note that the noise in these measurements is quite low for the young observers and so allows us to assess the validity of the instrument without the confounding effects of increase in noise and repeatability which is expected for older observers. On the basis of this experiment with simulating filters, it can be claimed that the physical characteristics of the instrument do not introduce age-related artifacts.

The next step in the evaluation procedure was to test *bona fide* observers of different ages who have the same MP value. It is apparent, as expected, that the central and peripheral minima are shifted equally along the *x*-axis in *Figure 1*. That is, the central and peripheral minima data points should be parallel to each other and linear with age as predicted by the well-known characteristics of the aging media (Pokorny *et al*., 1987). Age-related or media-associated artifacts would be manifest as a loss of linearity of these data. Note that the absolute MP values obtained with this instrument (in this case all observers had a value of 0.31) correlate highly with those of a spectral reflection technique (van der Veen *et al*., 2009). This ensures that the calculations of MP against which the central and peripheral data are tested, are valid.

Apart from the temporal frequency ramping, a further novelty of the MPS 9000 is the age-estimate technique. There are occasional cases where older observers are unable to perform the peripheral flicker setting or the test has to be repeated. This may be due to reduced flicker sensitivity in the periphery, the Troxler Effect, or fragmented vision loss associated with ocular disease such as macular degeneration. By using data from the literature (Pokorny *et al*., 1987; van de Kraats and van Norren, 2007) the peripheral green-blue setting can be estimated according to the age of the observer. This ignores any neural loss of green-blue sensitivity (but see below). The validity of the technique was tested with a large number of subjects: the measured and the estimated MP levels showed a highly significant correlation ($r^2 = 0.84$). Although there is a close association between the estimate and the measurement, the data must be used with caution, as discussed below. The facility is particularly valuable in cases where there are persistent problems making the peripheral setting. Note that as described in the Results, the regression line has a non-zero y-intercept, suggesting that when only the central measurement is taken, low MP is over-estimated and higher MP is under-estimated (see above for examples of this).

It is important to emphasise that the central-only facility should be used only when subjects encounter difficulty obtaining a peripheral setting despite frequent attempts. The peripheral measurement is important. Apart from providing a reference point from which to estimate the MPOD, it effectively minimises the effect of any criterion bias in the subjects' flicker settings; that is if they are conservative for central viewing they will be conservative for peripheral viewing and so on. Of course, if the main concern is whether or not the central measurement has changed, as in the case of patients wishing to enhance their MP, an accurate reference point giving an accurate MPOD is less important. In these cases using the age-based peripheral measurement is as good as any other reference point and the *change* in MPOD will be an appropriate indication of the effect of any enhancement regime.

Finally, previous flicker-based techniques assume that flicker sensitivities in the centre and periphery are the same. The adverse effects of this assumption can be avoided by using different temporal frequencies for the two viewing conditions as described in Hammond *et al*. (2005). The older observer offers a particular challenge in this respect, in that it is likely that flicker sensitivity differences between centre and periphery are particularly common in these cases. The modified HFP technique used in the MPS 9000 overcomes the problem in two ways. First, the device performs a preliminary flicker-sensitivity test (based on the mean of five settings) and sets the starting luminance contrast settings accordingly. Second, because the flicker rate is gradually reduced from above CFF until flicker is detected, the observer automatically selects the correct temporal frequency for his or her green-blue isoluminant point. When sensitivity is low, CFF at isoluminance will be low and the V-shaped curve will simply shift downwards and when it is high the curve is shifted upwards. Thus any effects due to differences in flicker sensitivity between central and peripheral viewing are minimized because the calculation of MPOD is based on the difference along the *x*-axis between the minima of the two curves.

Acknowledgements

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References

Ambati, J., Ambati, B. K., Yoo, S. H., Ianchulev, S. and Adamis, A. P. (2003) Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies. *Surv. Ophthalmol.* **48,** 257–293.

Avery, R. L., Pieramici, D. J., Rabena, M. D., Castellarin, A. A., Nasir, M. A. and Giust, M. J. (2006) Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. *Ophthalmology* **113,** 363–372.

Beatty, S., Boulton, M., Henson, D., Koh, H. H. and Murray, I. J. (1999) Macular pigment and age related macular degeneration. *Br. J. Ophthalmol.* **83,** 867–877.

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,** 115–134.

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.

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.

Bland, J. M. and Altman, D. G. (1999) Measuring agreement in method comparison studies. *Stat. Methods Med. Res.* **8,** 135–160.

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

Delcourt, C., Carriere, I., Delage, M., Barberger-Gateau, P. and Schalch, W. (2006) Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract: the POLA Study. *Invest. Ophthalmol. Vis. Sci.* **47,** 2329–2335.

Evans, J. R. (2001) Risk factors for age-related macular degeneration. *Prog. Retin. Eye Res.* **20,** 227–253.

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 Snodderly, D. M. (1998) Preservation of visual sensitivity of older subjects: association with macular pigment density. *Invest. Ophthalmol. Vis. Sci.* **39,** 397–406.

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.

Hogg, R. E. and Chakravarthy, U. (2006) Visual function and dysfunction in early and late age-related maculopathy. *Prog. Retin. Eye Res.* **25,** 249–276.

Hogg, R. E., Zlatkova, M. B., Chakravarthy, U. and Anderson, R. S. (2007) Investigation of the effect of simulated lens yellowing, transparency loss and refractive error on in vivo resonance Raman spectroscopy. *Ophthalmic Physiol. Opt.* **27,** 225–231.

Kanis, M. J., Berendschot, T. T. and van Norren, D. (2007) Influence of macular pigment and melanin on incident early AMD in a white population. *Graefes Arch. Clin. Exp. Ophthalmol.* **245,** 767–773.

Klein, R., Peto, T., Bird, A. and Vannewkirk, M. R. (2004) The epidemiology of age-related macular degeneration. *Am. J. Ophthalmol.* **137,** 486–495.

Koh, H. H., Murray, I. J., Nolan, D., Carden, D., Feather, J. and Beatty, S. (2004) Plasma and macular responses to lutein supplement in subjects with and without age-related maculopathy: a pilot study. *Exp. Eye Res.* **79,** 21–27.

Kvansakul, J., Rodriguez-Carmona, M., Edgar, D. F., Barker, F. M., Köpcke, W., Schalch, W. and Barbur, J. L. (2006) Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance. *Ophthalmic Physiol. Opt.* **26,** 362–371.

Landrum, J. T., Bone, R. A., Joa, H., Kilburn, M. D., Moore, L. L. and Sprague, K. E. (1997) A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp. Eye Res.* **65,** 57–62.

Mellerio, J., Ahmadi-Lari, S., Van Kuijk, F., Pauleikhoff, D., Bird, A. and Marshall, J. (2002) A portable instrument for measuring macular pigment with central fixation. *Curr. Eye Res.* **25,** 37–47.

Nolan, J. M., Stack, J., O, O. D., 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.

Pokorny, J., Smith, V. C. and Lutze, M. (1987) Aging of the human lens. *Appl. Opt.* **26,** 1437–1440.

Reading, V. M. and Weale, R. A. (1974) Macular pigment and chromatic aberration. *J. Opt. Soc. Am.* **64,** 231–234.

Richer, S., Stiles, W., Statkute, L., Pulido, J., Frankowski, J., Rudy, D., Pei, K., Tsirpursky, 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 (St. Louis, MO)* **75,** 216–230.

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,** 1413–1420.

Snodderly, D. M. (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am. J. Clin. Nutr.* **62,** 1448S–1461S.

van de Kraats, J. and van Norren, D. (2007) Optical density of the aging human ocular media in the visible and the UV. *J. Opt. Soc. Am.* **24,** 1842–1857.

van der Veen, R. L. P., Berendschot, T. T., Hendrikse, F., Carden, D., Makridaki, M. and Murray, I. J. (2009) A new desktop instrument for measuring Macular Pigment Optical Density based on a novel technique for setting flicker thresholds. *Ophthalmic Physiol. Opt.* **29,** 127–137.

Werner, J. S. and Schefrin, B. E. (1993) Loci of achromatic points throughout the life span. *J. Opt. Soc. Am. A* **10,** 1509–1516.

Zlatkova, M. B., Coulter, E. E. and Anderson, R. S. (2006) The effect of simulated lens yellowing and opacification on blue-on-yellow acuity and contrast sensitivity. *Vision Res.* **46,** 2432–2442.