

An evaluation of a novel instrument for measuring macular pigment optical density: the MPS 9000

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ABSTRACT.

Purpose: Of the antioxidants found in the human retina, only the macular carotenoid quantities can be estimated noninvasively (albeit in a collective fashion), thus facilitating study of their role in that tissue. The aim of this study was to evaluate concordance between macular pigment optical density (MPOD) values recorded on a commercially available instrument, the MPS 9000, with those of an already validated heterochromatic flicker photometry instrument. Also, we assessed and compared test–retest variability for each instrument.

Methods: Macular pigment optical density at 0.5° retinal eccentricity was measured using two different heterochromatic flicker photometers, the MPS 9000 and the Macular DensitometerTM, in 39 healthy subjects. Test–retest variability was evaluated separately for each instrument by taking three readings over a 1-week period in 25 subjects.

Results: There was a moderate positive correlation for MPOD at 0.5° of retinal eccentricity between the MPS 9000 and the Macular Densitometer described by the linear equation $y = 0.763x + 0.172$ ($r = 0.68$, $p < 0.001$, $r^2 = 0.46$); however, a paired-samples t -test showed a significant difference in terms of mean values, with a bias of lower MPOD values being yielded by the MPS 9000 ($t = -4.103$, $p < 0.001$). Bland–Altman analysis indicated only moderate agreement between the two instruments, reflected in 95% limits of agreement of 0.1 ± 0.27 . Inter-session repeatability, expressed as a coefficient of repeatability, ranged from 0.18 to 0.21 [mean (\pm SD): 0.19 (0.02)] for the MPS 9000 and from 0.11 to 0.12 [mean (\pm SD): 0.12 (0.01)] for the Macular Densitometer.

Conclusion: The results demonstrate that the MPS 9000 consistently yields MPOD readings, which are lower than that found with the Macular Densitometer, and exhibits substantial test–retest variability.

Key words: age-related macular degeneration – heterochromatic flicker photometry – Macular Densitometer – macular pigment – MPS 9000

Introduction

Age-related macular degeneration (AMD) is the most frequent cause of blindness among individuals ≥ 55 years in developed countries (Leibowitz et al. 1980; Attebo et al. 1996; Friedman et al. 2004), and with increasing longevity, the incidence of AMD is rising. The therapeutic options for AMD are limited, though improving, in particular for neovascular AMD. (Ciulla et al. 1988; Macular Photocoagulation Study Group 1993; Avery et al. 2006). Although vision loss with neovascular AMD is more sudden and severe, the non-neovascular forms, including the atrophic type, are more prevalent and account for approximately 90% of cases (Richer et al. 2004). At present, there is no consensus with respect to the management (including risk analysis and/or prevention) of these more common non-neovascular forms of the condition, which may, at least partly, reflect our incomplete understanding of AMD's aetiopathogenesis.

In the absence of effective treatment strategies for non-neovascular AMD, interest has focused on prevention and/or retardation of progression. Macular pigment (MP), composed of lutein (L) and zeaxanthin (Z), two hydroxycarotenoids, which are entirely of dietary origin (Bone et al. 1997; Johnson et al. 2005) and the retinal metabolite of L; meso-Zeaxanthin (meso-Z), is believed to be associated

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with reduced risk of development and progression of AMD. Macular pigment can be augmented, not only by eating food rich in these carotenoids, such as spinach, but also by dietary fortification with one of the many commercially available food supplements (Bone et al. 2003; Connolly et al. 2010). Epidemiological studies have observed an inverse association between the prevalence of AMD and a diet rich in L and Z (Eye Disease Case-Control Study Group 1993; Seddon et al. 1994), and furthermore, eyes with AMD have typically been shown to exhibit significantly lower levels of MP when compared to those without AMD (Eye Disease Case-Control Study Group 1993; Beatty et al. 2001; Bone et al. 2001; Bernstein et al. 2010), although this relationship was not observed in the Muenster Aging and Retina Study (Dietzel et al. 2011).

The putative capacity of MP to play a role in preventing or retarding the progression of AMD rests on its ability to limit photo-oxidative injury in the inner retina through its prereceptor absorption of short wavelength light (Snodderly et al. 1984a,b; Snodderly 1995) and/or the antioxidant properties of these carotenoids as they act as free radical scavengers in the retina (Snodderly 1995). The optical density and spatial distribution of MP have been shown to vary dramatically between individuals (Pease et al. 1987; Bone et al. 1992; Hammond et al. 1995), with consequential large inter-individual variation in prereceptor short wavelength light absorption and antioxidant activity in the retina.

Several methods for measuring the optical density of MP have been developed, thereby enabling investigators and/or eye care professionals to detect changes in MP concentration and distribution over time and therefore monitor the response to dietary modification or fortification. Unsurprisingly, there is a growing demand for a valid, reproducible, user-friendly instrument that measures macular pigment optical density (MPOD).

Heterochromatic flicker photometry (HFP) was the first, and remains the most widely used, technique for measuring MPOD *in vivo* (Snodderly et al. 1984a,b; Pease et al. 1987; Hammond et al. 1997, 2005; Berendschot et al. 2003; Nolan et al. 2008; Rougier et al.

2008; Stringham et al. 2008). Heterochromatic flicker photometry is a psychophysical method, which requires the subject to make iso-luminance matches between green (not absorbed by MP) and blue (strongly absorbed by MP) flickering lights, which is typically perceived as the point of cessation (or detection) of flicker. The technique typically employs a stimulus-surround configuration, where the stimulus consists of a target presented in counterphase flicker (alternating blue to green). The log ratio of the amount of blue light absorbed centrally, where MP peaks, to that absorbed at a peripheral retinal locus gives a measure of the individual's MPOD. This method has been validated against the absorption spectrum of MP *in vitro* (Bone et al. 1992; Hammond et al. 2005). The MPS 9000 is a relatively new HFP instrument that has been developed for clinical use (van der Veen et al. 2009). It is evident from the literature, however, that while based on the same basic optical principles of HFP, significant design and methodological differences do exist. We report a concordance study between the newly available commercial instrument, the MPS 9000, and the validated and conventional research instrument for measuring MPOD, the Macular Densitometer. We also measured and compared the inter-session repeatability for the two instruments.

Materials and Methods

This study was conducted at Dublin Institute of Technology (DIT), Dublin, Republic of Ireland. Eighty-nine subjects, aged 21–61 years, were recruited by word of mouth and were randomly assigned to either the concordance [39 subjects; mean age 29 (± 11)] or repeatability [50 subjects; mean age 34 (± 10)] arms of the study. Informed consent was obtained from each volunteer after the provision of a detailed information sheet. Ethical approval was granted by the research ethics committee at DIT, and the experimental procedures adhered to the Declaration of Helsinki. Inclusion criteria required participants to be aged 18 years or older, have no clinical signs of ocular pathology and have logMAR visual acuity (VA) of better than 0.2 in the study eye. All

subjects were naïve to both the instruments and to the process of measurement of MP.

The study eye was selected on the basis of corrected distance visual acuity (CDVA); the eye with the better CDVA being selected, and in cases of equal CDVA, the dominant eye was selected. A computer-generated LogMAR test chart (Test Chart 2000 Pro; Thompson Software Solutions, 74 Pine Grove, Hatfield, AL97BW, UK) was used to determine CDVA at a viewing distance of 4 m, using a Sloan ETDRS letterset. Subjects were requested to wear nontinted normal distance correction spectacles, if required. An ocular health examination was conducted to rule out any ocular pathology.

Macular pigment optical density was measured at 0.5° eccentricity on each instrument, on the same day, in 39 subjects to determine instrument concordance. To assess test–retest variability for each instrument, 25 subjects had MPOD measured on three occasions over a 1-week period on each instrument, 50 subjects were recruited for this part of the investigation, 25 were randomly assigned to the MPS 9000 and 25 were randomly assigned to the Macular Densitometer. All data were collected by a single operator.

The instruments used in this study were the MPS 9000, (Tinsley Precision Instruments Ltd, Croyden, Essex, UK), and the Macular Densitometer (Macular Metrics II, Rehoboth, MA, USA). The instrument used first in the concordance arm of the study was randomly selected on a case-by-case basis to minimize the risk of introducing bias attributable to a learning or fatigue effect from either instrument.

MPS 9000 (M|POD/QuantifEYE)

The MPS 9000 is a small, portable HFP instrument, capable of measuring MPOD at a single retinal locus (0.5° retinal eccentricity). The instrument uses a foveal target of 1° diameter (edge located at 0.5° retinal eccentricity) with the reference location at 8° retinal eccentricity (van der Veen et al. 2009). Testing was carried out according to the manufacturer's instructions. Prior to the first session, a short practice test was carried out to familiarize the participant with the

technique. Once the subject successfully completed the practice run, the subject's sensitivity to flicker was determined by a built in pretest routine, which enabled the appropriate initial luminance contrast of the two light sources to be established. This short (30 seconds) pretest flicker sensitivity routine was used to ensure the participants were in the middle of their flicker sensitivity range when performing the main task, as flicker sensitivity varies between individuals.

During the main test, the frequency of the blue (465 nm) and green (530 nm) light sources were automatically ramped down from 55 Hz for a series of luminance ratios of the two light sources. Initially, the observer viewed the target centrally and pressed a button when flicker was detected. This sequence of obtaining a flicker threshold for each blue-to-green ratio continued until a flicker response curve was obtained, where the minimum represents the equalization of the blue and green luminance. The procedure of obtaining the flicker detection for a series of blue-to-green ratios was repeated, after an additional short practice run, for peripheral viewing, with the subject fixating a red disc at a reference point of 8° horizontal eccentricity. The central and peripheral minima were used to calculate MPOD. The formation of the central and peripheral flicker response curves was monitored by the experienced examiner throughout the course of the examination to ensure reliability of the results based on the formation of a characteristic curve shape. Macular pigment optical density was calculated on the basis of a single, reliable measurement using both the central and peripheral stimuli. If a reliable measurement could not be obtained, the subject was re-instructed and afforded one additional and immediate opportunity to provide a reliable result. Failure to achieve a reliable result within two such measurement cycles resulted in exclusion from the study.

Macular Densitometer

The Macular Densitometer is a validated MPOD measurement instrument capable of determining a spatial profile of MP, by the measurement of MPOD at various retinal eccentricities

between 0.25 and 3° (Wooten et al. 1999). For the purpose of this study, readings were taken centrally at 0.50° using a 1° disc, [commonly used as it has been shown to have the highest repeatability of results (Snodderly et al. 2004)], matching that used in the MPS 9000, and a reference location at 7° using a 2° target (van der Veen et al. 2009). The Macular Densitometer was calibrated daily, and testing was carried out according to the manufacturer's instructions.

Prior to using the Densitometer, all subjects were shown an explanatory video describing the method for recording null flicker matches. The subject's critical flicker frequency (CFF) was then measured, and the optimal flicker frequency (OFF) determined using a defined test algorithm designed to minimize variance between readings, in a process that has become known as customized HFP. If a subject could not reach null flicker, the investigator increased the flicker frequency in increments of 1 Hz, until null flicker was perceived. Alternatively, if a subject exhibited a wide variation in null flicker readings (>10% of mean radiance at null flicker), the flicker frequency was decreased in increments of 1 Hz, until an acceptable null flicker range was achieved. An acceptable null flicker range was defined as one where the null flicker radiance values achieved by the subject were within 5% of the mean null flicker radiance at that test locus. Once the OFF was determined, the subject was required to find the middle zone of no flicker by turning a dial that adjusts the ratio of blue (458 nm) to green (530 nm). The desired end-point when using the Densitometer was a point of zero or 'null' flicker. For a detailed description of each instrument and instructions for use, please refer to van der Veen et al. (2009) and Wooten et al. (1999).

Statistical analysis

The statistical software package SPSS 18.0 for windows was used for data analysis. Mean MPOD for the MPS 9000 and the Macular Densitometer was compared using paired-samples *t* test. Bland-Altman analysis and plots, as well as the limits of agreement, were used to quantify the agreement between the two instruments.

Inter-sessional repeatability is expressed as a coefficient of repeatability, which was calculated as the standard deviation of the mean difference between measurements, and multiplied by 1.96. Coefficients of repeatability were calculated for (visit 1-visit 2), (visit 2-visit 3) and (visit 1-visit 3) for each instrument. A one-way repeated measures ANOVA was conducted to test for a learning or fatigue effect that might confound the test-retest analysis.

Results

The data were analysed (i) to compare measurements taken at 0.5° on the two instruments and (ii) to assess inter-sessional repeatability of each instrument. Two subjects were excluded from the instrument concordance analysis, and one subject from the instrument inter-sessional repeatability analysis, on the basis that they were deemed unable to perform the MPS 9000 task satisfactorily on the initial or repeat assessments. Data analysis is conducted and presented for the remaining 37 subjects in the concordance analysis, and 49 subjects in the inter-sessional repeatability analysis.

Instrument concordance

A scatterplot, graphically representing the relationship between MPOD values at 0.5° eccentricity obtained with each instrument, is shown in Fig. 1 ($r = 0.68$, $p < 0.001$).

A paired-samples *t*-test comparing the mean MPOD, as measured on each instrument, yielded a statistically significant difference between instruments ($t = -4.103$, $p < 0.001$), demonstrating a bias of lower MPOD values obtained on the MPS 9000, reflected in an average difference in MPOD values of 0.1 log unit between the two instruments (Fig. 2). The 95% limits of agreement between instruments were 0.1 ± 0.27 .

Inter-sessional repeatability

A one-way repeated measures ANOVA was conducted to assess repeat MPOD measurements for a learning or fatigue effect for each instrument. Mauchly's test of sphericity was not significant ($p > 0.05$) for either instrument. There was no significant

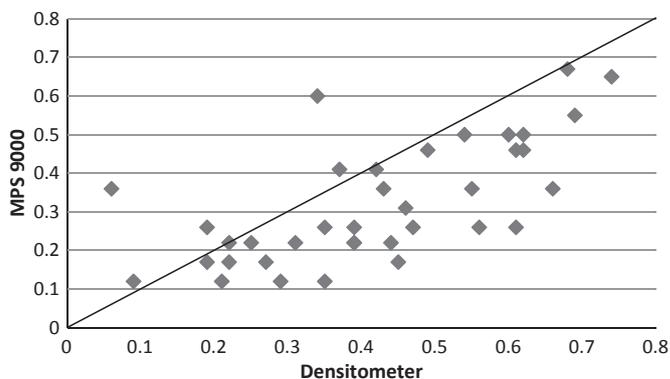


Fig. 1. Relationship between macular pigment optical density readings at 0.5° retinal eccentricity obtained with each instrument, with the line $y = x$ superimposed.

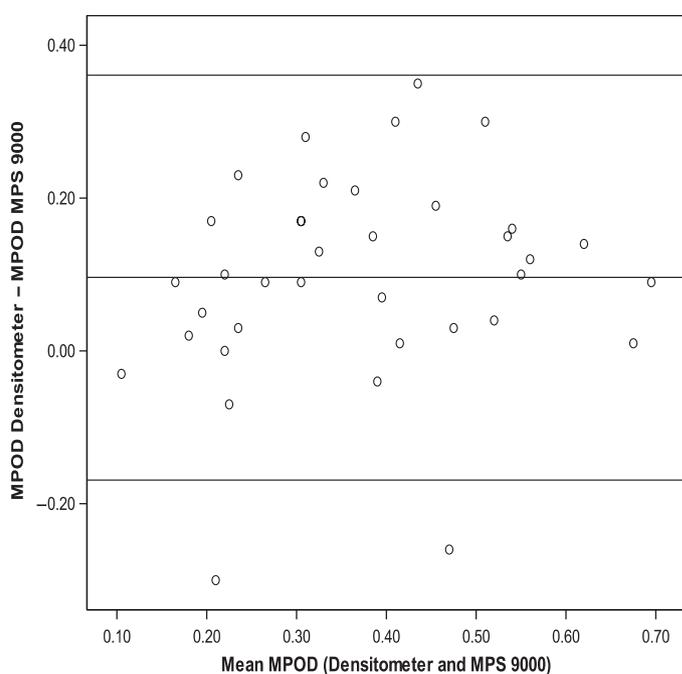


Fig. 2. Bland-Altman plot for macular pigment optical density values at 0.5° retinal eccentricity, showing 95% limits of agreement between the MPS 9000 and Macular Densitometer.

difference in repeat MPOD measurements for either the MPS 9000 or the Macular Densitometer, indicating the absence of any learning or fatigue effect [MPS 9000 ($F = 0.09$, $p = 0.92$); Macular Densitometer ($F = 2.556$, $p = 0.09$)]. Given that the p value for the Macular Densitometer is close to significance, a *post hoc* paired-samples t test was conducted comparing visit 1 and visit 3. No significant difference between the initial and final measurements was observed ($t = 0.000$, $p = 0.999$), providing further reassurance of the absence of a meaningful learning effect using this device.

A Bland-Altman plot was constructed to assess agreement between repeat measures taken on the MPS

9000 (Fig. 3). The coefficient of repeatability for the MPS 9000 ranged from 0.18 to 0.21 (mean 0.19 ± 0.02 ; see Table 1).

A Bland-Altman plot was also constructed to assess agreement between repeat measures taken on the Densitometer (Fig. 4). The coefficient of repeatability for the Macular Densitometer ranged from 0.11 to 0.12 (mean 0.12 ± 0.01 ; see Table 1). Inter-session repeatability results for each instrument are presented in Table 1.

Discussion

The Macular Densitometer has been validated in previous studies (Wooten

et al. 1999; Snodderly et al. 2004; Stringham et al. 2008), and the HFP technique for measuring MPOD has also been validated against the absorption spectrum of MP *in vitro* (Bone et al. 1992; Hammond et al. 2005). The MPS 9000 is a new commercial technology designed to measure MPOD and employs the HFP technique. Despite the use of HFP, the validity of this novel instrument has yet to be determined, and as such, the current study, which assesses the accuracy and repeatability of this new commercial instrument in relation to the current research standard HFP instrument, the Macular Densitometer is timely and necessary.

In the current study, mean MPOD was $0.32 (\pm 0.15)$ for the MPS 9000 and $0.42 (\pm 0.18)$ for the Macular Densitometer, values that are consistent with previous studies (Ciulla et al. 2001; Snodderly et al. 2004; Loane et al. 2007; Nolan et al. 2008; Makridaki et al. 2009; Bartlett et al. 2010; Loughman et al. 2010). The correlation between the MPS 9000 and the Macular Densitometer in this study was found to be positive, statistically significant, and similar to that previously reported comparing the MPS 9000 to the Macular Pigment Reflectometer (van der Veen et al. 2009). However, the mean difference between instruments was statistically significant, with a bias of lower MPOD in association with the MPS 9000, reflected in 95% limits of agreement of 0.1 ± 0.27 , indicating only moderate agreement between the two sets of readings (Bland & Altman 1986). The underestimation in MPOD values yielded by the MPS 9000 is in the range 0.05–0.15, but only in approximately 36% of subjects, with differences between respective measurements ranging from 0.35 to -0.3 , a 0.65 log unit range. It should be pointed out however that there are a number of exceptions to the trend for lower MPOD values on the MPS 9000 compared with the Macular Densitometer. In five subjects, the MPS 9000 demonstrated higher MPOD values when compared to the Macular Densitometer (see Fig. 1), and in two of these cases, the difference is substantial (0.26 and 0.30, respectively). Clinically, these two cases could not be discarded as both subjects were deemed to have understood and per-

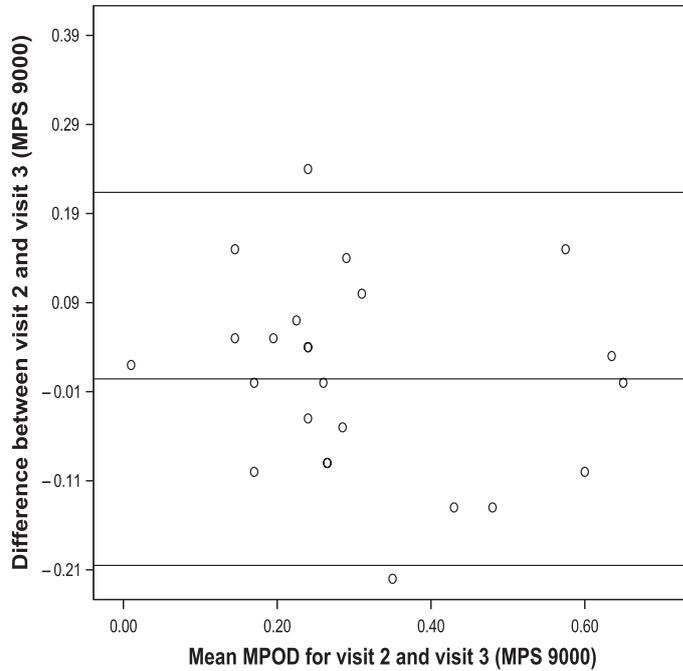


Fig. 3. Bland–Altman plot showing 95% limits of agreement for repeat measures at visit 2 and visit 3 for the MPS 9000.

formed the MPOD measurement to an acceptable standard on both devices. From a statistical viewpoint however, these may be regarded as outliers. Re-analysis of the data excluding these two cases does improve the observed correlation and agreement between devices to more acceptable levels ($r = 0.778$, and the coefficient of repeatability = 0.20). In other words, and contrary to the observations of van der Veen et al. (2009), the observed discrepancy between instruments is not systematic and is therefore not amenable to adjustment by means of a correction factor.

Inter-sessional repeatability is an important consideration for any prospective MP measurement device. The test–retest variability of the Macular Densitometer and the MPS 9000 has been investigated previously (Snodderly et al. 2004; Gallaher et al. 2007; van der Veen et al. 2009; Bartlett et al. 2010; De Kinkelder et al. 2011). The Macular Densitometer has been

shown to demonstrate good test–retest and intraclass correlation, with a coefficient of variation ranging from 17 to 22% (Snodderly et al. 2004; Gallaher et al. 2007). The MPS 9000 has also been reported to exhibit good repeatability, with limits of agreement ranging from 0.15 to 0.18 (van der Veen et al. 2009; De Kinkelder et al. 2011), but these results could not be substantiated in a recent study, which reported coefficients of repeatability and reproducibility ranging from 0.25 to 0.33 (Bartlett et al. 2010). The observed discordance between studies might be explained, at least in part, by methodological differences, including the use of more robust, averaged data in the research setting of the former study (van der Veen et al. 2009), when compared with data collected in a manner more reflective of a typical clinical setting in the latter study (Bartlett et al. 2010).

The mean coefficient of repeatability for the MPS 9000 in the current

study was 0.19 (± 0.02), and ranged from 0.18 to 0.21, which is consistent with previous findings (van der Veen et al. 2009; De Kinkelder et al. 2011), and has significantly better repeatability than that determined by Bartlett et al. (2010), whose interpretation of results is somewhat problematic, and simply not scientifically justified or sustainable. They do, nonetheless, still suggest a substantial amount of variability between sessions of MPOD measurement. For the purposes of comparability, the repeatability of the Macular Densitometer was also assessed. The mean coefficient of repeatability for the Macular Densitometer was 0.12 (± 0.01) and ranged from 0.11 to 0.12, substantially better than the MPS 9000. Indeed, the range of MPOD values across all three measures was <0.1 for 92% of subjects and <0.05 for 44% of subjects, using the Macular Densitometer, and this compares with only 54% and 25%, respectively, for the MPS 9000.

The MPS 9000 device is not described in sufficient detail in the published literature to provide a comprehensive analysis of the potential reasons for the inter-instrument observed differences in MPOD. Some of the design features of the instrument could potentially explain the observed differences. It is not clear, for example, whether a correction factor has been applied to account for differences in the spectral output of the chosen LEDs compared with the absorption spectrum of MP. The blue LED peak output for the MPS 9000 is given as 465 nm (van der Veen et al. 2009, De Kinkelder et al. 2011), although it is listed as 470 nm in the product literature. This compares to MP peak absorption at 458 nm, which matches the LED output employed in the Macular Densitometer. If such a correction factor has been employed for the MPS 9000, it is not clear whether this correction is based on LED manufacturer provided spectral

Table 1. Inter-sessional MPOD variability (mean \pm SD) and coefficient of repeatability for the MPS 9000 and Macular Densitometer.

Instrument	Mean (\pm SD) MPOD			Coefficient of repeatability		
	Visit 1	Visit 2	Visit 3	Visit 1–visit 2	Visit 2–visit 3	Visit 1–visit 3
MPS 9000	0.31 (± 0.15)	0.32 (± 0.16)	0.32 (± 0.17)	0.18	0.21	0.18
Macular Densitometer	0.40 (± 0.15)	0.38 (± 0.16)	0.40 (± 0.16)	0.11	0.12	0.12

MPOD, macular pigment optical density.

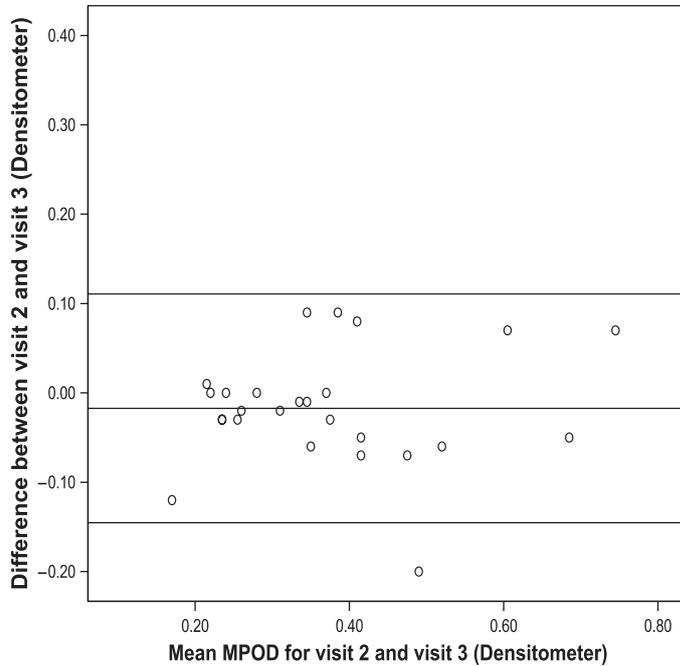


Fig. 4. Bland–Altman plot showing 95% limits of agreement for repeat measures at visit 2 and visit 3 for the Macular Densitometer.

outputs or independent spectral analysis of the specific LED outputs, which would be preferred. If this correction has not been applied or has been incorrectly applied, it certainly could explain the difference in MP optical density values derived by the two devices. Aside from the above unknown characteristics of the MPS 9000, there are a number of significant differences between the devices and the respective methodologies, which may explain both the observed lack of concordance and the disparity in terms of inter-session repeatability.

The MPS 9000 employs a 1° stimulus for both the central and peripheral measurements, whereas the Macular Densitometer employs a 2° stimulus for the peripheral measurement only and a 1° target centrally. Invariably, subjects reported difficulty completing the peripheral measurement, when using the MPS 9000, whereas no such difficulty was reported for the Macular Densitometer. It is likely that this difference in peripheral stimulus size is contributing to the greater relative difficulty experienced by subjects using the MPS 9000 and may explain the exclusion of three subjects unable to complete the peripheral measurement using this instrument. The difference in eccentricity of the peripheral reference target (7° for the Macular

Densitometer and 8° for the MPS 9000) could be a potential source of differences in the derived MP values (as the calculation of MP is based on the log ratio of central versus reference values). As the MPS 9000 employs a more eccentric reference stimulus, it might be expected that this technique would consequently derive higher MP values, if the effect was significant. The MPS 9000, however, appears to underestimate MPOD in comparison with the Macular Densitometer, so it is unlikely that the difference in reference location can explain the mean difference between devices.

Another distinction between the two instruments rests on the role of subject performance. The Macular Densitometer affords significant control to the subject, who can adjust the ratio of blue to green until a null flicker sensation is achieved, without a time restriction. The subject is simply instructed to use a method of adjustment or bracketing method to define the null flicker zone. The MPS 9000 employs a different technique, where a suprathreshold flicker rate is gradually reduced at a set rate of 6 Hz per second, and the subject responds by pressing a button to indicate the point at which flicker is detected. The rate of flicker decrease is a compromise

between testing time and differences in subject reaction times (van der Veen et al. 2009). Although reaction times are known to vary little across age (Porciatti et al. 1999), response times are significantly more complex. It would seem reasonable to suggest that subject threshold criteria could change during the course of a measurement session, particularly as task complexity increases from the central to peripheral target testing (Madden & Allen 1995; Hommel et al. 2004). Such a change in response criterion might not be easily detected by the examiner and could contribute to poor results.

Further, the MPS 9000 is unable to provide a useful measure of subject performance reliability. The only performance check an examiner can use is to determine that a ‘typical’ V-shaped flicker response curve is generated. The product literature describes that ‘irregularities in the data’ are typical and that the shape of the curves can vary between individuals. This makes interpretation of the curve, and reliability of the result, therefore dependent on examiner skill and training and subject to significant variation. Indeed, such dependency could represent a partial explanation for the poor coefficient of repeatability reported by Bartlett et al. (2010). It has been suggested that the number of subjects in the Bartlett paper with significant variation in test–retest MPOD values represents operator error (inappropriate acceptance of low-quality V-shaped flicker response functions), rather than measurement noise (Murray et al. 2011). This may be the case, but if so, this reinforces the observations herein that MPOD values obtained using the MPS 9000, may well be significantly affected by examiner skill level and training, and furthermore, that the limited means to determine patient performance acceptability would seem unreliable at best.

The technique basically produces a single central and peripheral end-point to determine MPOD. The MPOD value determined using the Macular Densitometer by comparison represents the average of multiple (typically four to six readings) end-points determined by the subject. Variation in performance, or lack of understanding of the task, becomes immediately obvious as a large standard deviation

in the radiance values produced and allows the examiner to ensure result reliability, to a degree that is simply not achievable with MPS 9000.

The variation in the stimulus-background configuration between instruments is also substantial and certainly has the potential to induce measurement discrepancies. For the Macular Densitometer, the configuration is a short-wave blue background, against which an incremental blue target is viewed. For the MPS 9000, the blue target is viewed against a spectrally broadband white light surround. While it is likely that both configurations effectively suppress the contribution of rods and S-cones, other HFP methods, such as those used by Beatty et al. (2000) and Bone & Sparrock (1971) which have employed a centre-surround stimulus configuration, have been shown to produce a spectral curve that is best fit with a significant rod contribution (Hammond et al. 2005). Such a centre-surround configuration could potentially suffer retinal adaptation effects, chromatic aberration effects, and off-axis lens effects induced by the +5D focusing lens. It is simply unclear whether the target-stimulus configuration, as employed in the MPS 9000, fulfils the basic principle of any technique for the measurement of MP, namely that such a technique 'should provide spectral absorption curves that match the extinction spectra of MP' (Hammond et al. 2005).

The current study was designed to evaluate the comparability and repeatability of MPOD measurements, as determined using the commercial MPS 9000 in relation to the conventional research standard Macular Densitometer. It is important to note that the experimental protocol was designed to be of clinical relevance and was compliant with manufacturer guidelines. The MPS 9000 appears to provide an unpredictable underestimation of MPOD when compared to the Macular Densitometer and demonstrates poorer repeatability. Our analysis suggests that the fundamental principles and technique of the MPS 9000 seem generally robust, but that the unacceptable test-retest variability observed here and elsewhere (Bartlett et al. 2010) may largely be as a consequence of the (i) absence of a user-friendly means to assess subject

performance variability during the test procedure, (ii) increased difficulty associated with the peripheral task and (iii) dependency on examiner training and skill level. In the presence of such design features, we would recommend that best clinical practice using the MPS 9000 would require multiple measures of MPOD. Results should be discarded where large discrepancies such as those obtained by Bartlett et al. (2010) are found and where results are more consistent, the average MPOD should be used.

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