Apparent motion photometry: evaluation and reliability of a novel method for the measurement of

macular pigment

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Keywords: age-related macular degeneration; apparent motion photometry; macular pigment; macular

pigment optical density.

Word count: 2633

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ABSTRACT

Background/aims

Macular pigment (MP) is thought to protect the macula against exposure to light and oxidative stress, both of which may play a role in the development of age-related macular degeneration. Our aim was to clinically evaluate a novel cathode ray tube (CRT) based method for measurement of macular pigment optical density (MPOD) known as apparent motion photometry (AMP).

Methods

We took repeat readings of MPOD centrally (zero degrees) and at three degrees eccentricity for 76 healthy subjects (mean (\pm SD) 26.5 \pm 13.2 years, range 18 to 74 years.

Results

The overall mean MPOD for the cohort was 0.50 ± 0.24 at zero degrees, and 0.28 ± 0.20 at three degrees eccentricity; these values were significantly different (t = -8.905, p < 0.001). The coefficients of repeatability were 0.60 and 0.48 for the zero and three degree measurements respectively.

Conclusions

Our data suggest that when the same operator is taking repeated zero-degree AMP MPOD readings over time, only changes of more than 0.60 units can be classed as clinically significant. In other words, AMP is not suitable for monitoring changes in MPOD over time, as increases of this magnitude would not be expected even in response to dietary modification or nutritional supplementation.

INTRODUCTION

Macular pigment (MP) is thought to protect the most important area of the retina against life-long exposure to light and oxidative stress [1] both of which may play a role in the development of age-related macular degeneration (AMD).[2] the most important cause of low vision in the developed world.[3] Although there are several lines of treatment that offer the possibility of amelioration [4] the condition is currently incurable. It has been suggested that MP acts as an antioxidant and screener of high-energy blue light.[5] Macular pigment may prevent light-initiated oxidative damage to the retina and therefore protect against subsequent age-related macular disease.[6] Therefore, it is important to develop methods for MP measurement in order to be able to offer advice on increasing MP levels.

Heterochromic flicker photometry (HFP), is a psychophysical technique that has proved popular,[7] and allows a subject to match the luminance of two differently coloured lights in a test field that flickers between the two by adjusting the luminance of one. When the two lights are equiluminant the flicker is greatly reduced or absent. To measure MPOD, the subject makes a heterochromatic match between a light absorbed by the MP, i.e. blue light, and a light not so absorbed, such as green. The match is made in the fovea where MP levels are high and again at a parafoveal location where it is assumed there is no MP. Comparing the two matches allows the MPOD to be calculated. Unfortunately, HFP has disadvantages such as the fact that it is a conceptually difficult task for subjects, and there is no assessment of fixation, and these have been discussed in detail elsewhere.[8-10]This means that an alternative approach is worth investigating. The aim of this study was to clinically evaluate a novel cathode ray tube (CRT) based method for measurement of MPOD, which is commercially available as part of the Metropsis psychophysical vision test suite (Cambridge Research Systems Ltd., 80 Riverside Estate, Sir Thomas Longley Road, Rochester, Kent ME2 4BH England).

The use of a CRT display driven by sophisticated software offers a way to overcome many of the problems of HFP. The spectral power distribution of the blue phosphor of a CRT matches the absorption spectrum of the MP such that light that is absorbed by MP is generated. The long wavelength light that

comes from the red phosphor can be used as the non-absorbed light. Anstis and Cavanagh described an apparent motion technique whereby four square wave gratings, each 90 degree phase advanced from the previous grating, are presented sequentially.[11] The first grating is a chromatic grating composed of alternate red and blue bars. The luminance of the blue is fixed whilst the red luminance can be varied. The second grating is a purely luminance modulated achromatic grating, modulated around the mean luminance of the blue/red chromatic grating. The sequence of gratings appears to move in one direction or the other, the direction being solely dependent upon the relative subjective luminance of the two components in the chromatic gratings. In this case, when the red luminance is greater the grating appears to drift upwards and when the blue luminance is greater the grating appears to shift downwards. At subjective equiluminance the stimulus appears to pulsate or flicker with no apparent motion, or with some parts moving one way and others moving in the opposite direction. The advantage of this stimulus is that judging the direction of apparent motion is conceptually easy for most naive subjects. We will refer to this method as apparent motion photometry (AMP).

We are currently not aware of any other study that has investigated the repeatability of AMP. Repeatability is the ability to duplicate measurements obtained by a measuring technique on an intersession basis.[12] Our aim was to determine the inter-session measurement variability, or measurement noise, of AMP in order to be able to identify what a clinically significant change in MPOD would be when using this technique. This is of particular interest since AMP might be used to monitor the effect of nutritional supplementation or dietary modification on MPOD over time.

MATERIALS AND METHODS

Subjects

Seventy-six healthy subjects were recruited from the staff, students and patients of Aston University

Optometry department and public service eye clinics. Ethical approval was obtained from the University's

Office of Human Research. Written consent was obtained after each participant had been fully informed

of the nature of the study according to the code of ethics in the Declaration of Helsinki protocol. None had

a history of ocular disease, amblyopia or ocular surgery. All had a visual acuity of at least 0.10 log MAR (approximately 6/7.5 in Snellen notation) in each eye with habitual refractive correction where necessary.

Materials

Stimuli were presented on a calibrated Mitsubishi Diamond Pro 2070SB monitor, viewed at 80 cm, driven by a Metropsis Precision Vision Testing System (Cambridge Research Systems Ltd, Rochester, UK) which has 14 bit luminance video resolution and precision temporal control, guaranteeing stimulus veracity. Each subject's gaze direction was continually measured with a video eye tracker (Cambridge Research Systems Ltd, Rochester, UK).

Protocol

Stimuli were presented on the monitor and viewed at 80 cm. One reference field, and two test fields were used to obtain MPOD readings at zero and three degrees eccentricity from the fovea. The seven degree reference field, assumed to be outside the extent of MP, was taken as baseline reference for MPOD calculation. The zero and three-degree test fields were compared with this reference to obtain an MPOD value at each of those locations. The stimuli presented at three and seven degrees eccentricity were composed of a radial grating in a 90 degree arc concentric to a white fixation cross and with the width and spatial frequency scaled to reduce with reducing eccentricity. The stimulus presented at zero eccentricity (central MPOD) and was composed of horizontal gratings forming a vertical rectangle 0.3 by 1.2 degrees. The frequency of presentation of the grating sequence was optimised for each subject to maximise the perception of motion. Using a rotary control, each subject was able to vary the presentation rate so as to achieve the strongest motion perception to a typical eccentric stimulus with the luminances of the red and blue components of the chromatics gratings markedly different. In order to saturate the S cones, stimuli were presented on a full field uniform background using only the field blue phosphor at a luminance of 12 cd/m², typically around 300 Trolands. The luminance of the blue component of the chromatic gratings was fixed at 10 cd/m². For each new test field position the red component initially had a luminance of 24 cd/m² and was varied by a 2AFC weighted up/down staircase algorithm controlled by the subject pushing a button on a response box to indicate if the movement was up or down. Computer software automatically

calculated the MPOD using the following equation: MPOD = log (*EfB/EfR*) – log (*EpB/EpR*) where, at subjective equiluminance, *EfB* is the luminance of the blue chromatic grating component at an eccentricity of *f* degrees within the macula, *EfR* the luminance of the red chromatic grating component at an eccentricity of *f* degrees within the macula, *EpB* the luminance of the blue chromatic grating component at an eccentricity of 7 degrees and *EpR* the luminance of the red chromatic grating component at an eccentricity of 7 degrees both outside the macula.

When the red luminance was at or near that of the blue, motion ceased or became anomalous. Subjects were instructed to always press the down button unless they were sure the motion was upward. Because the final MPOD value was calculated as a ratio of the luminance values recorded for the foveal and parafoveal locations, asking subjects to adopt this method did not affect the overall result. To obtain convincing apparent motion the mean luminance and contrast of the achromatic grating mimics that of the chromatic grating.

In order to reduce adaptation effects each test field was presented for only 500 msec. Correct fixation is vital if valid MPOD profiles are to be obtained; the subject's head was partly restrained by a chin and head rest and the subject's gaze direction was continually measured with the video eye-tracker. This was used to inhibit stimulus presentation until fixation was correct. Fixation had to be within ±0.5 degrees of the fixation point. If the subject broke fixation during stimulus presentation the stimulus was extinguished and the presentation repeated once correct fixation was re-established. Initially it was found that some subjects could not see coherent motion in any test field and thus could not make measurements of their MPOD. We ascertained that younger subjects required higher stimulus presentation frequencies than older, and the software was changed to allow subjects to optimise as described above all aspects of grating presentation before a series of measurements was commenced. Generally, subjects under 40 years found sequence presentation frequencies in the range of 15 to 20 Hz satisfactory whilst subjects older than 40 years chose lower frequencies but the frequency chosen was not very critical. Once the grating parameters had been optimised, subjects found the procedure easy and most naive subjects could complete a MP profile with readings at each test field in less than 10 minutes. Data were collected

by one operator (HB) in two sessions separated by one week. Prior to the first session a trial run was completed, to allow each participant to practice the test. Practice run data were not included in the analysis. The study design permitted assessment of the test repeatability (HB1 versus HB2).

Data analysis and display

Microsoft Windows XP and SPSS software was used for data analysis. Graphs were produced using Sigma Plot software (version 6) for Microsoft Windows XP.

RESULTS

We measured MPOD for 76 healthy subjects (mean (\pm SD) 26.5 \pm 13.2 years, range 18 to 74 years). Our sample consisted of 26 males and 50 females. The overall mean central MPOD for the cohort was 0.50 \pm 0.24, calculated by averaging the mean of the two central readings for each subject. The overall mean MPOD at three degrees eccentricity was calculated in the same way, and was 0.28 \pm 0.20. The zero and three degree MPOD values were significantly different (t = -8.905, p < 0.001). There was no correlation between AMP central MPOD readings and age (r = -0.051, p = 0.665) or between three-degree MPOD readings and age (r = 0.105, p = 0.367). The mean central MPOD reading for females (n = 50) was 0.53 \pm 0.25 and for males (n = 26) was 0.44 \pm 0.21; there was no significant gender difference (t = 0.-1.719, p = 0.090). The mean three degree MPOD reading for females was 0.29 \pm 0.21 and for males was 0.27 \pm 0.18, and again, there was no significant gender difference (t = -0.327, t = 0.745). There was significant positive correlation between the first and second readings for zero degree (t = 0.419, t = 0.001) and three-degree (t = 0.487, t = 0.001) data sets.

Analysis of inter-session repeatability involved calculating the difference in the MPOD for each subject obtained by one observer between two test sessions. The degree of inter-session repeatability is the range over which 95% of the differences lie i.e. the 95% limits of repeatability are equal to the mean difference ±1.96 x standard deviation of the differences.[13] The limits of repeatability are shown in table 1 and were also plotted as the difference versus the mean of the MPOD in the two test sessions (see figures 1 and 2).

	Repeatability	
	Zero degrees	Three degrees
Mean difference	-0.04	0.01
Standard deviation of mean differences	0.31	0.24
Coefficient of repeatability/reproducibility	0.60	0.48

Table 1: Coefficient of repeatability for the zero and three-degree apparent motion photometry derived macular pigment optical density (MPOD) scores

Insert figures 1 and 2 about here

DISCUSSION

We have measured MPOD on 76 healthy subjects using a CRT based AMP technique that has adopted the apparent motion of the grating phenomenon described by Anstis and Cavanagh.[11] The coefficient of repeatability values indicate the amount of change that can occur between readings and still be classed as instrument 'noise'. Our data suggests that when the same operator is taking repeated zero-degree AMP MPOD readings over time, only increases or decreases of more than 0.60 units can be classed as clinically significant (see figure 1), and when the same operator is taking repeated three-degree AMP MPOD readings over time, only increases or decreases in MPOD of more than 0.48 units can be classed as clinically significant (see figure 2).

The coefficient of repeatability for the central MPOD reading was 0.60. This is comparable with the results a similar studies that have assessed the repeatability and reproducibility of the Macuscope[™] macular pigment densitometer, which uses the psychophysical method of HFP to measure MPOD, One study found a coefficient of repeatability of 0.58 for this instrument,[14] and another reported coefficients of variance of 36.1% and 23% for right and left eyes respectively.[15]

The mean central MPOD value was 0.50 ± 0.24 . Other studies that used HFP to measure MPOD have reported average values in normal cohorts of 0.211 ± 0.13 (n= 280, age range: 18-50 years),[16] 0.28 ± 0.21 (n= 280, age range: 18-50 years),[17] 0.289 ± 0.156 (n = 46, age range: 21-81 years),[18] 0.319 (n = 100, age range: 22-60 years),[19], 0.43 ± 0.23 (n = 1648, age range: 53-86 years),[20] 0.47 ± 0.14 (n = 38, age range: 19-46 years),[14] and from 0.58 ± 0.29 to 0.72 ± 0.27 (n = 24, mean age: 38.1 ± 10.6 years).[15]The wide range of mean MPOD values may be the result of differing test protocols and stimulus characteristics.

We also report a three-degree MPOD value of 0.28 ± 0.20 , which was significantly lower than the central value (t = -8.0.5, p < 0.001). This decline with eccentricity supports work on primate monkeys using microspectrophotometry that found a peak in MPOD at the fovea with a sharp decline towards four degrees of eccentricity,[21 22] as well as a high-performance liquid chromatography study on donor eyes that reported negligible levels of MPOD at seven degrees eccentricity.[23]

There has been considerable interest in determining how far from the fovea the MPOD falls to negligible levels because in psychophysical measurements of MPOD the luminance of the light absorbed by MP at each eccentricity is compared against a baseline luminance from a match made in a part of the retina that is assumed to have no MPOD. Some workers have used a point at four degrees eccentricity as this reference,[24] but MPOD profiles have been reported where measurable pigment extends to five, six, seven degrees or even further.[25] There now seems to be a consensus that seven to eight degrees is a reasonable eccentricity to choose for the baseline [9] and this is easily achieved with the display used in this study.

We also found that the optimum frequency for a coherent motion illusion chosen by subjects could vary quite widely from day-to-day and that there was a trend for this frequency to deccrease with age. For our subjects there was no significant correlation between frequency and age but the majority of subjects (93%) were under the age of 40 years. For some subjects the range of luminances over which anomalous motion was reported varied. We countered this issue by instructing the subject to respond down unless they perceived clear upwards motion and consider this to be a valid strategy in determining a reversal point in the staircase procedure.

In conclusion, our results suggest that if central MPOD is being monitored over time to assess the effect of an intervention, then any change less than 0.60 units should not be considered clinically significant as they are very likely to be due to measurement noise. In other words, AMP is not suitable for monitoring changes in MPOD over time, as increases of this magnitude would not be expected even in response to dietary modification or nutritional supplementation.

ACKNOWLEDGEMENTS

Cambridge Research Systems provided the equipment used in this study. Hannah Bartlett was funded by a grant from the Macular Disease Society. The authors declare no conflict of interest.

WHAT IS ALREADY KNOWN ABOUT THIS TOPIC

Heterochromic flicker photometry has been used to assess MPOD in the clinical and research environments, but is a conceptually difficult task. Our group has found coefficient of repeatability values of 0.58 for the Macuscope™, and 0.33 for the MPS 9000, which are both commercially available instruments. The AMP technique for MPOD measurement is thought to be an easier task, and so this study has been designed to assess its repeatability.

WHAT THIS STUDY ADDS

Heterochromatic flicker photometry (HFP) has been used in research environments to assess macular pigment optical density (MPOD), and this psychophysical technique is considered to be the gold standard for macular pigment measurement despite the fact that it is a conceptually difficult task for patients to perform. This study is the first to independently evaluate the alternative apparent motion photometry technique for MPOD measurement, which is commercially available as part of the Metropsis psychophysical vision test suite (Cambridge Research Systems Ltd., 80 Riverside Estate, Sir Thomas Longley Road, Rochester, Kent ME2 4BH England), and is thought to be a conceptually more accessible task. The coefficient of repeatability for the central (zero-degree) measurement is 0.60, which compares well with our reported coefficient of repeatability for the MacuscopeTM, which is 0.58. This information is important for practitioners who may be using the Metropsis suite to assess the longitudinal effect of nutritional supplementation or dietary modification on MPOD.

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AUTHORSHIP

Both named authors were involved in the conception and design of the study, analysis and interpretation of data, and revising it critically for important intellectual content. Both named authors gave final approval of the version published. HB collected the data. HB and FE drafted the final manuscript.

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FIGURE LEGENDS

Figure 1: Difference in zero-degree MPOD reading between sessions one and two, compared with the mean (n = 76). The mean bias is represented by the solid line, and the 95% confidence limits are represented by the dotted lines.

Figure 2: Difference in three-degree MPOD reading between sessions one and two, compared with the mean (n = 76). The mean bias is represented by the solid line, and the 95% confidence limits are represented by the dotted lines.