Macular Pigment Optical Density in Singapore Population

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MACULAR PIGMENT OPTICAL DENSITY IN SINGAPORE POPULATION

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List of abbreviations

AD	:	Alzheimer's disease
AF	:	Autofluorescence
AMD	:	Age-related macular degeneration
ApoE	:	Apolipoprotein E
ARMS2	:	Age-related maculopathy susceptibility 2
AL	:	Axial length
BCMO1	:	Beta-carotene monoxygenase 1
BF	:	Factor B
BMI	:	Body mass index
CFF	:	Critical flicker fusion
CSC	:	Central serous chorioretinopathy
C2	:	Complement component 2
C3	:	Complement component 3
CD 36	:	Cluster determinant 36
DHA	:	Docosahexaenoic acid
EPA	:	Eicosapentaenoic acid
HDL	:	High-density lipoprotein
HFP	:	Heterochromatic flicker photometry
HPLC	:	High-performance liquid chromatography
FR	:	Fundus reflectometry
L	:	Lutein
LED	:	Light-emitting diode
LB	:	Lycium barbarum
MAP	:	Macular assessment profile
MP	:	Macular pigment
MPOD	:	Macular pigment optical density
MPS II	:	Macular pigment screener II
MMD	:	Macular Metrics Densitometer
MVOS	:	Maxwellian view optical system
MZ	:	Mesozeaxanthin
OCT	:	Optical coherence tomography
OD	:	Optical density
RPM	:	Revolutions per minute
RPE	:	Retinal pigment epithelium
RS	:	Raman spectometry
Rx	:	Refractive error
SR-BI	:	Scavenger receptor class B type 1
SLO	:	Scanning laser ophthalmoscope

SNPs	:	Single nucleotide polymorphisms
SP	:	Spatial profile
T	:	Transmittance
Z	:	Zeaxanthin

Abstract

Cher Huiyun Joanna; The University of Manchester

Macular pigment optical density in Singapore population; Master of Philosophy, 2015

Introduction

Macular pigment (MP) is a yellow pigment found at the center of primate macula. It is believed to protect the macula against oxidative damage initiated by blue light and free radicals. Little is known if different ethnicity with same iris colour affects MP level and its distribution.

Although Macular Metrics Densitometer (MMD) is a validated instrument to measure macular pigment optical density (MPOD), it must be operated by a trained personnel and the subject must clearly understand the task. In search of a better and faster instrument to measure MPOD, Macular Pigment Screener II (MPS II) was developed.

This study aims to: (1) measure MP spatial profile (SP) in Singapore Chinese, Malay, and Indian using MMD; (2) compare MPOD obtained with MPS II and MMD.

Methods

MP SP in 161 healthy volunteers, aged 21 to 63, were measured (72 Chinese, 47 Malay, and 42 Indian) in Khoo Teck Puat Hospital using MMD from 2013 to 2015. MPOD was also measured in 65 Chinese subjects at 0.50° retinal eccentricity using both MMD and MPS II on the same day.

Results

The mean ± SD age of the study population is 30.6 ± 9.6, with 60 males and 101 females. Mean MPOD in the population at 0.50° retinal eccentricity was 0.58 ± 0.23, with the range 0.10 to 1.13. MPOD does not have any significant correlation with age ($p = 0.15$), gender ($p = 0.15$) and body mass index (BMI) ($p = 0.32$), except myopia ($p = 0.02$). There is no significant difference in MPOD with ethnicity (MPOD: Chinese = 0.57 ± 0.21; Malay = 0.60 ± 0.22; Indian = 0.59 ± 0.27, $p = 0.73$). Majority of the subjects have exponential MP SP (Malay = 78%, Chinese = 73%, Indian = 62%). Overall 22% to 38% of the subjects have atypical SP in form of central dip, secondary peak and plateau. There is a difference in MP SP among 3 ethnic groups ($p = 0.05$), when the plateau SP is excluded. Indian ethnicity demonstrated a higher number of atypical SP as secondary peaks at 1.00° retinal eccentricity. There is a good degree of correlation of MPOD between MPS II and MMD (MMD: 0.57 ± 0.21, MPS II: 0.48 ± 0.17; $p < 0.0001$, R = 0.72).

Conclusion

There is a significant difference in MP SP among the 3 ethnic groups, with Indian ethnicity demonstrating a higher number of atypical SP as secondary peaks. A larger sample size will be required to validate these observation in the future. MPS II is a good tool to screen MP, however, investigators need to be aware that MP readings are lower in MPS II compared to MMD.

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Acknowledgement

Chapter 1 : Introduction

1.1 What is macular pigment (MP)?

The primate central retina, macula lutea, contains concentrated yellowish pigment (Snodderly et al., 1984a), known as macular pigment (MP). The yellowish pigment is xanthophyll carotenoids including lutein (L), zeaxanthin (Z), and mesozeaxanthin (MZ) (Nolan et al., 2013; Canovas, 2010). Handelman et al. (1991) and Snodderly and Hammond (1999) suggested that an accurate way to estimate the MP is by measuring its Optical Density (OD). The OD of MP is calculated by log(1/T), where T is the MP's transmittance. T represents the amount of incident light at a specified wavelength that passes through the MP. Figure 1.1.1 illustrated the possibility of having OD of MP more than 3.
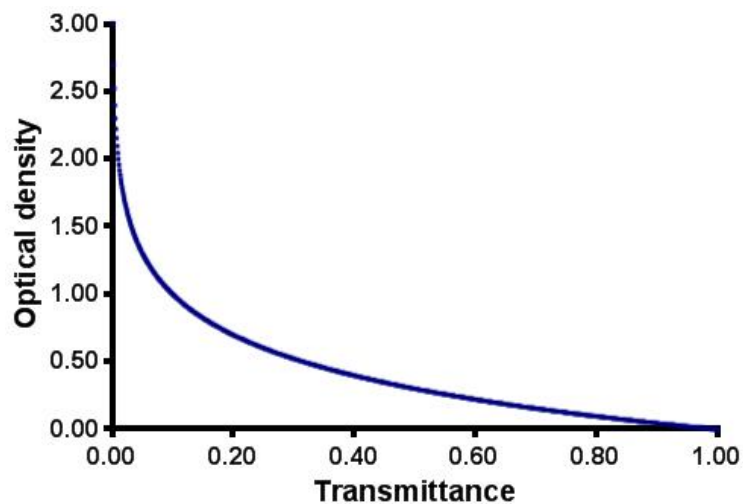
Figure 1.1.1. Relationship between optical density and transmittance.

1.2 Occurrence of MP

There are more than 600 carotenoids present in the nature, about 40 to 50 are found in the fruits and vegetables, and 14 had been detected in human body. However, only L, Z and MZ were found in the retina. Interestingly, MZ was absent in the blood and it is a chiral isomer of Z. MZ is believed to be converted from L in the retina (Johnson et al., 2005).

Carotenoids extracted from 33 fruits and vegetables were analysed with high-performance liquid chromatography (HPLC) (Sommerburg et al., 1998). Egg yolk and corn had the highest L and Z concentration. Although most green leafy vegetables have high L concentration, Z concentration is very low (Sommerburg et al., 1998). Nolan et al. (2015) found the presence of MZ in salmon skin, sardine skin, trout skin and trout flesh using HPLC.

The relationship of L and Z between retinal and brain level were studied in 12 rhesus monkeys using HPLC (Vishwanathan et al., 2013). Vishwanathan et al. (2013) found that L and Z concentration in the macula is positively correlated with L and Z concentration in the cerebellum, occipital cortex and pons.

1.3 Chemical structure of Lutein (L), Zeaxanthin (Z) and Mesozeaxanthin (MZ)

There are two major types of carotenoids: hydrocarbon carotenoids and xanthophyll carotenoids. Xanthophyll carotenoids are also known as oxycarotenoid due to the additional hydroxide molecule on the ring (Figure 1.3.1). Hydroxycarbon carotenoids include beta-carotene and lycopene, while xanthophyll carotenoids include L, Z, and MZ (Nolan et al., 2013).

The carotenoid molecule is made up of long polyene chain with alternating single and double carbon bond (Britton, 1995). Unlike hydroxycarbon carotenoids, both ends of the xanthophyll carotenoids consist of two hydroxyl groups. The chemical structure of L and Z are similar to each other except for the presence of one double bond in their structural isomer (Bone et al., 1985; Snodderly, 1995). MZ is a stereoisomer of Z that is believed to be originated from L in the macula (Bernstein e al., 2010; Bone et al., 1997). This is possible when the double bond in the L molecule migrates and the spatial configuration of the hydroxyl group remains the same (Bone et al., 1997). Beta-carotene, Z and MZ contains 2 beta-rings, while L contains one beta-ring and 1 epsilon-ring (Cunningham and Gantt, 2001). Because of the unique chemical structure of each carotenoid, they have different antioxidant and light absorption properties (Nolan et al., 2013).
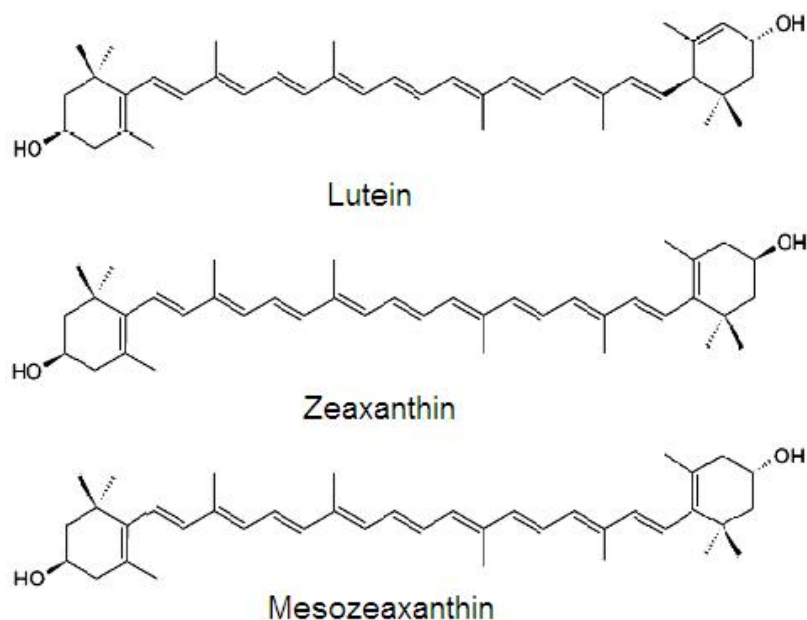
Figure 1.3.1. Chemical structures of Lutein (L), Zeaxanthin (Z) and Mesozeaxanthin (MZ).

1.4 Absorption spectrum of MP

Absorption spectrum of MP at fovea and parafovea was studied using Maxwellian view optical system (MVOS) by Hammond and Fuld (1992). After dark adaptation and using a background light of 450 nm, contribution of short-wave sensitive cones is eliminated. Experimental animal studies done on monkey found that fovea and parafovea has a peak absorption at approximately 460 nm and 530 nm, respectively (Snodderly et al., 1984; Hammond and Fuld, 1992).

The peak absorption of MP is affected by the number of conjugated double bond and number of beta-ring or epilson-ring in the carotenoid. As shown in Table 1.4.1, carotenoid with lesser conjugated double-bond absorbs shorter wavelength (Melendez-Martinez et al., 2007).

Carotenoid	Number of conjugated double bond	Number of beta- / epilson-ring	Peak absorption (nm)
Lutein	10	1	448
Zeaxanthin	11	2	454

Table 1.4.1. Peak absorption of MP in relation to number of conjugated double bonds and beta- or epilson-rings.

1.5 MP distribution

MP distribution on monkey retinas was studied with micro-spectrophotometry and two-wavelength micro-densitometry by Snodderly et al. (1984b). MP density was highest in the receptor axon layer, especially cone axons of the foveola, and inner plexiform layer of the parafovea. There is a general decrease in MP with retinal eccentricity (Snodderly et al., 1984b). MP distribution was studied again on macaques and squirrel monkeys using HPLC and found that MP has a peak concentration within central 1 to 2 mm of the fovea and declines gradually with eccentricity (Snodderly et al., 1991).

Bone et al. (1985) was the first to discover the presence of L and Z in human retina, using HPLC. L occupied a concentric area with radius of 2.5 mm and Z occupied a concentric area with radius of 0.75 mm from the fovea (Bone et al., 1988). In 1997, Bone et al. mapped out

the distribution of L, Z and MZ using HPLC in adult and infant human retinas. His results revealed that L increases, while Z and MZ decrease with radial distance from the fovea. Adult retinas had less L, more MZ and relatively similar amount of Z compared to infant retinas. In other words, L dominates the peripheral macula, while Z and MZ dominate the center macula.

Similarly, MPOD measured using in-*vivo* methods, such as Macular Metrics Densitometer (MMD) demonstrated healthy human subjects' MP decrease gradually with retinal eccentricity and eventually become optically undetectable at 6 to 8° (Yu et al, 2012, Raman et al, 2011). On the other hand, Delori et al. (2006) measured MP distribution with AF technique demonstrated an elliptical distribution of MP (i.e. more horizontally and inferiorly).

Hammond et al. (1997c) measured human MP spatial profile (SP) using three-channel MVOS at 6 locations: 0°, 0.5°, 1°, 2°, 3°, and 4° retinal eccentricities. Majority of the MP SP showed that MP decreases gradually with eccentricity (i.e. exponential decay with eccentricity). The authors also found some unique MP SP including possible secondary peaks, whereby MPOD is 0.05 higher at 1° and 2° compared to the fovea. MP distribution was also wider in subjects with overall higher MPOD.

1.6 Functions of MP

MP is believed to prevent oxidative damage of the macula *via* its antioxidant properties, which prevent free-radical reactions, and absorption of short wavelength (blue) light at a pre-receptoral level thus protecting the photoreceptors from photo-oxidative damage (Berstein et al. 2010; Hammond et al., 1998; Snodderly et al., 1984a). MP may improve visual performance by filtering blue light which is easily scattered and poorly focused (Loughman et al., 2012a; Loskutova et al., 2013). It may also reduce chromatic aberration, visual discomfort and disability glare (Hammond et al., 1998; Hammond and Caruso-Avery, 2000; Wenzel et al., 2003; Bernstein et al., 2010; Loskutova et al., 2013; Stringham and Snodderly, 2013).

Khachik et al. (1997) found oxidative products of L and Z in human and Rhesus monkey retinas using HPLC. This suggested that L and Z may act as antioxidants, by singlet oxygen quenching and free radical scavenging, to protect the retina.

Ham et al. (1979) found that light with wavelengths between 488 nm and 458 nm causes both thermal and photochemical damage. Wavelength shorter than 441.6 nm causes photochemical injury to the retina. The MP filters off blue light, thus protecting macula from photochemical damage.

Stringham and Snodderly (2013) compared the effect of MP and visual discomfort using three-channel MVOS with a 1000 Watt xenon arc lamp. Five male and one female, aged 21 to 34, were recruited. Subjects with high MP were found to be more tolerant against brighter light in the fovea relative to parafovea. The authors hypothesized that MP act as an optical filter which reduces visual discomfort and glare caused by brighter light, and improves visual acuity.

Stringham et al. (2011) compared the effect of MP on photostress recovery, disability glare, and visual discomfort. MP was measured using MMD while glare was induced by very bright white light-emitting diodes (LEDs). MPOD was measured at 0.25°, 0.50°, 1.00°, and 2.00° retinal eccentricities. Disability glare and photostress recovery was tested by identifying of a 1.00° Gabor patch's orientation. Higher MPOD was found to have significantly lower photostress recovery time, lower disability glare, and lesser visual discomfort.

Hammond et al. (2014) tested photostress recovery, chromatic contrast and glare disability after one year of L and Z supplementation in 57 subjects (placebo 58 subjects). MPOD was measured using MVOS as well. Subjects with higher MPOD and increased MPOD after supplementation were found to have significant improvement on photostress recovery and chromatic contrast. Although there was a faster recovery against glare disability in subjects with supplementation, it did not reach statistical significance.

Chapter 2 : Methods for measuring MP

MP can be measured by *in-vitro* or *in-vivo methods*. *In-vitro* methods include HPLC, micro-spectrophotometry, and micro-densitometer whereas i*n-vivo* methods include psychophysical method and imaging method. Table 2.1 summaries the *in-vivo* subjective (psychophysical) methods and objective (imaging) methods for measuring MP.

Subjective methods	Objective methods
1. Maxwellian view optical system (MVOS)	1. Raman spectrometry (RS)
2. Maculometer	2. Autofluorescence (AF)
3. Macular Metrics Densitometer (MMD)	3. Fundus reflectometry (FR)
4. Macular pigment screener II (MPS II)	
5. Macular assessment profile (MAP) test	

Table 2.1. In-vivo methods for measuring MP.

2.1 Psychophysical method

Psychophysical method depends on the MP's spatial distribution and spectral absorption characteristics. Heterochromatic Flicker Photometry (HFP) principle is adopted in psychophysical method for measuring MP.

In HFP, a test stimuli of alternating blue (460 nm) and green (550 nm) is presented. MP absorbs blue light maximally but not green light. Utilising blue background and flicker (in MMD) eliminate the involvement of rods and S-cones during MP measurement and obtain signals solely from the L- and M-cones. The observer alters the amount of blue and green light until the isoluminant flicker becomes stable. MPOD is obtained by calculating the difference between the maximal blue light absorption in the fovea and negligible absorption in parafovea (Snodderly et al., 1984a; Snodderly et al., 2004).

HFP method made two assumptions (also illustrated in Figure 2.1.1):

1. The relative sensitivity of the retina to blue and green light is the same at both fovea and parafovea.

2. When minimum flicker is detected on this heterochromatic light, same amount of light must be absorbed by L- and M-cones at fovea (where maximum MP is found) than the parafovea (where negligible MP is found - reference point) (Snodderly and Hammond, 1999).
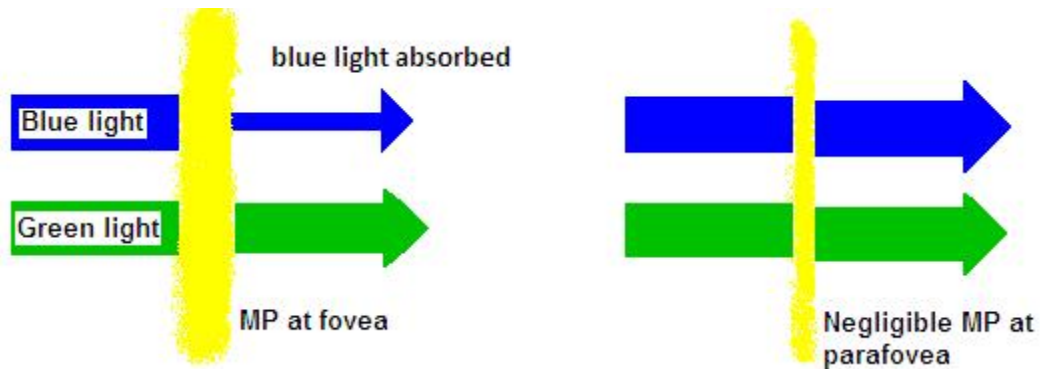
Figure 2.1.1. HFP principle.

MPOD is then calculated using the formula below:

$$MPOD = \log_{10} 1/T_{MP} = \log (B\ fov\ /\ B\ ref)$$

T_{MP} being the transmittance of MP, B fov being the amount of blue light absorbed by the fovea, and B ref being the amount of blue light absorbed by the reference point in the parafovea (Snodderly and Hammond, 1999).

The advantages of using HFP include the ability to measure MP on living eye, minimal effect from subject's misalignment compared to objective method, and no pupil dilation is required. MPOD is relatively unaffected by head movement or media opacity such as cataract (Ciulla and Hammond, 2004; Stringham et al., 2008). However, the accuracy of HFP result depends on subjective interpretation of the flicker. Some subjects may not be able to do this test reliably (Ciulla and Hammond, 2004).

The major disadvantage of using HFP is subject-dependent. Since the subject needs to have a relatively good vision to look at the target, older subjects, amblyope, or subjects with eye diseases may experience fatigue easily when performing the test. Similarly, subjects who are unable to understand the instruction may have difficulty performing the test accurately (Ciulla and Hammond, 2004).

Large test target, corresponding to a location further away from the foveal center, produces a lower MPOD. Since different HFP designed with different target size, MP measurement may be different. There are problems comparing different instrument using HFP method because they may have different target sizes, fixation targets, light sources, and background illumination.

There are several instruments that utilises the principle of HFP such as MVOS, maculometer, MMD, MPS II and MAP test.

2.1.1 Maxwellian view optical system (MVOS)

The three-channel, MVOS (Arizona State University West, USA) is able to measure MPOD at any customised retinal eccentricity using the smallest possible test target of 12 arc minute to 1 degree and has a reference at 5.5° using 1.2° size test target. MVOS consists of a xenon arc and a grating monochromator to produce monchromatic light which eliminate stray light. MPOD is calculated by comparing the sensitivity between tested location and the reference point (Hammond et al., 1997c; Wooten et al., 1999).

2.1.2 Maculometer

Maculometer (University of Westminster, UK) measures MPOD at 0.50° retinal eccentricity and has a reference point at 5° on the parafoveal region. When taking measurement from the fovea, the subject is instructed to minimise the visibility of the flicker by adjusting the luminance of the blue light. This is repeated at the parafovea while the subject fixates at the red target 5° away. MPOD was calculated after 4 to 8 readings were measured at fovea and parafovea (Neelam et al., 2005b; Neelam et al., 2006).

2.1.3 Macular Metrics Densitometer (MMD)

MMD (Macular Metrics Corp., USA) was validated against *in-vivo* methods (Wooten et al., 1999). Its accuracy and repeatability was compared to other objective methods such as RS in many studies (Neelam et al., 2005a; Hogg et al., 2006; Canovas et al., 2010; Loughman et al., 2012b). MMD measures MPOD at 0.25°, 0.50°, 1.00°, and 1.75° retinal eccentricities with a reference point at 7° on the parafoveal region. It uses HFP technique while subject changes the luminance of the stimulus until the flicker is minimised (Kirby et al., 2009).

2.1.4 Macular Pigment Screener II (MPS II)

MPS II (Elektron Technology, UK) is a small portable instrument which can be connected to a desktop or laptop computer. The MP screener automatically measure MPOD using the MPS II software in the computer. It measures MPOD at 0.50° retinal eccentricity and has a reference point at 8° on the parafoveal region. While MP is measured using MPS II, the task of the subject is to look at the fixation target and press the response button once the stationary target appears to be flickering (Loughman et al., 2012b).

2.1.5 Macular assessment profile (MAP) test

MAP test (Eizo Nanao Corporation, Ishikawa, Japan) uses the Eizo T566 monitor, which is customised to filter off the unwanted red phosphors, leaving only blue and green phosphors for MP measurement. MAP is capable to measure MP at 7 locations: 0°, 0.8°, 1.8°, 2.8°, 3.8°, 6.8° and 7.8° retinal eccentricities. (Barbur et al., 2010; Huntjens et al., 2014).

2.2 Comparison between MMD and MPS

Loughman et al. (2012b) evaluated the repeatability and correlation of the two HFP instruments, MPS 9000 (Hartest Precision Instruments, UK), a similar model of MPS II, and MMD. MPOD was tested at 0.50° in 39 healthy subjects and the test was repeated on 25 subjects within a week. MMD was found to have better repeatability than MPS 9000. Although there is a positive correlation between the 2 instruments, lower MPOD was found in MPS 9000 when compared to MMD.

2.3 Imaging method

Imaging methods have an advantage over HFP method when measuring MPOD in a diseased eye because the patient may not be able to perform the test. In a study by Neelam et al. (2005b), MPOD was measured in 120 healthy subjects using maculometer and RS. Although MP measured by maculometer is slightly higher than the results obtained by Raman spectometry (RS), the comparison of both techniques fall within the Bland-Altman 95% limit of agreement. The author also reported that there were 4 in 100 subjects who had difficulty to perform the test. Since RS showed a good correlation and acceptable test-retest repeatability with maculometer in healthy subjects, they concluded that RS could be used to measure MP in healthy young volunteers.

As different instrument measures MPOD by different principles, MPOD measured with different technique would produce different results. Ten healthy subjects had MPOD measured by both AF and MMD. MPOD measured by AF was consistently higher than MMD in all eccentricities (0.25°, 0.50°, 1°, and 1.75°). MPOD obtained by AF is inevitably higher than MMD because the measured area absorbs fluorophore from RPE melanin or retinal hemoglobin (Canovas et al., 2010).

2.3.1 Autofluorescence (AF)

Autofluorescence (AF) is based on the fluorescence of lipofuscin in the human retinal pigment epithelium (RPE) which is emitted at a spectral range of 520 to 800 nm and excited *in vivo* from 400 to 570 nm. In the fovea, excitation light within the absorption range of the MP is partially absorbed by the carotenoids and caused a central zone of minimal fluorescence (Canovas et al., 2010; Lima et al., 2010; Lima et al., 2013). MPOD is obtained by measuring the intensity of fluorescence at 2 wavelengths, one absorbed maximally and another absorbed minimally by MP (Delori, 2004).

The advantage of AF imaging is the ability to generate a MP distribution map. The disadvantages of AF method include difficulty in obtaining a good AF image and pupil dilation is required. In order to obtain an optimum image, the instrument must be properly aligned and focused. Pupil must be dilated to at least 6mm prior to the test, and the subject must have steady fixation (Delori, 2004; Trieschmann et al., 2006).

2.3.2 Fundus Reflectometer (FR)

Scanning laser ophthalmoscope (SLO), fundus reflectometer (FR), or fundus camera attached to charge-couple device are some of the instrument that uses FR to measure MPOD. This is done by comparing the reflectance at specific wavelength at the fovea and the peripheral retina. Among all the chromophores found in different parts of the eye such as red blood cells, photoreceptors, RPE, choroid, and sclera, only MP and lens absorb the blue light. The sum of signals from lens and MP generate the reflectance map (Berendschot et al., 2000; Chen et al., 2001).

2.3.3 Raman spectometry (RS)

Raman spectometry (RS) utilises argon laser at 448 nm to excite the macular L and Z for about 2 seconds. MPOD is obtained by measuring the return backscattered light intensity captured by the Raman detector (Bernstein et al., 2004). Even though RS can obtain the result quickly and easily, it is not widely used because of the signal strength reduction due to aging, small pupil and media opacity (Gellerman et al., 2002; Neelam et al., 2005b; Hogg et al., 2006).

MP obtained using imaging method such as AF, FR, and RS requires pupil dilation to obtain good image quality.

Chapter 3 : MP and diseases

Since MP serves as antioxidant, diseases related to oxidative stress may be associated with lower levels of MP (Lima el al, 2010; Loane et al., 2010). MPOD was measured and studied in the following diseases: age-related macular degeneration (AMD), central serous chorioretinopathy (CSC), glaucoma, cataract, diabetes mellitus, and Alzheimer's disease (AD).

3.1 Age-related macular degeneration (AMD)

AMD was found to be more prone in affecting the parafoveal region (particularly 2° to 4° retinal eccentricity). Interestingly, the area with highest concentration of MP (fovea to 2° retinal eccentricity) was being spared. This shows that there is a possibility that MP may protect the retina from oxidative damage (Swann and Lovie-Kitchin, 1991; Hammond et al., 1997c). Before performing clinical trials on the prevention of AMD progression, many studies were done in the past 2 decades to study the relationship between MPOD and AMD.

Curio et al. (1996) believed that the MP declines inevitably as rods and cones were loss in AMD. Their hypothesis was later proven by Bone et al. (2001) using HPLC, who found lower L and Z concentration in donor eye with AMD compared to without AMD. Similarly, the carotenoid concentration was reported to be 70% higher in rod outer segment compared to other retinal membrane using HPLC by Rapp et al. (2000).

A large-scale clinical trial was done using the AREDS 2 supplement containing 10 mg L, 2 mg Z, 250 mg Docosahexaenoic acid (DHA), and 650 mg Eicosapentaenoic acid (EPA). The AREDS 2 results were launched recently after following up the AMD subjects for 5 years. L and Z were suggested to substitute beta-carotene in the new formulation of the multivitamin tablets since beta-carotene was found to increase the risk of lung cancer in both current and ex-smokers. The new formulation with L and Z showed additional 10% reduction in risk of progression to advanced AMD, 11% reduction in risk of progression of neovasular AMD, and 16% reduction in risk of vision loss compared to those receiving supplements without L and Z (Chew et al., 2013). Although MPOD was not measured in the subjects who participate in the AREDS 2 study, MPOD may increase after supplementation of L and Z.

Table 3.1.1 summarises the relationship between AMD and MPOD. Both HFP (Beatty et al., 2001) and imaging method (Kaya et al., 2012) found lower MP in eye at risk and with AMD compared to healthy subjects. However, it is unknown if lower MP contributed to the risk of AMD since both healthy and affected eyes have similar level of MP (Hammond and Fuld, 1992). Although both Berendschot et al. (2002) and Kaya et al. (2012) studied the effect of AMD to MPOD using FR, only Kaya et al. studied the age dependence of MPOD. MPOD difference may be due to age but not AMD as the healthy subjects are younger. To date, it is still unknown if AMD affects MPOD as different study find different result.

Author / Year	Sample size	Method	Result / Conclusion
Beatty et al., 2001	46 healthy and 9 subjects with monocular advanced AMD (the eye without AMD was tested)	Modified MVOS with green/blue light from LED and 10 watt halogen bulb for background illumination.	Lower MPOD in eyes that are at risk for AMD.
Berendschot et al., 2002	289 healthy subjects and 146 AMD subjects	Fundus reflectometer (FR)	No difference in MPOD and melanin OD between healthy and AMD subjects.
Kaya et al., 2012	96 AMD subjects (age range 50 to 89 years) and 85 healthy subjects (age range 21 to 79 years)	Fundus reflectometer (FR)	AMD subjects have lower MPOD compared to healthy subjects. MPOD is age dependent in AMD subjects, but not in healthy subjects.

Table 3.1.1. Relationship between AMD and MPOD.

3.2 Central serous chorioretinopathy (CSC)

MPOD of 70 chronic CSC patients and 41 acute CSC patients were compared with 94 healthy subjects using AF. Optical coherence tomography (OCT) was used to measure central retinal thickness in normal and CSC subjects. Lower MPOD was reported in eyes with chronic CSC together with the fellow eye. Since there is no difference in MPOD between both eyes (Hammond and Fuld, 1992), lower MPOD might have contributed to the risk of having CSC. Sasamoto el al. (2010) proposed low MPOD may be a risk factor of CSC and could aggravate thinning of central retina.

3.3 Glaucoma

Igras et al. (2013) measured MPOD with MMD at 0.50° eccentricity and compared the MP values between 40 glaucoma patients and 54 healthy subjects. Glaucoma patients were found to have lower MPOD compared to healthy subjects. The authors suggested that ocular blood circulating is reduced in glaucoma patient. Carotenoid reaching the retina may be affected and causing lower MPOD.

3.4 Cataract

The effect of cataract on MPOD was tested on 41 cataract patients without other eye disease. MP measurement with AF was performed before and after cataract removal. MPOD was higher after cataract removal and dense nuclear sclerosis was associated with failure measurement (Sasamoto et al., 2011). Imaging method is preferred as subjects with poor visual acuity may find it difficult to see the flicker in HFP method. However, accurate MP measurement is almost impossible to obtain in cataract patients as media opacity blocks and reduces the signal from imaging technique. The reduction in signal might have produced a lower MP measurement due to light scattering.

MP measured using 1.00° target from the three-channel, MVOS showed that older subjects (age range 55 to 78 years) with cataract showed lower MPOD when compared to younger (age range 24 to 31 years) healthy subjects (Hammond et al., 1997a). The lower MPOD could be due to age difference instead of the cataract. Using the same measuring method, Ciulla and Hammond (2004) did not find any significant difference in MPOD between the elderly with or without severe cataract. The authors argued that MP measurement using HFP method will not be affected by poor visual acuity from cataract as the relative sensitivity between the central fovea and reference point in the parafovea are the same.

3.5 Diabetes mellitus

Fourteen healthy subjects were compared with type 2 diabetic patients with non-proliferative diabetic retinopathy (n = 12) and without diabetic retinopathy (n = 17). Their MPOD was measured using modified confocal SLO. The diabetic patients, with or without retinopathy, had lower MPOD compared to healthy patients. Furthermore, subjects with higher HbA1c levels had lower MPOD. There are 3 possible causes to the reduced MPOD in diabetic patients: First, the media opacity in diabetic patients may affect the measurement especially when Lima et al uses objective method to measure MPOD. Second, oxidative stress and poor glycemic control in diabetes leading to fluctuating or poor vision which affects MP measurement, and lastly, diabetic patients may have reduced bioavailability of L and Z in the macula (Lima el al, 2010).

3.6 Alzheimer's disease (AD)

Alzheimer's disease (AD) is more commonly found in elderly. Since L and Z is also found in the brain and is positively correlated to L and Z in the macula (Vishwanathan et al., 2013), there is a possibility that MP might be affected in AD. Nolan et al. (2014) studied the effect of AD and their MP, serum L and Z, and visual function. Thirty six mild to moderate AD patients and 33 control subjects, aged 64 to 94, were recruited. Their MP was measured with AF using Heidelberg Spectralis. Visual function was assessed by their best corrected visual acuity and contrast sensitivity using computerized LogMAR ETDRS test chart. Their retina health was also assessed with a 45° macula centred colour photograph using Visucam 200. Results showed that AD patients have lesser MP, poorer vision, lower serum L and Z, and higher occurrence of AMD compared to control subjects.

3.7 MP measurement for biomarker in some diseases

The studies stated above shows that there is a possibility that MP is lower in some eye diseases and degenerative or systemic diseases. The detection of lower MP may indicate the following possibilities: Poor absorption and/or bioavailability of L and Z to blood and retina; Patients have intrinsic anatomical abnormalities in the retina which lead to lower MP accumulation. Further research need to be done to study MP level in these diseases.

Chapter 4 : Factors affecting MP level

4.1 Diet

As different ethnicity have different dietary habit, level of carotenoid intake can be different and affect the MP level. Johnson et al. (2005) divided 18 rhesus monkeys into 3 groups and manipulated their diet. Upon study completion, all monkeys were sacrificed, and L, Z and MZ from the retina were analysed with HPLC. Results are summarised in table 4.1.1. This proved that L must be originated from diet and not Z.

Diet type vs presence of macular carotenoids	L in macula	Z in macula	MZ in macula	Remarks
Diet with L only		X		
Diet with Z only	X		X	All-trans Z is found in the macula
Xanthophyll-free diet	X	X	X	

Table 4.1.1. Summary of the manipulated diet and their effect in monkeys (Johnson et al., 2005). X for absent and   for present in macula.

Since primates, including human beings, cannot synthesize carotenoids *de novo,* L and Z have to depend entirely on dietary sources (Bernstein et al., 2010). Hammond et al. (1997b) measured MPOD on 13 healthy subjects before and after 4 weeks of dietary modification that included addition spinach, corn, or both to their usual meal. MPOD was monitored with MVOS using a test stimulus of 1.00°. MPOD increased in most subjects after dietary modification.

4.2 Family history of age-related macular degeneration (AMD)

Nolan et al. (2006) measured MPOD using maculometer in 625 subjects without family history of AMD and 175 subjects with family history of AMD. All subjects recruited are healthy, without AMD. MPOD is significantly lower in subjects with family history of AMD.

4.3 Smoking

Cigarette smoking is known to be a risk factor for AMD, particularly neovascular AMD. It is believed to reduce the antioxidant level in the body hence increase oxidative damage to the biological system (Hammond et al, 1996a).

An age-matched study done by Hammond et al. (1996a) recruited 34 smokers and 34 non-smokers to compare their MPOD difference. MPOD measured at 0.50° eccentricity with MVOS was found significantly lower in smokers than non-smokers.

Nolan et al. (2006) measured MPOD with 1° test target using maculometer on 828 healthy subjects from age 20 to 60. Current and past smokers had significantly lower MPOD than never smokers. Furthermore, heavy smokers had significantly lesser MPOD compared to light smokers and never smokers (Hammond and Caruso-Avery, 2000).

4.4 Age

Aging is believed to be one of the factors which attribute to lower MP. This could be due to two reasons. First, insufficient uptake or excessive depletion of the retinal carotenoids in elderly (Beatty et al., 2001). Second, elderly may have inadequate systemic absorption of L and Z. Tucker et al. (1999) assessed dietary carotenoid with dietary questionnaires and found lower serum L and Z concentration in elderly.

Neelam et al. (2005a; 2005b) found a significant decline in MP using both RS and maculometer in subjects above 55 years old. The authors stated that age-related miosis might have contributed to the lower MP in elderly using RS.

On the other hand, MPOD measured with MVOS did not have a significant change with age, even when elderly develop cataracts or AMD (Ciulla and Hammond, 2004). Interesting, Delori (2004) found an increase in MPOD at fovea with age using AF imaging.

Hammond et al. (1997c) measured MP and its SP using three-channel MVOS over a period of 16 years. Both MPOD and its SP did not have significant difference with age. On the other hand, MP distribution measured by Delori et al. (2006) using AF is found to be broader with age. MP is also found to be more at the parafovea compared to the fovea. Neelam et al. (2014) measured MP SP using MMD and found a central dip in older and higher body mass index (BMI) subjects. The authors believed that this atypical MP SP may be due to the unique foveal architecture. However, they did not investigate the foveal architecture in this study. MP SP with increasing age was also studied using MMD by Beirne (2014). MPOD was measured at 4 locations: 0.25°, 0.50°, 1.00°, and 1.75° retinal eccentricities. MPOD is

significant higher at 1.75° retinal eccentricity with increasing age but no significant relationship at 0.25°, 0.50°, and 1.00° retinal eccentricities.

4.5 Iris colour

Since melanin and carotenoids may protect retina from degenerative diseases, different ethnicity, with different iris colour, may have different MP level. Hammond et al. (1996c) evaluated the relationship between MPOD and 3 groups of iris colours: blue or gray (Group 1), green or hazel (Group 2), black or brown (Group 3). MPOD was measured with MMD at 0.50° eccentricity in 95 healthy subjects. MPOD was found to be significantly higher in Group 3 compared to Group 1. This is supported by other published papers done by Hammond and Caruso-Avery (2000), Stringham (2011), Wolf-Schnurrbusch et al. (2007), and Kirby et al. (2010). They hypothesized that the lower MPOD could be due to the greater transmission of short-wavelength light into lighter iris eyes, which results in increased oxidative stress to the retina.

4.6 Foveal architecture

Snodderly et al. (1984b) was the first to propose that foveal architecture such as foveal width, depth, slope and thickness might influence the MP density and its distribution. Since MP is found primarily in the cone axons, wider fovea may store more MP.

Based on Snodderly's hypothesis, Nolan et al. (2008) and Kirby et al. (2009) carried out a study to find out if foveal architecture (using OCT) affects SP of MP (using MMD). Both of them found out that thicker and wider fovea have higher MPOD (Nolan et al., 2008; Kirby et al., 2009).

It is common that the spatial distribution of MP declines gradually with eccentricity. Interestingly, some subjects have higher MPOD at 0.50° compared to 0.25° from the fovea, and other subjects have higher MPOD at 1.00° compared to 0.50° from the fovea. This atypical MP SP is known as "central dip" and "secondary peak" respectively (Kirby et al., 2009; Kirby et al., 2010). Kirby et al. (2009) studied the fovea architecture with the presence of secondary peak. Subjects with secondary peak showed significantly wider fovea in females. The steeper slope of MP distribution was also found to have steeper foveal depression.

4.7 Gender

Females may have lower MPOD as they tend to have more adipose tissue than males. Body fat can reduce the carotenoid levels present in serum and retina due to poorer transportation when lipid is deposited in the blood vessel wall (Yu et al, 2012). Hammond et al (2002) stated that MPOD was 38% higher in males compared to females. There was a significant

positive relationship between MPOD and serum L and Z in both genders. However, males have a stronger correlation than females (Hammond et al., 2002).

Similarly, Neelam et al. (2014) measured MPOD using MMD in 95 Chinese healthy subjects revealed significant lower MPOD in female gender at 0.25°, 0.50°, 1.00° and 1.75° degree fovea eccentricities. Neelam et al. (2014) believed that the absorption and storage of MP may be different between both males and females.

On the other hand, Nolan et al found that both males and females with higher percentage of body fat had lower MPOD but results were only significant in males (Nolan et al, 2004).

Interestingly, MP distribution is also slightly different between the males and females. Delori et al. (2006) measured MP distribution using 2 wavelength AF and found out that females are more prone to ring-like profile (i.e. secondary MP peak), shoulders, or plateaus with MP peaks at approximately 0.7° and 0.5° retinal eccentricities.

4.8 Lipoprotein and serum carotenoids

The ratio of serum L to Z ranges from 2.7 to 4.5:1 depending on individual dietary habit, lifestyle, and genetics (Landrum and Bone, 2001). It is believed that L and Z, from the diet, are transported by lipoprotein in the serum to the macula by attaching itself to the lipoprotein (Loane et al., 2010).

Higher concentration of serum high-density lipoprotein (HDL) was associated with higher serum L concentration. However, concentration of serum cholesterol and HDL had no relationship with MPOD. Furthermore, higher concentration of serum triglyceride was correlated to lower MPOD (Loane et al., 2010). Many studies reported that serum L and Z concentration had a significant positive relationship with MPOD measured with HFP method (Neelam et al, 2005b; Nolan et al, 2007; Sandberg et al, 2010). A randomized, placebo-controlled, clinical trial on 44 healthy subjects using supplement containing 10.6 mg MZ, 5.9 mg L and 1.2 mg Z were compared to the control group. Both serum L and Z and MPOD measured with MMD increased after 6 months supplementation (Connolly et al., 2011). On the other hand, Neelam et al. (2014) found a significant positive relationship between MPOD and serum L, but not in serum Z.

Serum L and Z concentration should be similar in spouses, since they tend to consume similar diet. Wenzel et al. (2007) recruited 25 married couples and analysed their MPOD with MMD. The diet were analysed with a dietary questionnaire. Interestingly, MPOD was different between spouses, even though dietary intake and serum L and Z concentration

were similar. These results showed that other determinants such as difference in lipoprotein concentration and genes might have influenced the MP level.

4.9 Genetics

MP is found to be associated with different types of genes related to carotenoids transport, uptake, and metabolism (Meyers et al., 2013).

Since carotenoids cannot be synthesized *de novo* and the only way to obtain L and Z is through diet (Bernstein et al., 2010), bioavailability of L and Z to the blood and retina is crucial. Bioavailability of Z by the retina cells was significantly reduced when the scavenger receptor class B type 1 (SR-BI) had a small interfering RNA transfection, presence of A allele at lipid transporters cluster determinant 36 (CD 36), or presence of CT allele at the beta-carotene monoxygenase 1 (BCMO1) (During et al., 2008; Borel et al., 2011). With reduced Z absorption by the retina cells, MPOD is lower as well.

Single nucleotide polymorphisms (SNPs) gene such as the CFH complement component 2 (C2), factor B (BF) and complement component 3 (C3) were proposed to be associated with increased risk of AMD due to inflammation. MPOD was measured in 302 healthy subjects with MMD at 0.25°, 0.50°, 1.00°, and 1.75° eccentricities (Loane at el., 2011). Subject with both CFH Y402H and age-related maculopathy susceptibility 2 (ARMS2) allele had a significantly lower MPOD. However, C2, C3, and BF gene showed no statistically association with MPOD.

Loane et al. (2010) found a strong association between Apolipoprotein E (ApoE) gene and MPOD. Out of the 4 types of allele (1, 2, 3, and 4) in ApoE gene, 4 allele was found to be associated with lower risk of AMD, while 2 allele had higher risk of AMD. Some researchers believe that the ApoE gene affects the transportation and circulation of L and Z in serum and reduces retinal uptake of these carotenoids. (Baird et al., 2004; Zareparsi et al., 2004; Sun et al., 2011) Furthermore, subjects with 4 allele were found to have significantly higher MPOD. Interestingly, subjects with 2 allele were not found to have lower MPOD (Loane et al., 2010).

Meyers et al. (2013) found out that 21 SNPs from 11 genes were related to MPOD. The following 15 genotypes demonstrated significantly lower MPOD (Table 4.9.1). However, Meyers et al. (2013) did not find any significant difference in MPOD with CD 36.

Gene	SNPs	Genotype	How the gene cause lower MPOD?
GSTP1	rs675679	AA	Affect retinal uptake or intracellular trafficking of L and Z.
BCMO1	rs11645428	GG	Affect enzymes which involve in cleavaging of L and Z.
	rs6564863	GG	
ABCA1	rs1929841	CC	Affect HDL or cholesterol status
ABCG5	rs10179921	AG or AA	
LIPC	rs6078	AG or AA	
SCARB1	rs10744182	GG	Affect lipid, L and Z absorption.
	rs838879	AG and GG	
	rs437992	GG	
ELOBL2	rs1150561	GG	Affect synthesis or metabolism of long-chain fatty acids in blood or retina.
FADS1	rs174534	AA	
FADS2	rs2727271	AA	
RPE65	rs4926339	GG	This is a rare gene mutation which is known to cause autosomal recessive condition Sjorgren-Larsson Syndrome, which is linked to lower MP.
ALDH3A2	rs8069576	AA	

Table 4.9.1.Summary of 15 genotypes demonstrating lower MPOD (*Meyers et al., 2013*).

4.10 Ethnicity

There are many factors affecting level of MP, however, it is still unknown if ethnicity affects level of MP. From table 4.10.1, MPOD seems to be different between ethnicity. However, the MP measuring method, MP test location, age group and study protocol were not standardized between studies. Therefore, it is difficult to compare if MPOD is different between ethnicities.

MPOD measured by MMD seems to be higher in Chinese and Indian (Raman et al. 2011; Yu et al., 2012; Neelam et al., 2014) compared to White and Black population (Iannaccone et al., 2007). However, this difference in MPOD could be due to the older age group recruited by Iannaccone et al. (2007).

We cannot compare the ethnic difference in MPOD across different research paper as different studies have different protocols and methods of measuring MP. Even when the same principle is used, such as HFP, the size of the test target, wavelength of light, type of light source, background illumination is different. All the mentioned differences between instruments can produce a different MP level in the same subject.

At a glance, MPOD seems to be lowest when measured with MVOS (Hammond and Caruso-Avery, 2000; Ciulla et al., 2001) and highest with MMD (Raman et al., 2011; Yu et al., 2012; Neelam et al., 2014). To evaluate the relationship between ethnicity, study protocol and subjects demographic need to be standardised.

Author / Year	Subjects / Sample size (n)	Age range (years old)	Method	MPOD at 0.50° from the fovea (Mean ± SD)
Hammond and Caruso-Avery, 2000	American from Phoenix metropolitan area (n = 217)	17 to 92	Modified MVOS using LED light source	0.22 ± 0.13
Ciulla et al., 2001	White (n = 239) and African (n = 32) from America	18 to 50	MVOS	White 0.21 ± 0.13 African 0.23 ± 0.14
Iannaccone et al., 2007	White (n = 148) and black (n = 35) from Memphis and Pittsburgh	69 to 86	MMD; measuring 0.50° from fovea	White 0.37 ± 0.19 Black 0.22 ± 0.23
Nolan et al., 2008	White (n = 41) and non-white* (n = 18) from Ireland *non-white: 5 Indian, 6 Asian, 3 Hispanic/Spanish, and 4 Black	18 to 60	MMD	White: 0.34 ± 0.13 Non-white: 0.55 ± 0.28
Raman et al., 2011	South Indian from India (n = 161)	20 to > 60	MMD	0.50 ± 0.21
Yu et al., 2012	Chinese from Beijing (n = 281)	17 to 85	MMD	0.49 ± 0.18
Neelam et al., 2014	Chinese from Singapore (n = 95)	21 to 68	MMD	0.55 ± 0.19
Howells et al., 2013	Indian (n = 75) and Pakistani (n = 22) from UK, Aston	18 to 61	MPS 9000	Indian 0.44 ± 0.15 Pakistani 0.42 ± 0.12

Abell et al., 2014	Australian from Australia (n = 201)	21 to 84	MPS 9000	0.41 ± 0.20
Huntjens et al., 2014	South Asian* (n = 54) and White (n = 19) from UK, London *South Asian: Indian, Pakistan, or Bangladesh	16 to 34	MAP	South Asian: 0.80°: 0.46 ± 0.13 White: 0.80°: 0.37 ± 0.14 *measuring 0.80° from fovea
Wolf-Schnurrbusch et al., 2007	White (n = 67) and African from Germany (n = 51)	35 to 49	Modified confocal scanning laser ophthalmoscope	White 0.36 ± 0.13 DU African 0.59 ± 0.14 DU *DU: Density units

Table 4.10.1. Comparison of MPOD in different studies.

Chapter 5 : Rationale

In the western country, MPOD was shown to be different in African and white subjects by Iannaccone et al. (2007) and Wolf-Schnurrbusch et al (2007). Both articles showed that different ethnicity with different iris colour can affect MP level. However, it is not known if different ethnicity with the same iris colour has different MPOD. Therefore, the first aim of my study is to measure MPOD in Singapore population and analyse their MP SP, particularly on the 3 ethnic groups, Chinese, Malay and Indian. This study rules out iris colour as an independent factor which affect the MP level.

Howells and his colleagues (2013) studied the factors affecting MPOD in the South Asian subject, such as sunlight, diet, ethnicity, iris colour, and lifestyle. However, the MP SP was not measured in the study. Hammond et al. (1997c) measured the human MP SP using three-channel MVOS. The authors described MP SP as an exponential decay with eccentricity and they also found possible secondary peaks, valleys and shoulder in the MP profile. In 2006, Delori et al. measured the MP distribution using AF. Besides the typical exponential decay, a bimodal distribution (i.e. central peak of MP with a second ring of increased MP at 0.6° to 1.2° retinal eccentricity in the profile), shoulders and plateaus at 0.5° and 0.7° retinal eccentricities were also detected. According to Kirby et al. (2009), secondary peak is defined as having a higher or equal MPOD in the 0.50 or 1.00 eccentricity compared to 0.25 eccentricity using MMD. Huntjens et al. (2014) measured MP using MAP and classified the MP SP into 3 types: typical exponential, ring profile and central dip profile. Their results suggested that there are different types of MP SP, but the profile between ethnic groups have not been studied.

To date, there are no published paper about the relationship between ethnicity and MPOD done in Southeast-Asia. There is also no published article regarding Malay population's MPOD. Since Singapore population is made up of 76.8% of Chinese, 13.9% of Malay, 7.9% of Indian, and 1.4% of other ethnicities (Central Intelligence Agency, 2012), Singapore would be the best place to conduct this study. Since all Asian in Singapore have dark iris colour, the difference in MPOD would be mostly due to their diet, genetics, serum carotenoids, and lifestyle between ethnicity, but not iris colour.

Although MMD is a validated instrument, it must be operated by a trained personnel and the subject must clearly understand the task while giving a reliable result. In search of a better and faster instrument to measure MPOD, MPS II was developed. MPS II is a relatively new instrument which measure MPOD subjectively. It was claimed to be a fast MP screener.

MPS 9000, which is a similar model of MPS II, had been tested and compared with MMD by Loughman et al. (2012b). It was commented by the authors that MPS 9000 produces an

unpredictable lower MPOD and poorer repeatability. However, only 39 healthy subjects were recruited in this study. In our study, more subjects would be recruited to compare MP level using both MMD and MPS II. Their ease of use and factors affecting the measurement are investigated.

Chapter 6 : Methology

6.1 Subject recruitment

This cross-sectional study was conducted in the Department of Ophthalmology and Visual Sciences at Khoo Teck Puat Hospital over a 2 years period. The study was approved by the Research and Ethics Committee of the National Healthcare Group (Annex 1). All subjects signed an informed consent form (Annex 2), and all research procedures followed the tenets of the Declaration of Helsinki.

All subjects were age 21 years old and above with best corrected visual acuity of 6/12 or better, and absence of anterior and posterior segment diseases. Non-smoker and past smoker, who quitted more than 6 months, were recruited. Subjects with epilepsy, diabetes mellitus, undetermined ethnicity, current smokers, or those who cannot give informed consent, were not recruited in our study.

All healthy subjects without eye diseases who expressed willingness and desire to participate in the study were screened for eligibility. The following screening tests were included: visual acuity measurement, anterior and posterior segment examination using slit lamp biomicroscopy and fundus camera (Annex 3).

After an informed consent, each study subject completed a questionnaire regarding their sociodemographic, lifestyle, medical history, standard medication for chronic diseases (e.g. hypertension, diabetes, and cardiovascular disorders) and/or nutritional supplements history (Annex 4). Refractive status was taken from the subject's habitual glasses using focimeter.

6.2 MP measurement

MPOD was measured using MMD and MPS II. MPOD was calculated from the logarithmic ratio of the amount of blue light required to achieve null flicker at the fovea to the reference point where MP is presumed to be negligible.

In this study, MMD measured MP 0.25°, 0.50°, 1.00°, and 1.75° retinal eccentricities, while MPS II measured MP at 0.50° only. Five readings were obtained from MMD and MPS II respectively and recorded (Annex 5). The mean and standard deviation from the 5 readings were calculated and subjects with standard deviation equals to or more than 0.1 were excluded.

6.2.1 MMD

Before measuring MP, the subject's critical frequency flicker (CFF) threshold was measured using a 1° stimulus target in MMD. Examiner increased the flickering frequency of the stimulus until subject perceive a steady target. This was repeated 3 times and average was taken for calculation of CFF. Detailed instruction is described in Annex 7.

MMD was allowed to warm up for 10 minutes before daily calibration. MPOD was measured at 0.25°, 0.50°, 1.00°, and 1.75° retinal eccentricities with a reference point at 7° on the parafoveal region. Before the actual measurement of MP, CFF threshold was measured with the blue light at 0.50° target. The subject was presented with a flickering stimuli consisting of two superimposed lights. The stimuli temporally alternates in square-wave counterphase between 460nm (blue light) and 550 nm (green light).

During MP measurement, the subject was asked to look at a flickering blue/green light in the central 0.25°, 0.50°, 1.00°, and 1.75°, and adjust the knob until the flicker stops or become minimal (reading A). This happened when the blue and green lights were perceived as isoluminant. The task was repeated at an eccentric location that is known to contain negligible levels of MP (reading B). Reading A and B were used to calculate MPOD by the software using the formula: $MPOD = \log_{10} 1/T_{MP} = \log (A / B)$.

Detailed procedure and instruction are attached in Annex 7 to 9. MP SP was measured using MMD in all ethnic groups for comparison.

6.2.2 MPS II

MPS II was connected to a laptop with software running based on Microsoft Access. MPS II measured MPOD at 0.50° retinal eccentricity with a reference point at 8° from the fovea. While measuring MP using MPS II, the subject was asked to look at the fixation target and press the response button once the stationary target appears to be flickering. The subject was presented with 3 circular targets 8° apart. MPS II measured MPOD using a 1° circular stimulus, and a 3° circular fixation target 6° in the periphery. While MP was measured using MPS II, the subject was told to look at the fixation target and to press the response button once the stationary target appears to be flickering.

Instead of adjusting the blue-green intensity, the flicker frequency rate changed through a series of different blue-green ratio. One v-shaped curve was generated from the central test, while the second v-shaped curve was generated from the peripheral test. MPOD was calculated by an algorithm difference between the central and peripheral minimum points. Typical v-shape curve (Figure 6.2.2.1) was monitored and assessed for test reliability at the same time when the test was running. Test was repeated if poor quality curves, such as

broad-U shape curve (Figure 6.2.2.2) or multiple minimum points (Figure 6.2.2.3), were generated.
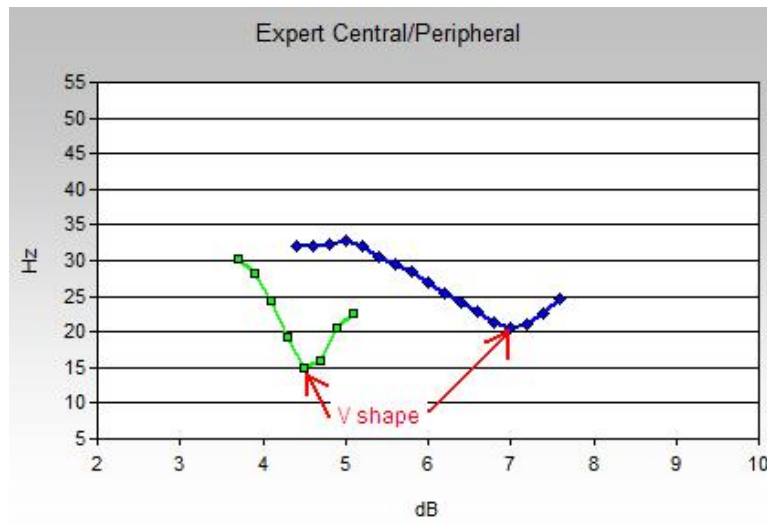
Figure 6.2.2.1. Reliable central (blue) and peripheral (green) curves.
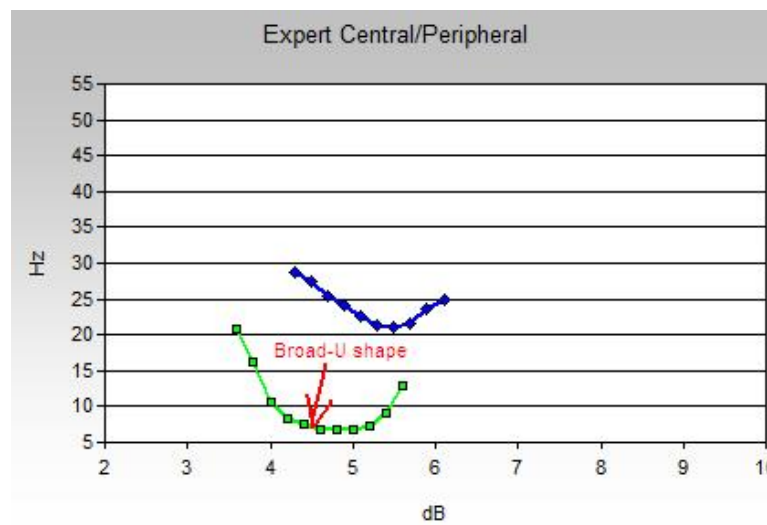
Figure 6.2.2.2. Unreliable broad-U shape peripheral curve.
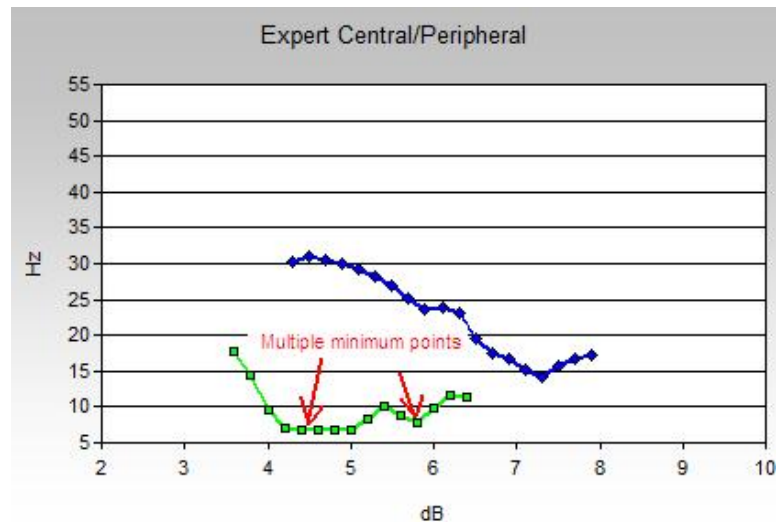
Figure 6.2.2.3. Unreliable multiple minimum points in the peripheral curve.

Detailed procedure and instruction are attached in the Annex 10 to 12. MPOD was only performed in Chinese subjects. Sixty five Chinese subjects were able to perform both MPS II and MMD.

6.3 Sample size calculation and statistical analysis

Two sample size calculations in our study were done using PASS 13 (NCSS Software). Paired study was done to compare MPOD between MMD and MPS II, and independent study was done to compare MPOD between ethnic groups.

1. Paired study: Prior data (Loughman et al., 2012b) indicated that the difference in the response of matched pairs was normally distributed with standard deviation 0.2. If the true difference in the mean response of matched pairs is 0.1, we will need to study 33 pairs of subjects to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

2. Independent study: In a previous study (Neelam et al., 2014) the response within each subject group was normally distributed with standard deviation 0.19. If the true difference in the experimental and control means is 0.1, we will need to study 58 experimental subjects and 58 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

GraphPad Prism 6 was used to analyse majority of our results. Demographics of the study population were examined using mean and standard deviation (SD). One-way ANOVA was used to compare variables between 3 ethnic groups. Unpaired t-test with Welch's correction was used to compare variables between 2 ethnic groups. Linear regression and Bland-Altman plots were used to compare the differences between MMD and MPS II. Other factors affecting MPOD were analysed using linear regression. Statistical significance level was set at 0.05 and power was set at 0.80.

Fisher exact test was done using Stata Statistical Software 2013 (StataCorp LP) to analyse if different ethnic group have any difference in MP SP.

Chapter 7 : Results

7.1 Subject demographic

In this study, 83 Chinese, 49 Malay, and 53 Indian were recruited. Table 7.1.1 shows the demographic data of the subjects recruited. Three Chinese, 2 Malay, and 11 Indian were excluded from the study as they were unable to perform the MP measurement reliably. Sixty five Chinese subjects were included for the comparison between MMD and MPS II.

There is a statistical difference in the subjects' age among the 3 ethnic groups as determined by one-way ANOVA ($F_{(2,158)} = 7.00$, $p = 0.001$). An unpaired t-test with Welch's correction reveals significantly older Indian subjects were recruited compared to Chinese ($p = 0.002$) and Malay ($p = 0.004$). There is no significant age difference between Chinese and Malay ($p = 0.95$).

There is a statistical difference in the subjects' BMI among the 3 ethnic groups as determined by one-way ANOVA ($F_{(2,160)} = 4.06$, $p = 0.02$). An unpaired t-test with Welch's correction reveals significantly Indian subjects recruited had higher BMI compared to Chinese ($p = 0.01$). There is no significant age difference between Chinese and Malay ($p = 0.15$) and Indian and Malay ($p = 0.19$).

Ethnic group	Gender	Sample size (n)	Age (mean ± SD)	Age range	BMI
Chinese	Female	42	27.6 ± 8.0	21 to 51	20.9 ± 3.6
	Male	30	29.6 ± 10.5	21 to 58	22.8 ± 3.2
	Total	80	28.4 ± 9.1	21 to 58	21.7 ± 3.5
Malay	Female	33	29.1 ± 8.4	21 to 50	22.6 ± 3.4
	Male	14	27.2 ± 8.4	22 to 48	22.7 ± 3.0
	Total	47	28.6 ± 8.3	21 to 50	22.6 ± 3.4
Indian	Female	26	35.2 ± 11.5	21 to 63	23.7 ± 4.6
	Male	16	34.4 ± 11.1	23 to 59	23.6 ± 3.5
	Total	42	34.9 ± 11.2	21 to 63	23.7 ± 4.1

Table 7.1.1. Demographics of the study population.

Out of the 80 Chinese, 47 Malay and 42 Indian subjects recruited, only 57 (71.3%) Chinese, 32 (68.1%) Malay and 25 (59.5%) Indian subjects wear glasses respectively. Subjects without glasses were excluded when MPOD was plotted against Rx. There is a statistical difference in the subjects' Rx between the 3 ethnic groups as determined by one-way ANOVA ($F_{(2,110)}$ = 3.60, p = 0.03). An unpaired t-test with Welch's correction reveals significantly higher degree of myopia in Chinese subjects compared to Indian (p = 0.02) There is no significant difference in myopia between Chinese and Malay (p = 0.41), and Malay and Indian (p = 0.10).

Ethnic group	Sample size (n)	Percentage (%)	Rx* (mean ± SD)
Chinese	57	71.3	-4.05 ± 2.47
Malay	32	68.1	-3.28 ± 2.26
Indian	25	59.5	-2.74 ± 2.67

Table 7.1.2. Refractive error (Rx) of the study population (*Rx is measured from subject's habitual glasses using focimeter and converted into spherical equivalent. Subjects who do not wear glasses are excluded from the above table.)

7.2 MPOD in 3 ethnic groups

The mean MPOD measured by MMD in each ethnic group is summarised in Table 7.2.1. There is no significant difference in MPOD in the 3 ethnic groups as determined by one-way ANOVA ($F_{(2,147)} = 1.033$, $p = 0.36$ at 0.25° retinal eccentricity; $F_{(2,158)} = 0.32$, $p = 0.73$ at 0.50° retinal eccentricity; $F_{(2,154)} = 1.25$, $p = 0.29$ at 1.00° retinal eccentricity; $F_{(2,152)} = 1.71$, $p = 0.18$ at 1.75° retinal eccentricity).

MPOD (mean ± SD)	F0.25 (n)	F0.50 (n)	F1.00 (n)	F1.75 (n)
Chinese	0.67 ± 0.22 (67)	0.57 ± 0.21 (72)	0.39 ± 0.16 (69)	0.20 ± 0.11 (65)
Malay	0.73 ± 0.23 (42)	0.60 ± 0.22 (47)	0.43 ± 0.16 (46)	0.21 ± 0.13 (44)
Indian	0.69 ± 0.27 (42)	0.59 ± 0.27 (42)	0.43 ± 0.19 (42)	0.24 ± 0.15 (42)
Total	0.70 ± 0.25 (151)	0.58 ± 0.23 (161)	0.41 ± 0.17 (157)	0.22 ± 0.13 (151)
ANOVA (p-value)	0.36	0.73	0.29	0.18

Table 7.2.1. MPOD in all ethnic groups at 4 locations measured by MMD.

Table 7.2.2 summarises the p-value from unpaired t-test between 2 ethnic groups at 4 measuring locations measured by MMD. Malay and Indian population seems to have higher macular pigment compared to Chinese at all 4 measuring locations, but it is not statistically significant as determined by unpaired t-test with Welch's correction.

Unpaired t-test	F0.25	F0.50	F1.00	F1.75
Chinese vs Indian	0.69	0.63	0.22	0.09
Chinese vs Malay	0.13	0.42	0.17	0.33
Indian vs Malay	0.39	0.87	0.96	0.46

Table 7.2.2. P-value from unpaired t-test between 2 ethnic groups at 4 measuring locations measured by MMD.

7.3 Gender and MPOD

Female seems to have lower MPOD than male using MMD in all 3 ethnic group, but it is not statistically significant in MPOD between genders as determined by unpaired t-test (Table 7.3.1).

Ethnic group	Gender	MPOD			
		F0.25 (n)	F0.50 (n)	F1.00 (n)	F1.75 (n)
Chinese	Female	0.64 ± 0.23 (38)	0.55 ± 0.22 (42)	0.38 ± 0.17 (40)	0.18 ± 0.13 (41)
	Male	0.70 ±0.21 (29)	0.59 ± 0.20 (30)	0.38 ± 0.16 (30)	0.20 ± 0.10 (28)
	P-value	0.28	0.39	0.89	0.38
Malay	Female	0.70 ± 0.21 (33)	0.56 ± 0.22 (33)	0.41 ± 0.16 (33)	0.20 ± 0.14 (31)
	Male	0.82 ± 0.25 (12)	0.68 ± 0.20 (14)	0.48 ± 0.14 (13)	0.25 ± 0.11 (13)
	P-value	0.17	0.10	0.17	0.24
Indian	Female	0.69 ± 0.29 (26)	0.58 ± 0.28 (26)	0.45 ± 0.21 (26)	0.24 ± 0.17 (26)
	Male	0.68 ± 0.26 (16)	0.60 ± 0.26 (16)	0.41 ± 0.18 (16)	0.23 ± 0.13 (16)
	P-value	0.93	0.81	0.54	0.71
Total	Female	0.67 ± 0.24 (93)	0.56 ± 0.24 (101)	0.41 ± 0.18 (99)	0.20 ± 0.15 (98)
	Male	0.72 ± 0.24 (57)	0.61 ± 0.22 (60)	0.41 ± 0.16 (59)	0.22 ± 0.11 (57)
	P-value	0.25	0.15	0.86	0.40

Table 7.3.1. Mean MPOD and p-value between genders.

7.4 Comparison between MMD and MPS II

Since MPS II is only used in Chinese subjects, MPOD measured by MMD at 0.50° retinal eccentricity, will be compared with the MPS II results (Table 7.4.1).

Chinese (n = 65)	MMD	MPS II
MPOD (mean ± SD)	0.57 ± 0.21	0.48 ± 0.17
Paired T-test	p < 0.0001	

Table 7.4.1. Mean and p-value of MPOD at 0.50° retinal eccentricity using MMD and MPS II.

Bland Altman plot was used to compare the difference in MP measurement using 2 instruments (Figure 7.4.1). There is an average difference of 0.09, with a limit of agreement of 0.37 and -0.17, between MMD and MPS II.
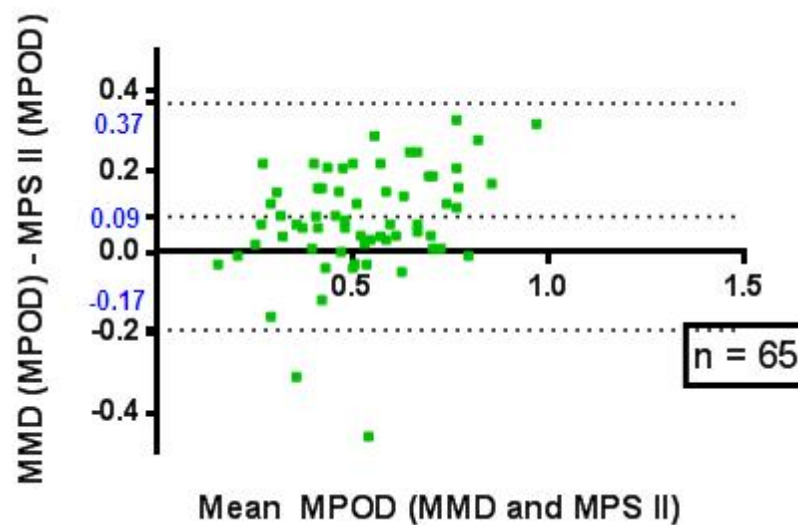

Figure 7.4.1. Difference in MPOD at 0.50° retinal eccentricity measured by MMD and MPS II.

As shown in Figure 7.4.2, MPOD measured using MMD is significantly higher and positively correlated with data obtained from MPS II ($p < 0.0001$, $R = 0.72$). In other words, the measured MPOD values from MMD were consistently higher than those obtained by MPS II.
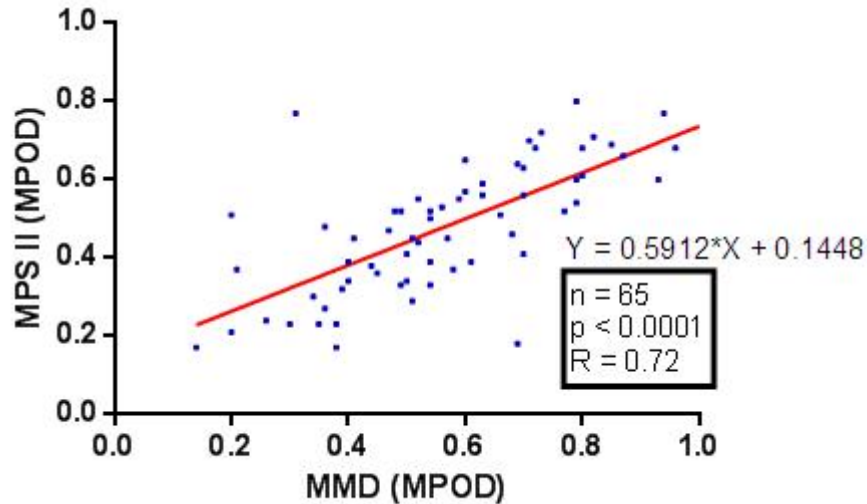
Figure 7.4.2. Relationship between MPOD at 0.50° retinal eccentricity measured by MMD and MPS II.

The coefficient of variability of the Chinese subject's performance in MPOD measurement were compared (Figure 7.4.3). There is no significant correlation between the standard deviation of MPOD measured by MMD and MPS II ($p = 0.82$, $R = 0.03$).
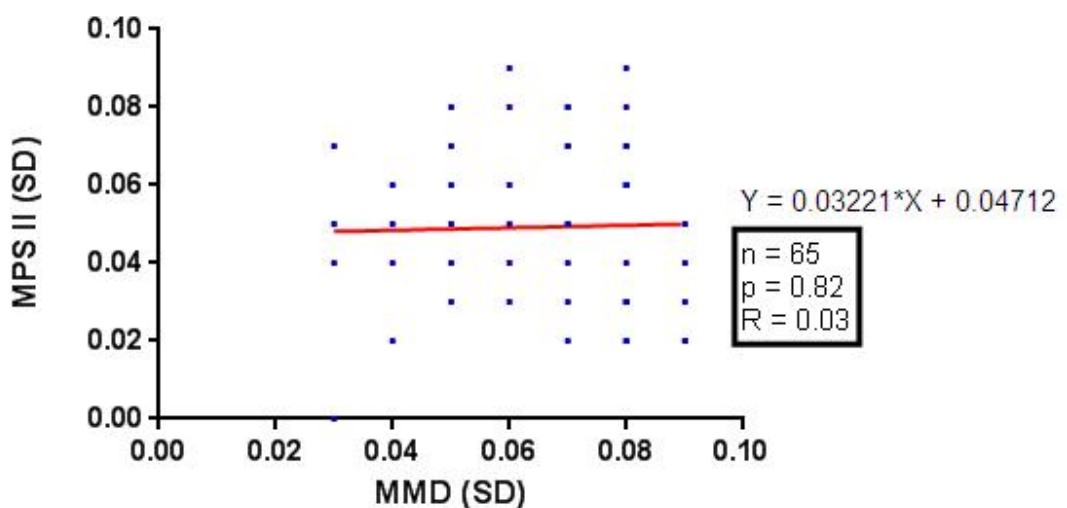
Figure 7.4.3. Relationship between standard deviation of the MP measurement in Chinese subjects when performing at 0.50° retinal eccentricity with MMD and MPS II.

There are two modes in MPS II: expert and standard mode. Expert mode calculates MPOD using a central target and a peripheral target, while standard mode calculate MPOD using the value measured at the central target and a European database obtained from a large population. Therefore, it is not necessary to measure the reference value from peripheral target.

The relationship between expert mode and standard mode is analysed. Figure 7.4.4 shows that MPOD using expert mode is strongly correlated with the results obtained by standard mode ($p < 0.0001$; $R = 0.97$).
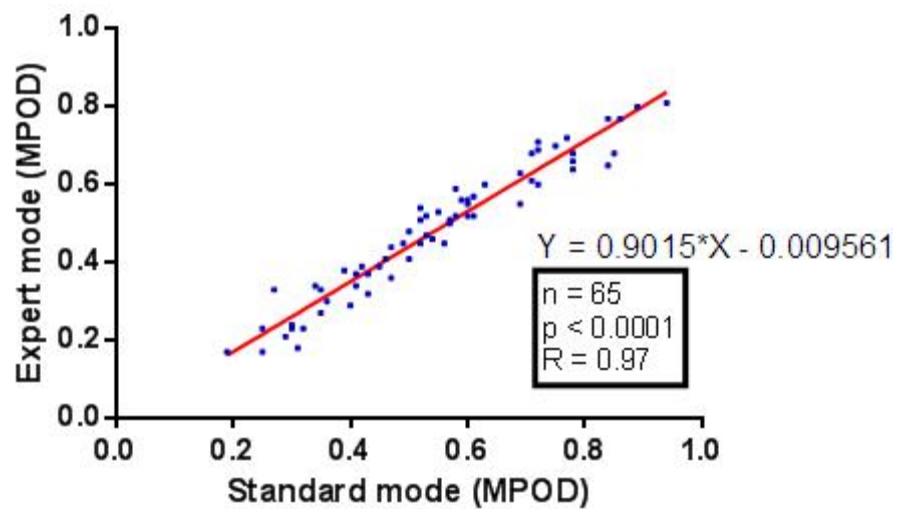
Figure 7.4.4. Relationship between MPOD measured using MPS II in standard mode and expert mode in Chinese subjects.

Time taken to measure MPOD using MMD and MPS II was compared using linear regression. Results reveal significantly longer measurement time in MPS II by subjects with higher MPOD (p = 0.02, R = 0.28). This relationship was not found in MMD (p = 0.60, R = 0.07). Time taken to measure MP using MPS II is dependent on the level of MP.
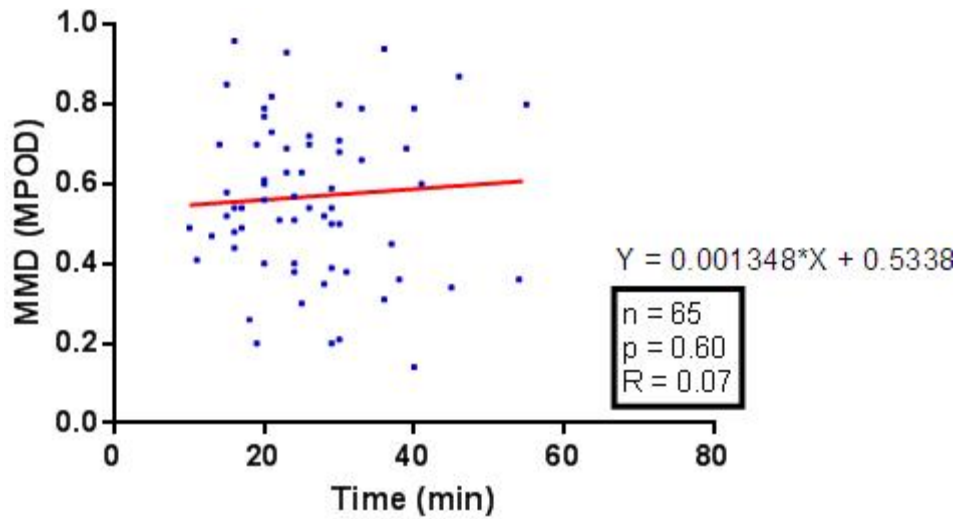
Figure 7.4.5. Relationship between time taken and MP measurement using MMD.
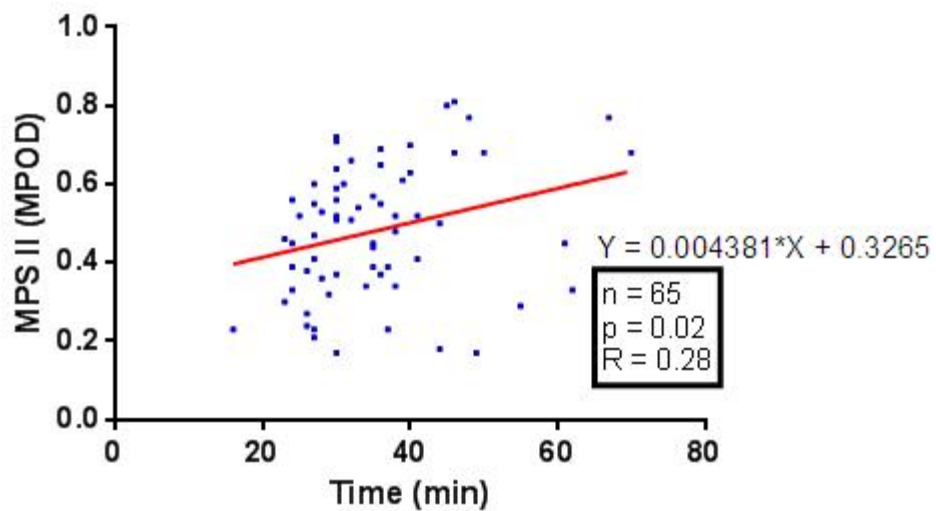
Figure 7.4.6. Relationship between time taken and MP measurement using MPS II.

7.5 Different types of MP SP

As shown in Figure 7.5.1, there are 4 different types of SP identified in the current study population. Description is as follows:

1. Exponential (Type A): Type A is the typical exponential curve whereby MP decreases with increasing retinal eccentricity.

2. Central dip (Type B): MP at 0.50° retinal eccentricity is higher than that at 0.25° retinal eccentricity.

3. Secondary peak (Type C): MP is lower at 0.50° retinal eccentricity compared to 0.25° retinal eccentricity and the MP level increases at 1.00° retinal eccentricity and decreasing at 1.75° retinal eccentricity.

4. Plateau (Type D): Type D has the same amount of MP at 2 adjacent locations of the fovea (0.25° and 0.50° retinal eccentricities).

Figure 7.5.1. Illustration of different types of MP SP.

Table 7.5.1 tabulates the frequency distribution of MP SP in all ethnic groups and genders. Majority of the population has a typical exponential MP SP, in which MP declines with eccentricity, in all ethnic groups. As there is a different sample size in each ethnic group, the frequency distribution 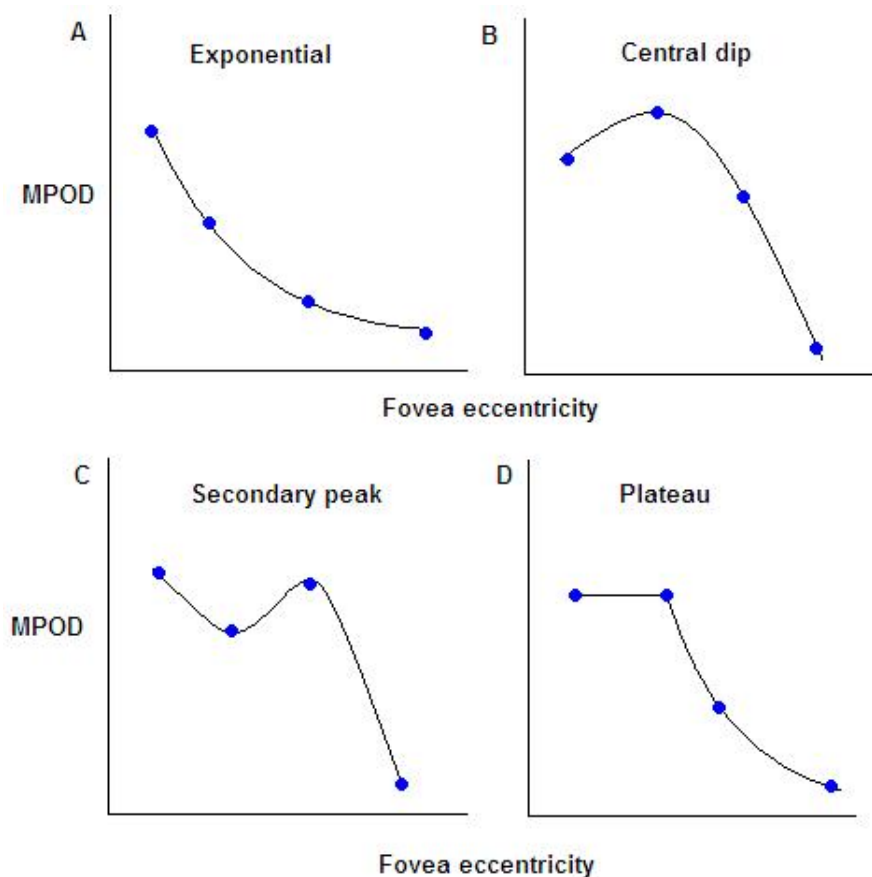is converted into percentage for easier comparison. Histograms (Figure 7.5.2 to 7.5.5) are drawn to illustrate the frequency distribution of MP SP in each ethnic group and gender.

Ethnic group	Gender (%[+])	Spatial profile							
		Type A		Type B		Type C		Type D	
		n	%*	n	%	n	%	n	%
Chinese	Female (57.1)	23	63.9	10	27.8	2	5.6	1	2.8
	Male (42.9)	23	85.2	2	7.4	1	3.7	1	3.7
	Total	46	73.0	12	19.0	3	4.8	2	3.2
Malay	Female (70.7)	20	69.0	4	13.8	3	10.3	2	6.9
	Male (24.3)	12	100.0	0	0.0	0	0.0	0	0.0
	Total	32	78.0	4	9.8	3	7.3	2	4.9
Indian	Female (61.9)	16	61.5	6	23.1	4	15.4	0	0.0
	Male (38.1)	10	62.5	5	31.3	1	6.3	0	0.0
	Total	26	61.9	11	26.2	5	11.9	0	0.0

Table 7.5.1. Frequency distribution of MP SP in all ethnic groups and genders.

[+]Percentage calculated from each gender to total subject from each ethnic group.

* Percentage calculated from sample size of each SP type to the total.

Fisher exact test revealed that the type of MP SP is borderline different in each ethnic group (p = 0.06). When Type D (plateau) is excluded from the analysis (due to its small number of subjects (5%)), Fisher exact test revealed that the type of MP SP is significantly different in each ethnic group (p = 0.05). Figure 7.5.2 shows that majority of the subjects have exponential MP SP (Malay = 78%, Chinese = 73%, Indian = 62%). Overall 22% to 38% of the subjects have atypical MP SP in form of central dip, secondary peak and plateau. Central dip, followed by secondary peak, was the most common atypical MP SP seen in our study. Both were commonly observed in Indian (Central dip: Indian = 26%, Chinese = 19%, Malay = 10%; Secondary peak: Indian = 12%, Chinese = 5%, Malay = 7%).
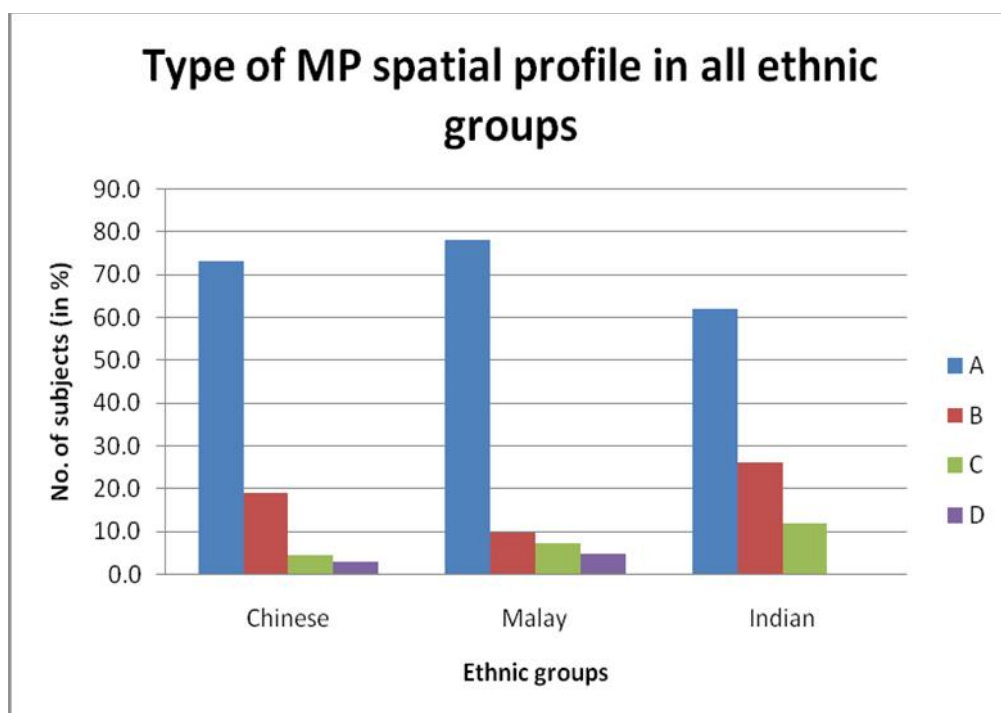
Figure 7.5.2. Frequency distribution of MP SP in all ethnic groups.

Females have higher percentage of subjects with central dip and secondary peak MP SP compared to males in all ethnic groups (except Indian males have higher percentage of subjects with central dip MP SP than Indian females).

Figure 7.5.3. MP SP difference between genders in Chinese.

Figure 7.5.4. MP SP difference between genders in Malay.
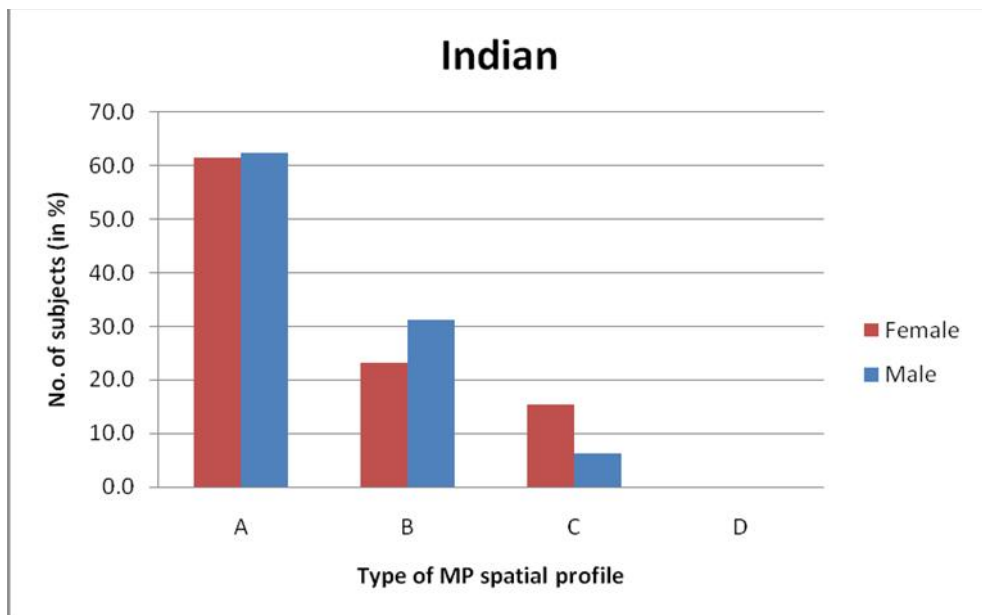
Figure 7.5.5. MP SP difference between genders in Indian.

Table 7.5.2 summaries the mean MPOD in different types of MP SP in each ethnic group.

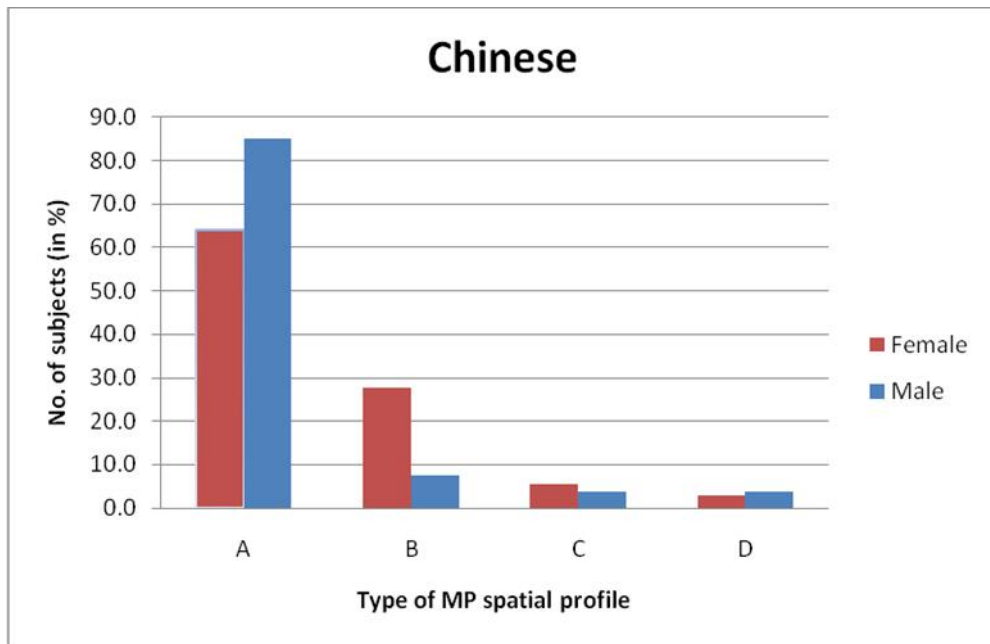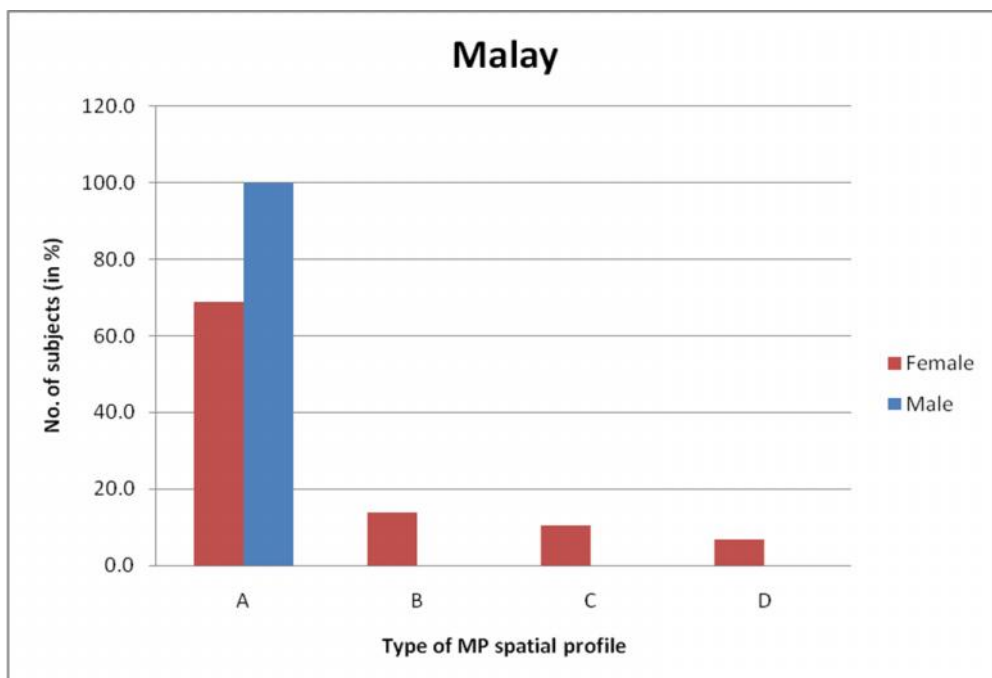| Spatial profile	Ethnic group	MPOD (mean ± SD)			
		F0.25	F0.50	F1.00	F1.75
Type A	Chinese	0.74 ± 0.22	0.60 ± 0.22	0.40 ± 0.16	0.19 ± 0.11
	Malay	0.78 ± 0.20	0.63 ± 0.20	0.43 ± 0.15	0.21 ± 0.12
	Indian	0.71 ± 0.24	0.57 ± 0.25	0.41 ± 0.21	0.21 ± 0.14
Type B	Chinese	0.52 ± 0.16	0.57 ± 0.13	0.40 ± 0.12	0.18 ± 0.11
	Malay	0.58 ± 0.37	0.71 ± 0.33	0.43 ± 0.27	0.30 ± 0.26
	Indian	0.62 ± 0.34	0.76 ± 0.26	0.47 ± 0.16	0.28 ± 0.16
Type C	Chinese	0.54 ± 0.03	0.42 ± 0.07	0.43 ± 0.07	0.23 ± 0.07
	Malay	0.61 ± 0.15	0.30 ± 0.11	0.31 ± 0.15	0.17 ± 0.16
	Indian	0.72 ± 0.34	0.33 ± 0.23	0.47 ± 0.19	0.26 ± 0.19
Type D	Chinese	0.56 ± 0.15	0.56 ± 0.15	0.26 ± 0.28	0.11 ± 0.15
	Malay	0.55 ± 0.12	0.49 ± 0.20	0.72 ± 0.24	0.72 ± 0.24
	Indian	NA	NA	NA	NA

Table 7.5.2. Mean MPOD of each MP SP types.

Table 7.5.3 shows the p-value between Type A and B in Chinese and Indian subjects from the unpaired t-test. There is a significant difference in MPOD between Type A and B at 0.25° retinal eccentricity in Chinese subjects (p = 0.0001), and Chinese and Indian subjects (p = 0.01). There is also a significant difference in MPOD between Type A and B at 0.50° retinal eccentricity in Indian subjects (p = 0.05).

Chinese and Indian with type A MP SP does not have any significant difference in all retinal eccentricities. There is a significant difference between Chinese and Indian with Type B MP SP at 0.50° retinal eccentricity (p = 0.04).

Ethnic group (MP SP type)	MPOD			
	F0.25	F0.50	F1.00	F1.75
Chinese (A vs B)	0.0001*	0.49	0.98	0.68
Indian (A vs B)	0.43	<0.05*	0.29	0.21
Chinese vs Indian (A)	0.66	0.57	0.90	0.53
Chinese vs Indian (B)	0.43	0.04*	0.21	0.08

Table 7.5.3. P-value of Chinese and Indian subjects with type A and B MP SP.

(*Malay is not included in the comparison because n = 4.)

7.6 Relationship between MPOD with age

The overall relationship between age and MPOD is not significant. However, once we divide subject into each ethnic group, MPOD, measured using MMD, is lower at 0.50° and 1.00° retinal eccentricities in Chinese subjects with increasing age (0.50° : p = 0.01, R = -0.32; 1.00° : p = 0.01, R = -0.30), and at 0.25° and 1.00° retinal eccentricities in Malay subjects with increasing age (0.25° : p = 0.03, R = -0.33; 1.00° : p = 0.04, R = -0.31). However, this relationship is not found in Indian. Similarly, an inverse relationship was observed between MPOD, measured using MPS II, and increasing age among the Chinese (p = 0.03, R = -0.26).

Age vs MPOD	F0.25	F0.50	F1.00	F1.75
Chinese MPS II	NA	n = 73 p = 0.03* R = -0.26	NA	NA
Chinese MMD	n = 67 p = 0.07 R = -0.22	n = 72 p = 0.01* R = -0.32	n = 70 p = 0.01* R = -0.30	n = 69 p = 0.15 R = -0.17
Malay MMD	n = 41 p = 0.03* R = -0.33	n = 47 p = 0.27 R = -0.16	n = 46 p = 0.04* R = -0.31	n = 44 p = 0.05 R = -0.29
Indian MMD	n = 42 p = 0.78 R = 0.05	n = 42 p = 0.50 R = 0.11	n = 42 p = 0.95 R = 0.01	n = 42 p = 0.63 R = 0.08
Total MMD	n = 150 p = 0.10 R = -0.14	n = 161 p = 0.15 R = -0.11	n = 158 p > 0.05 R = -0.15	n = 155 p = 0.42 R = -0.07

Table 7.6.1. Relationship between MPOD and age.

Figure 7.6.1. Relationship between MPOD and age in Chinese subjects at 0.50° retinal eccentricity using MPS II.

Figure 7.6.2. Relationship between MPOD and age in Chinese subjects at 0.25° retinal eccentricity using MMD.

Figure 7.6.3. Relationship between MPOD and age in Chinese subjects at 0.50° retinal eccentricity using MMD.

Figure 7.6.4. Relationship between MPOD and age in Chinese subjects at 1.00° retinal eccentricity using MMD.
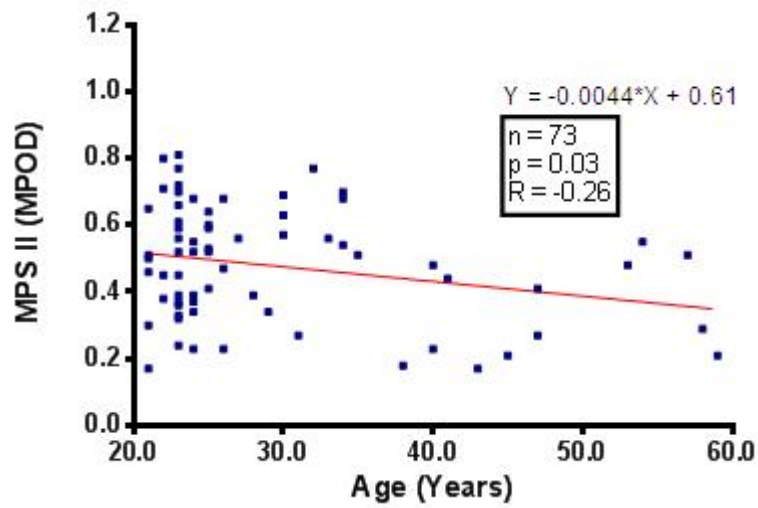
Figure 7.6.5. Relationship between MPOD and age in Chinese subjects at 1.75° retinal eccentricity using MMD.

Figure 7.6.6. Relationship between MPOD and age in Malay subjects at 0.25° retinal eccentricity using MMD.
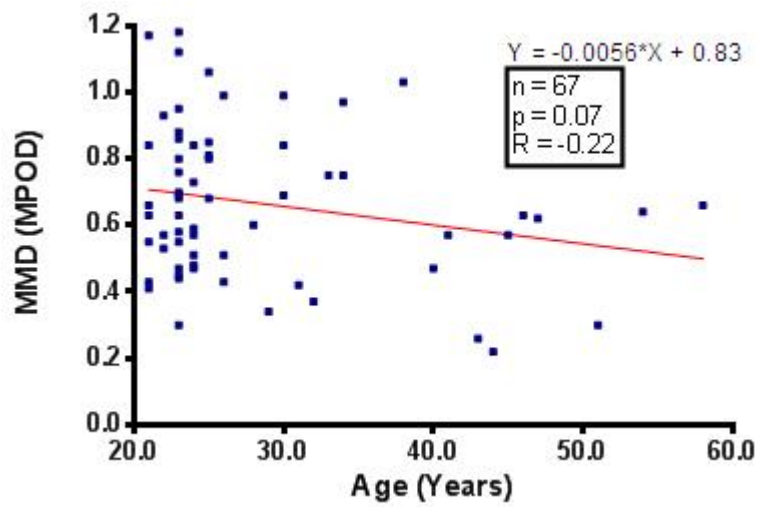
Figure 7.6.7. Relationship between MPOD and age in Malay subjects at 0.50° retinal eccentricity using MMD.

Figure 7.6.8. Relationship between MPOD and age in Malay subjects at 1.00° retinal eccentricity using MMD.

Figure 7.6.9. Relationship between MPOD and age in Malay subjects at 1.75° retinal eccentricity using MMD.

Figure 7.6.10. Relationship between MPOD and age in Indian subjects at 0.25° retinal eccentricity using MMD.
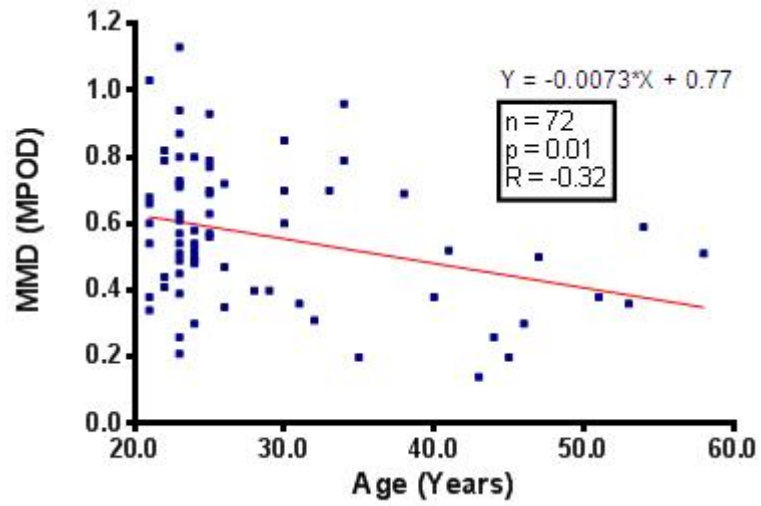
Figure 7.6.11. Relationship between MPOD and age in Indian subjects at 0.50° retinal eccentricity using MMD.

Figure 7.6.12. Relationship between MPOD and age in Indian subjects at 1.00° retinal eccentricity using MMD.

Figure 7.6.13. Relationship between MPOD and age in Indian subjects at 1.75° retinal eccentricity using MMD.

Figure 7.6.14. Relationship between MPOD and age in all subjects at 0.25° retinal eccentricity using MMD.

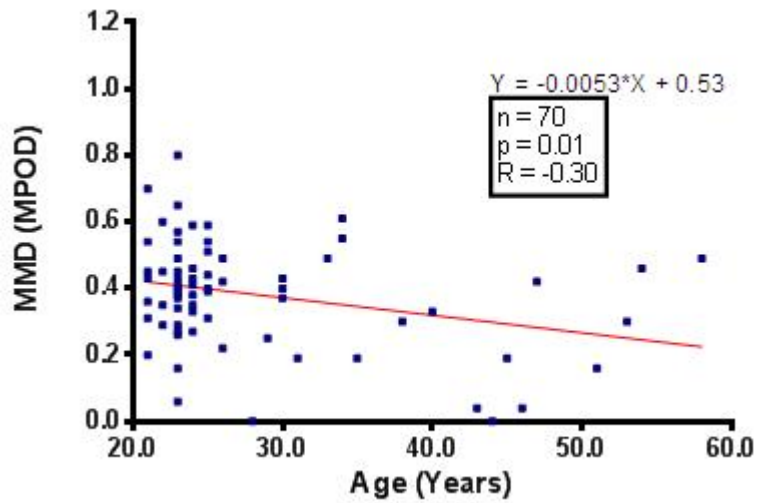*Figure 7.6.15. Relationship between MPOD and age in all subjects at 0.50° retinal eccentricity using MMD.*

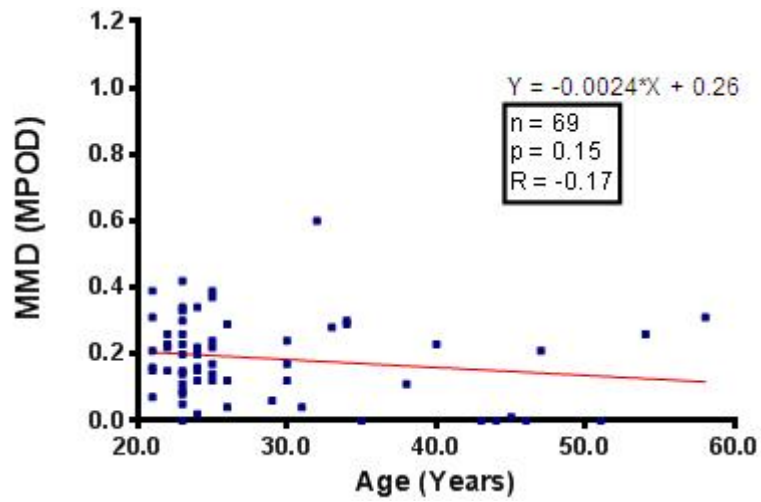*Figure 7.6.16. Relationship between MPOD and age in all subjects at 1.00° retinal eccentricity using MMD.*

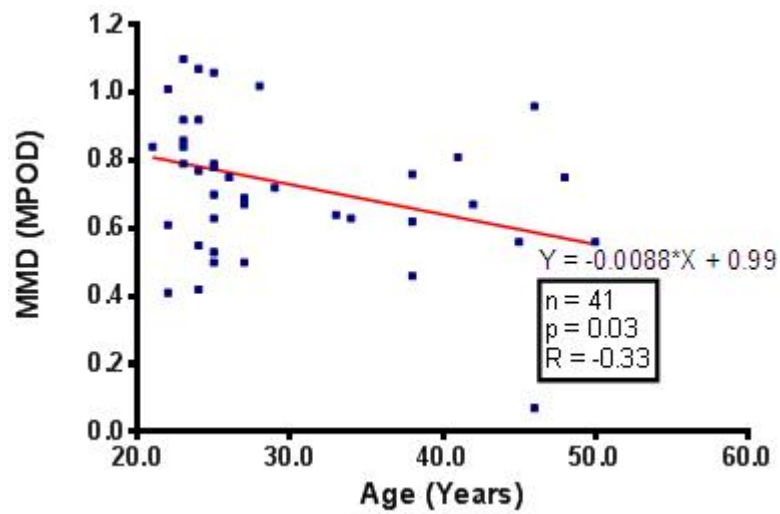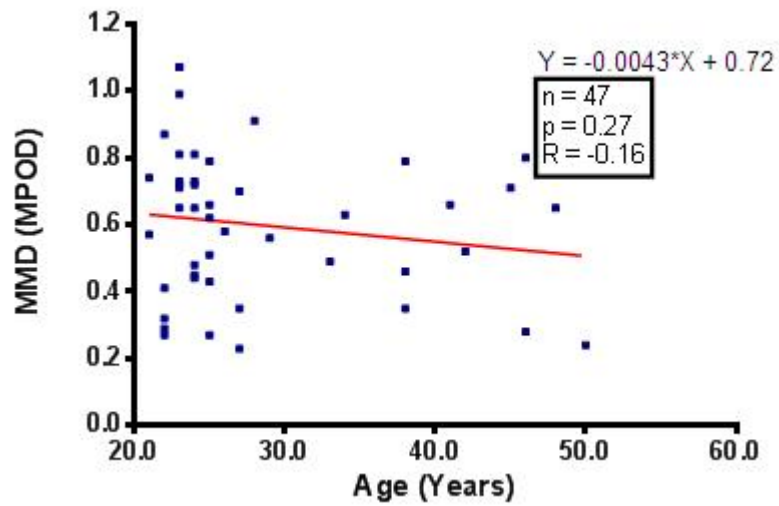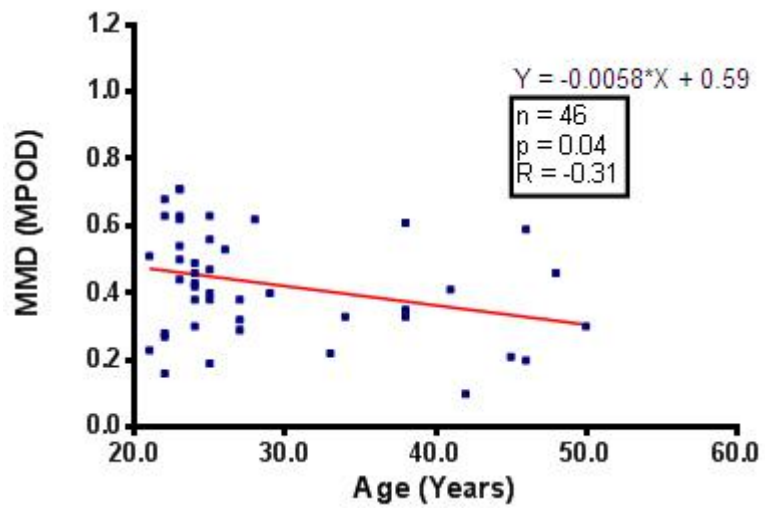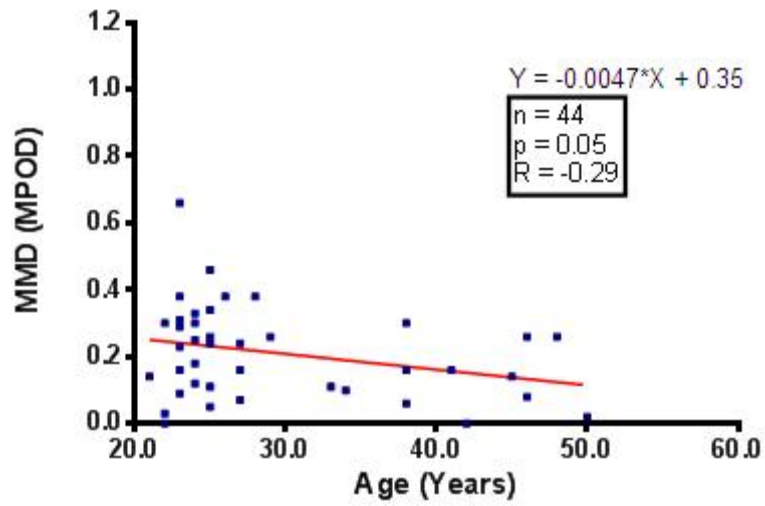*Figure 7.6.17. Relationship between MPOD and age in all subjects at 1.75° retinal eccentricity using MMD.*
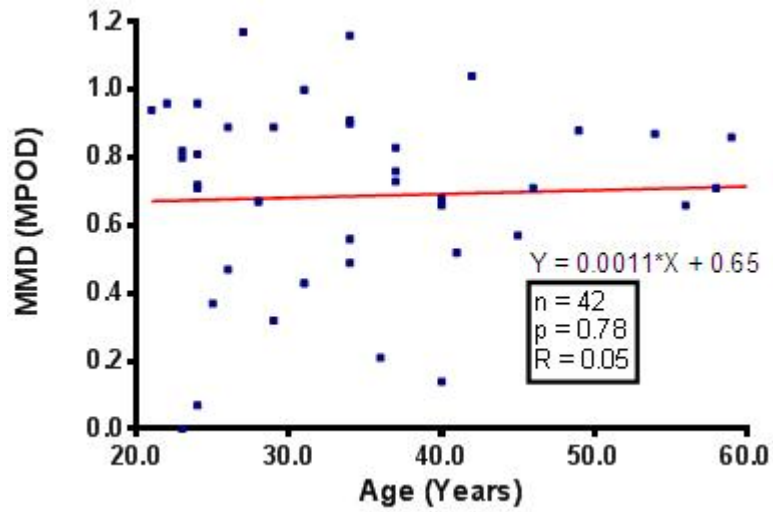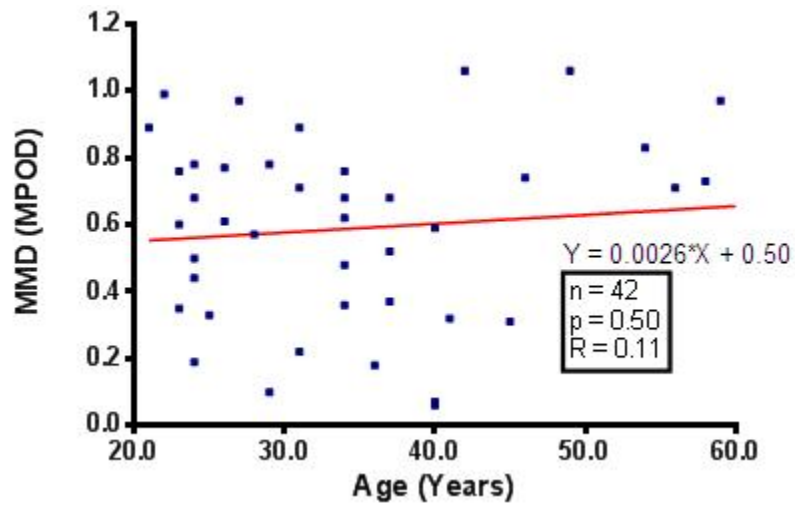
7.7 Relationship between MPOD with BMI

There is a significantly lower MPOD in subjects with higher BMI in the total study population at 0.25° retinal eccentricity ($p < 0.05$, $R = -0.16$). However, the correlation is weak. When the subjects were separated into their ethnicity, there is no statistical difference between BMI and MPOD at all retinal eccentricities with both MMD (in all 3 ethnicities) and MPS II (in Chinese).

BMI vs MPOD	F0.25	F0.50	F1.00	F1.75
Chinese MPS II	NA	n = 73 p = 0.17 R = -0.16	NA	NA
Chinese MMD	n = 67 p = 0.18 R = -0.16	n = 72 p = 0.08 R = -0.21	n = 70 p = 0.13 R = -0.18	n = 69 p = 0.33 R = -0.12
Malay MMD	n = 41 p = 0.67 R = -0.07	n = 47 p = 0.93 R = 0.01	n = 46 p = 0.70 R = -0.06	n = 44 p = 0.85 R = -0.04
Indian MMD	n = 42 p = 0.08 R = -0.27	n = 42 p = 0.82 R = -0.04	n = 42 p = 0.62 R = -0.08	n = 42 p = 0.85 R = 0.04
Total MMD	n = 150 p < 0.05* R = -0.16	n = 161 p = 0.32 R = -0.08	n = 158 p = 0.82 R = -0.07	n = 155 p = 1.00 R = 0.00002

Table 7.7.1. Relationship between MPOD and BMI.

Figure 7.7.1. Relationship between MPOD and BMI in Chinese subjects at 0.50° retinal eccentricity using MPS II.

Figure 7.7.2. Relationship between MPOD and BMI in Chinese subjects at 0.25° retinal eccentricity using MMD.

Figure 7.7.3. Relationship between MPOD and BMI in Chinese subjects at 0.50° retinal eccentricity using MMD.
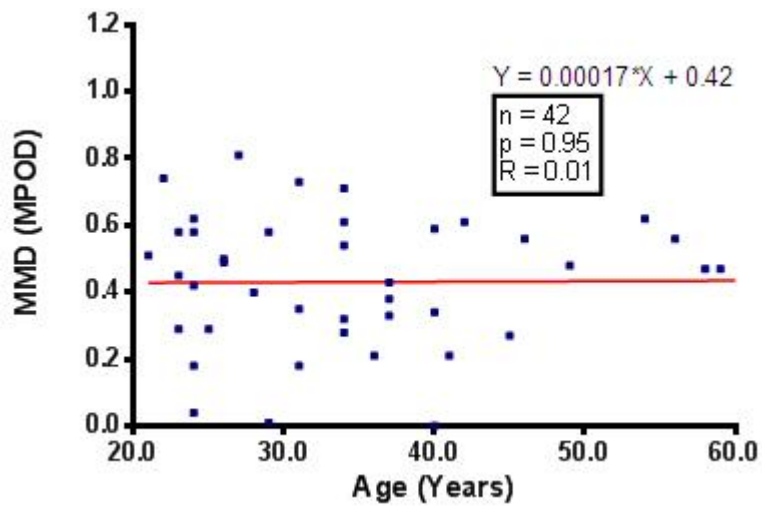

Figure 7.7.4. Relationship between MPOD and BMI in Chinese subjects at 1.00° retinal eccentricity using MMD.
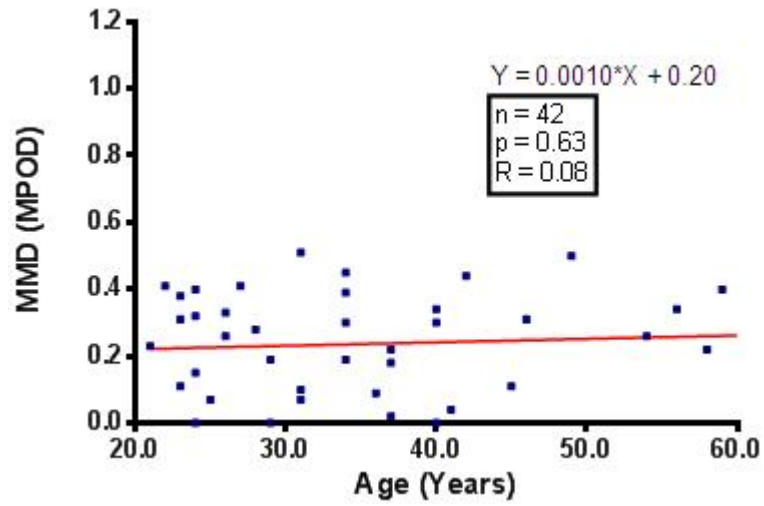
Figure 7.7.5. Relationship between MPOD and BMI in Chinese subjects at 1.75° retinal eccentricity using MMD.

Figure 7.7.6. Relationship between MPOD and BMI in Malay subjects at 0.25° retinal eccentricity using MMD.

Figure 7.7.7. Relationship between MPOD and BMI in Malay subjects at 0.50° retinal eccentricity using MMD.

Figure 7.7.8. Relationship between MPOD and BMI in Malay subjects at 1.00° retinal eccentricity using MMD.

Figure 7.7.9. Relationship between MPOD and BMI in Malay subjects at 1.75° retinal eccentricity using MMD.
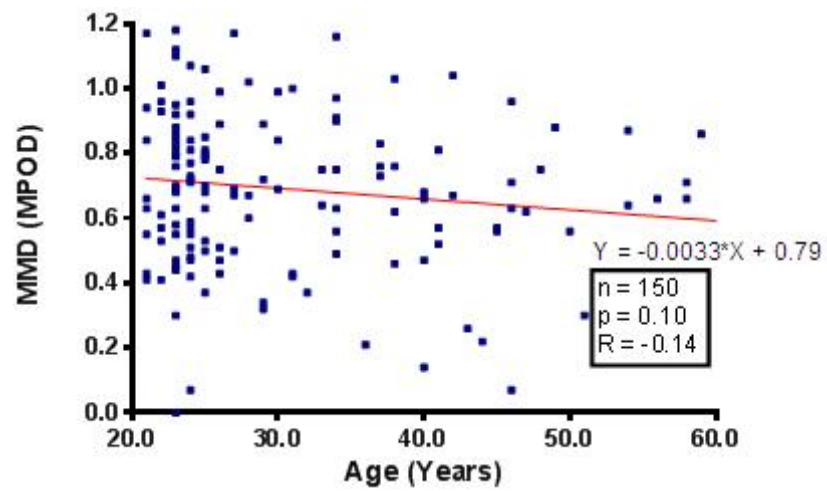

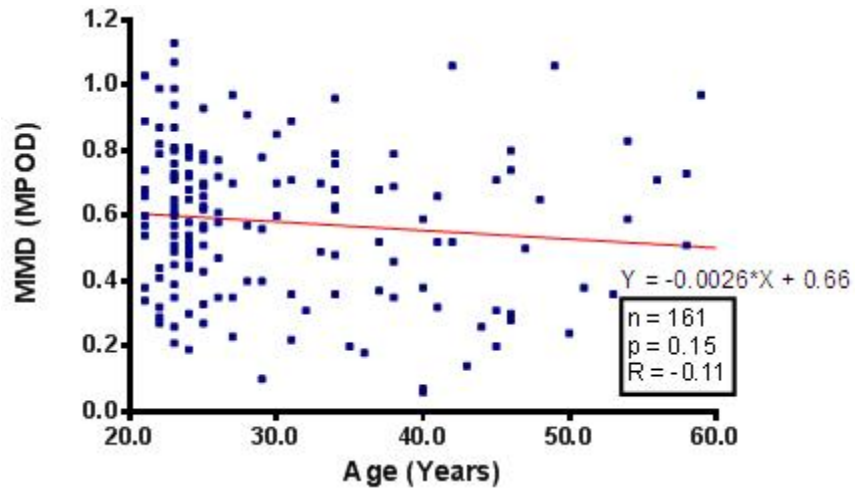*Figure 7.7.10. Relationship between MPOD and BMI in Indian subjects at 0.25° retinal eccentricity using MMD.*

Figure 7.7.11. Relationship between MPOD and BMI in Indian subjects at 0.50° retinal eccentricity using MMD.
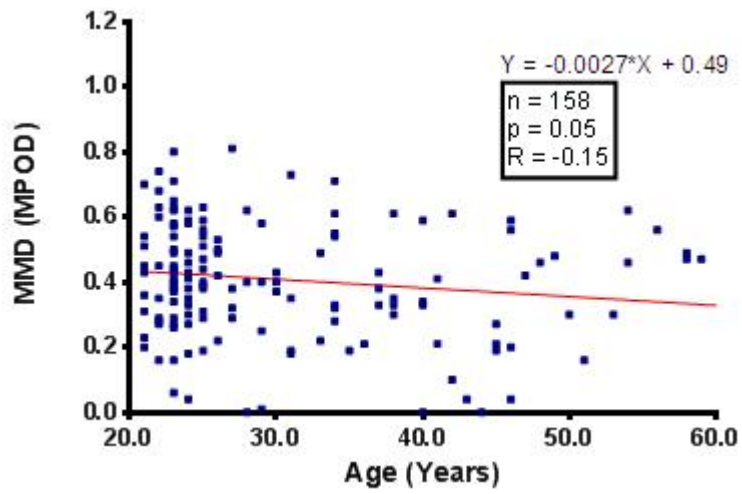

Figure 7.7.12. Relationship between MPOD and BMI in Indian subjects at 1.00° retinal eccentricity using MMD.
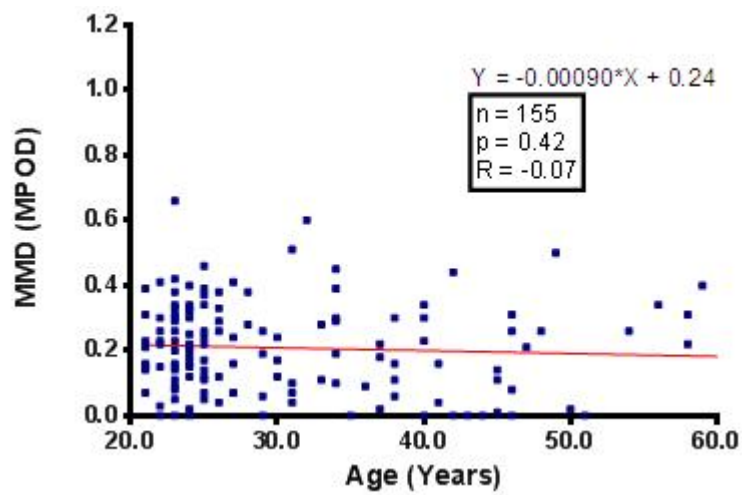
Figure 7.7.13. Relationship between MPOD and BMI in Indian subjects at 1.75° retinal eccentricity using MMD.

Figure 7.7.14. Relationship between MPOD and BMI in all subjects at 0.25° retinal eccentricity using MMD.

Figure 7.7.15. Relationship between MPOD and BMI in all subjects at 0.50° retinal eccentricity using MMD.

Figure 7.7.16. Relationship between MPOD and BMI in all subjects at 1.00° retinal eccentricity using MMD.

Figure 7.7.17. Relationship between MPOD and BMI in all subjects at 1.75° retinal eccentricity using MMD

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7.8 Relationship between MPOD with Rx

Rx is compared to MPOD at all 4 locations using MMD in all ethnic groups, and MPS II in Chinese. There is a significantly lower MPOD at 0.25°, 0.50° and 1.00° retinal eccentricity, using MMD with higher degree of myopia in Chinese subjects only ($p = 0.02$, $R = 0.32$ at 0.25° retinal eccentricity, $p = 0.03$, $R = 0.29$ at 0.50° retinal eccentricity and $p = 0.04$; $R = 0.28$ at 1.00° retinal eccentricity). This relationship is not found in Malay and Indian, or MPS II in Chinese. When all the subjects were analysed together, only MPOD at 0.25° and 0.50° retinal eccentricity are lower with higher degree of myopia.

Rx vs MPOD	F0.25	F0.50	F1.00	F1.75
Chinese MPS II	NA	n = 59 p = 0.30 R = -0.14	NA	NA
Chinese MMD	n = 54 p = 0.02* R = 0.32	n = 57 p = 0.03* R = 0.29	n = 55 p = 0.04* R = 0.28	n = 55 p = 0.81 R = -0.03
Malay MMD	n = 28 p = 0.69 R = -0.08	n = 31 p = 0.11 R = -0.06	n = 31 p = 0.50 R = 0.13	n = 30 p = 0.77 R = 0.06
Indian MMD	n = 27 p = 0.25 R = 0.23	n = 27 p = 0.26 R = 0.22	n = 27 p = 0.67 R = -0.08	n = 27 p = 0.84 R = -0.04
Total MMD	n = 109 p = 0.01* R = 0.25	n = 115 p = 0.02* R = 0.22	n = 113 p = 0.06 R = 0.17	n = 117 p = 0.61 R = 0.05

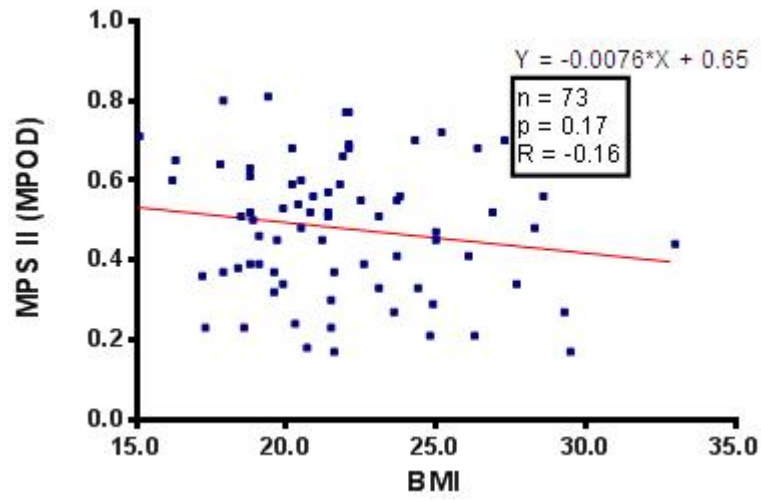*Table 7.8.1. Relationship between MPOD and Rx.*
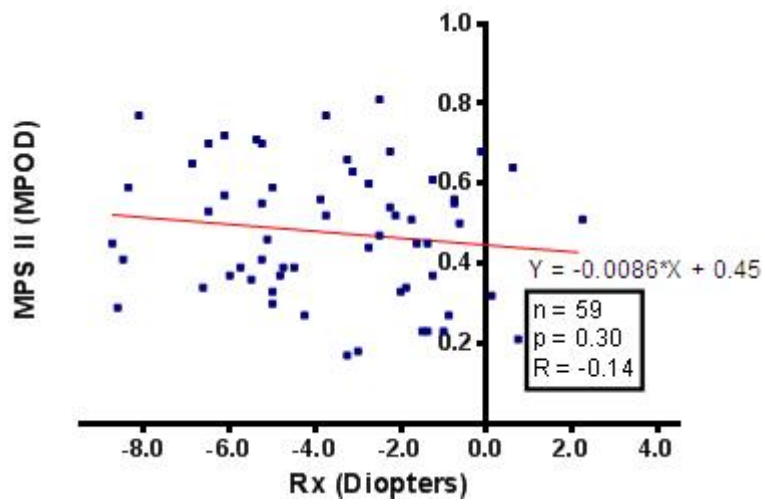
Figure 7.8.1. Relationship between MPOD and Rx in Chinese subjects at 0.50° retinal eccentricity using MPS II.
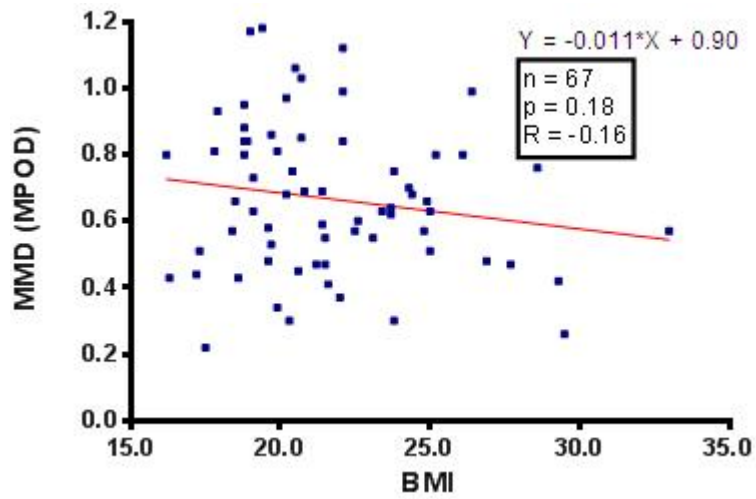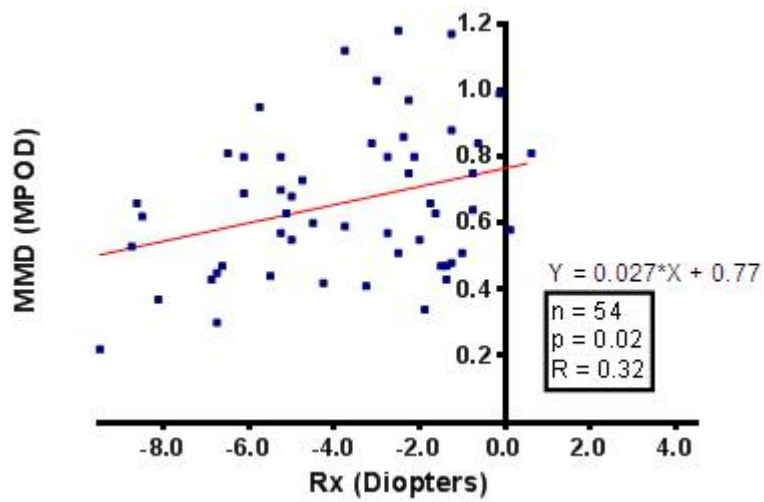

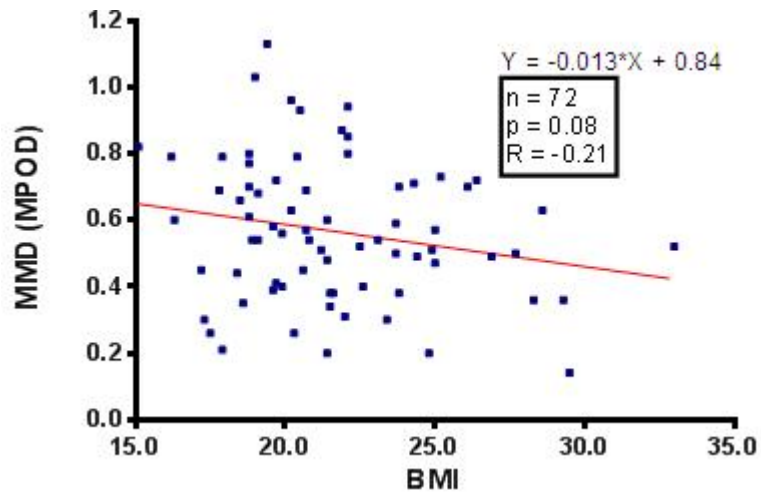*Figure 7.8.2. Relationship between MPOD and Rx in Chinese subjects at 0.25° retinal eccentricity using MMD.*
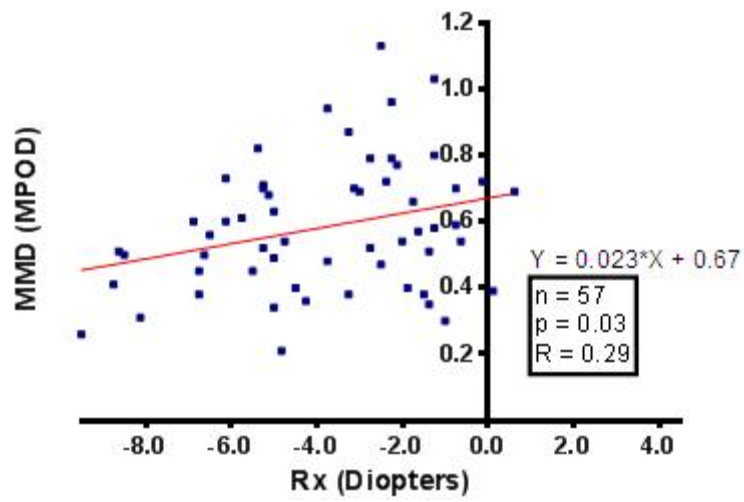
Figure 7.8.3. Relationship between MPOD and Rx in Chinese subjects at 0.50° retinal eccentricity using MMD.
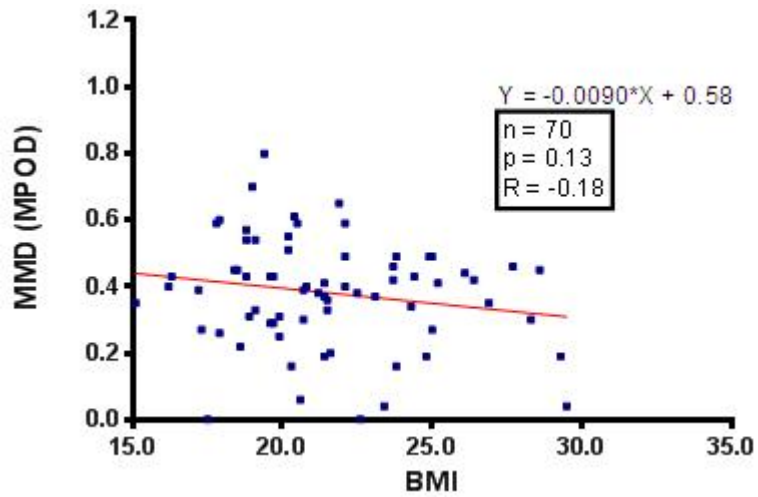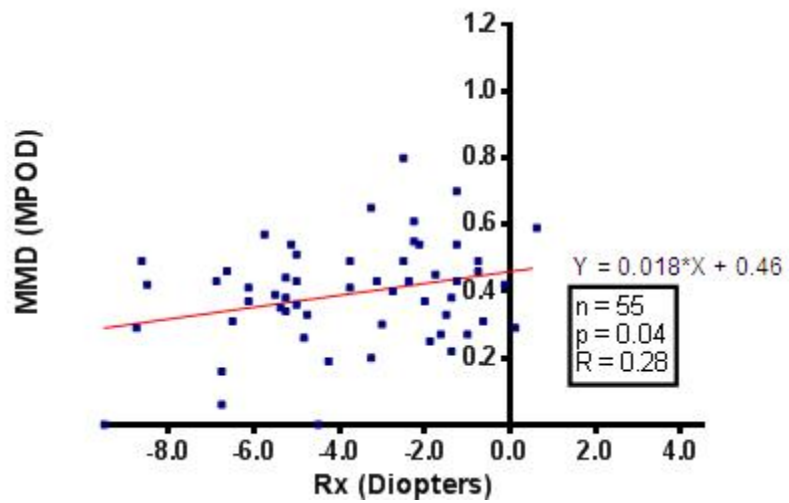

Figure 7.8.4. Relationship between MPOD and Rx in Chinese subjects at 1.00° retinal eccentricity using MMD.
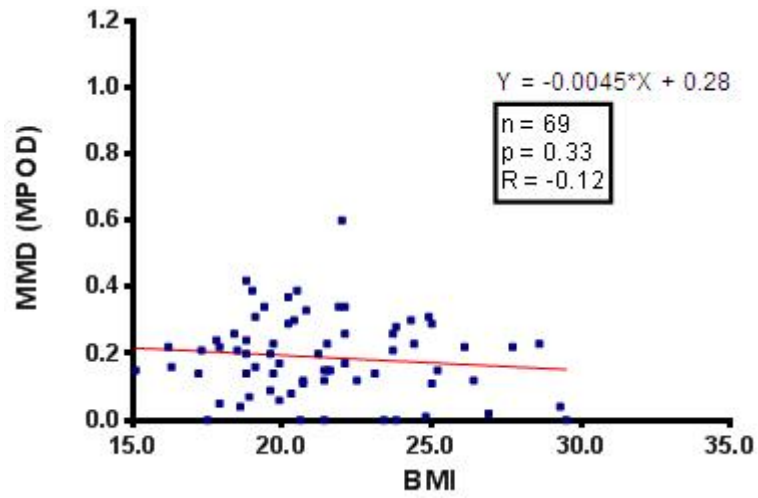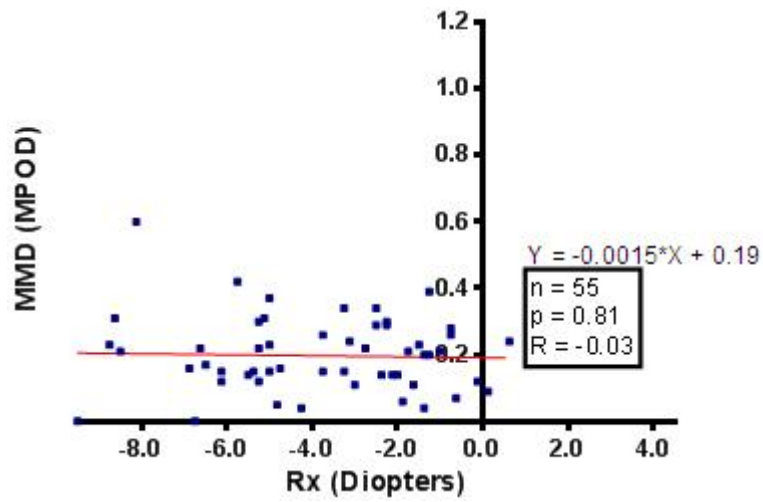
Figure 7.8.5. Relationship between MPOD and Rx in Chinese subjects at 1.75° retinal eccentricity using MMD.
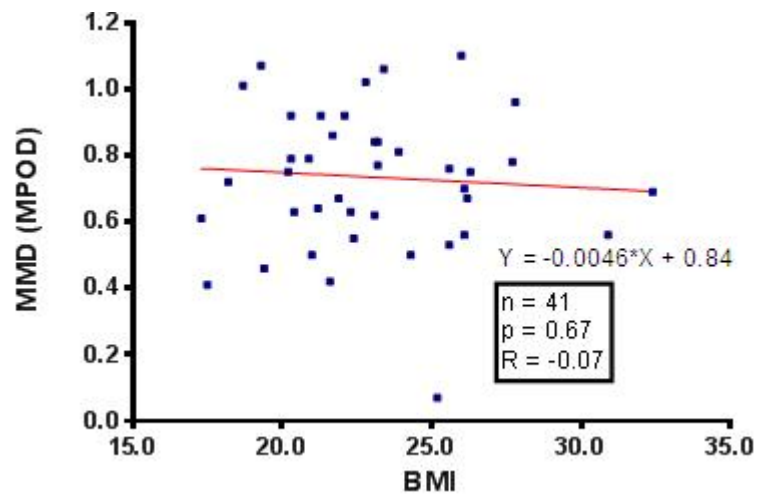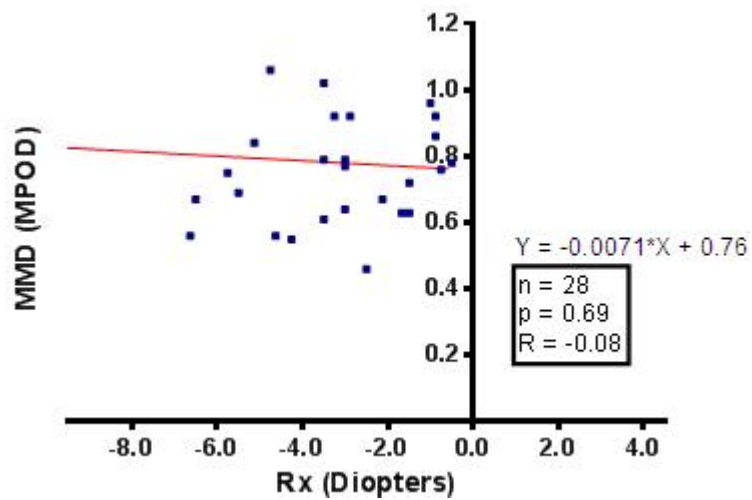

Figure 7.8.6. Relationship between MPOD and Rx in Malay subjects at 0.25° retinal eccentricity using MMD.
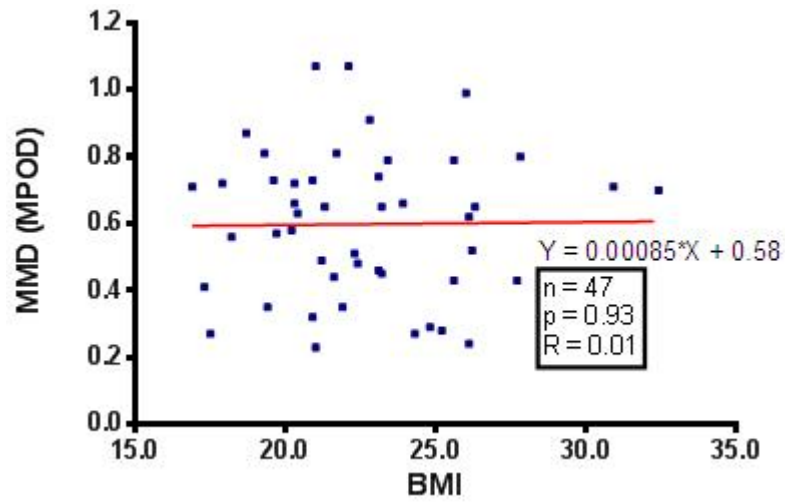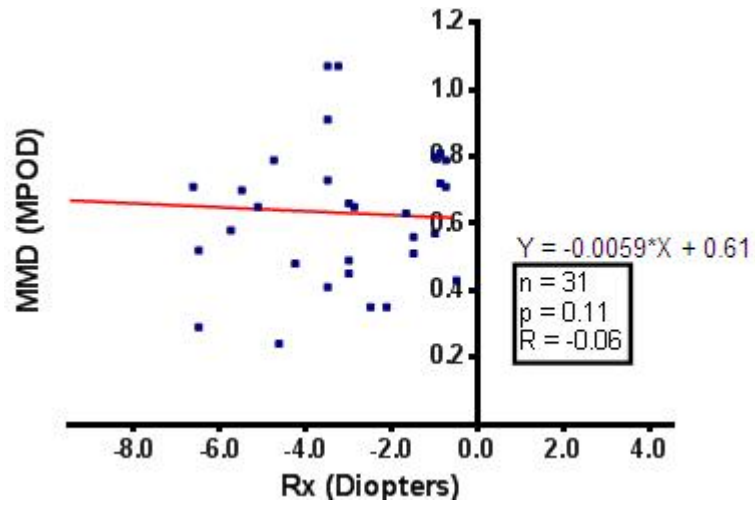
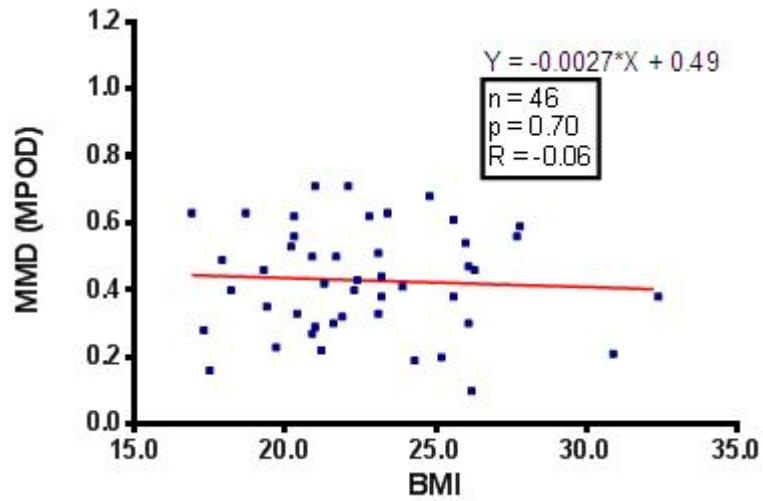*Figure 7.8.7. Relationship between MPOD and Rx in Malay subjects at 0.50° retinal eccentricity using MMD.*
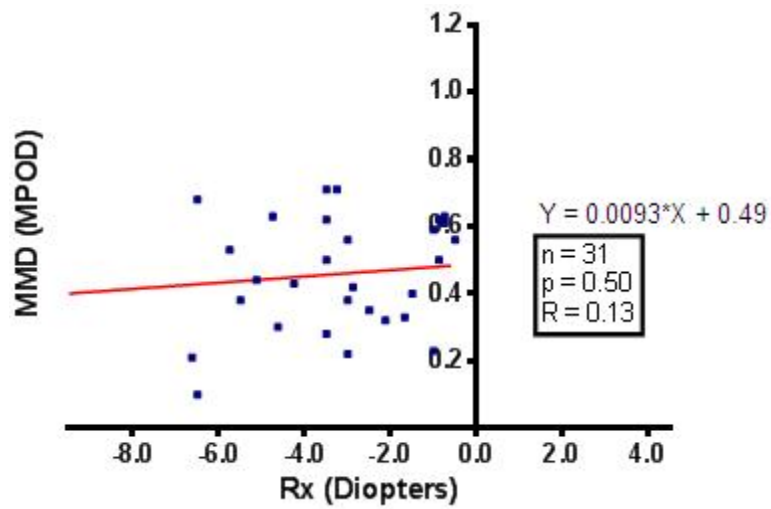
Figure 7.8.8. Relationship between MPOD and Rx in Malay subjects at 1.00° retinal eccentricity using MMD.
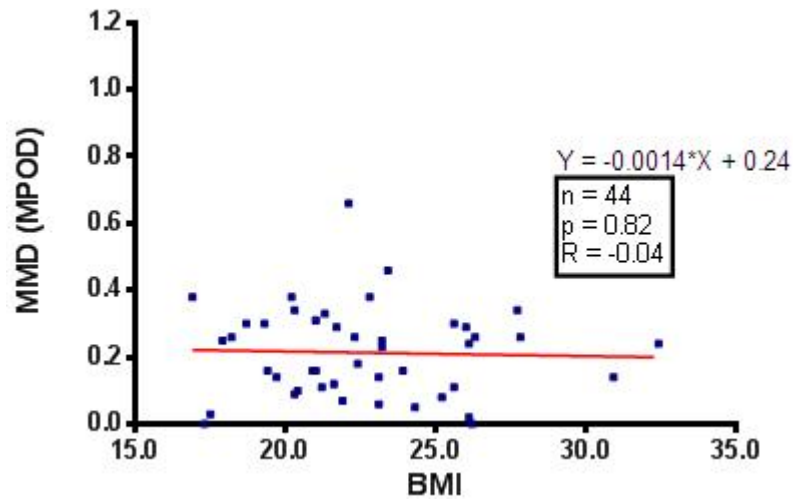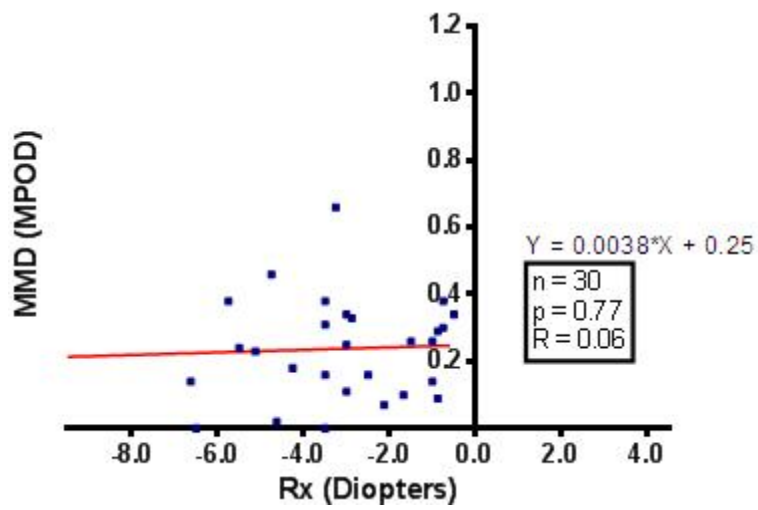
Figure 7.8.9. Relationship between MPOD and Rx in Malay subjects at 1.75° retinal eccentricity using MMD.
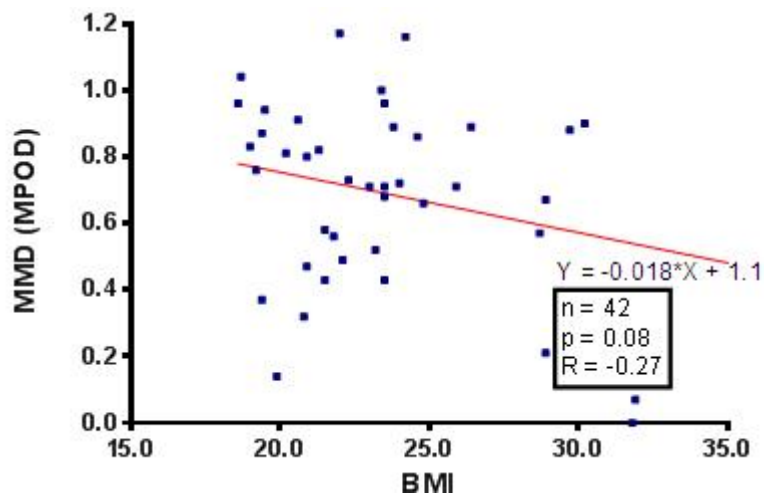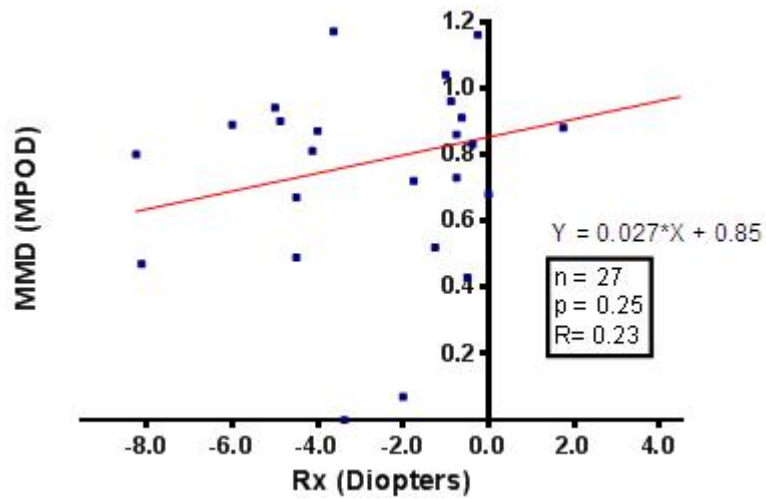

Figure 7.8.10. Relationship between MPOD and Rx in Indian subjects at 0.25° retinal eccentricity using MMD.
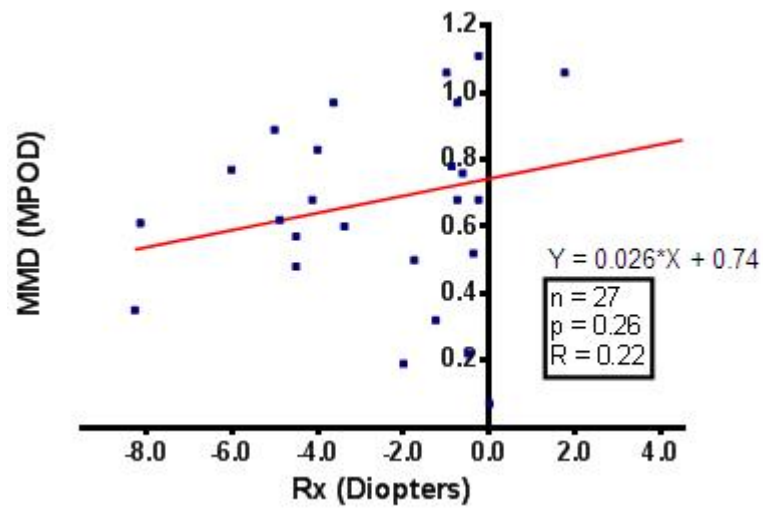
Figure 7.8.11. Relationship between MPOD and Rx in Indian subjects at 0.50° retinal eccentricity using MMD.
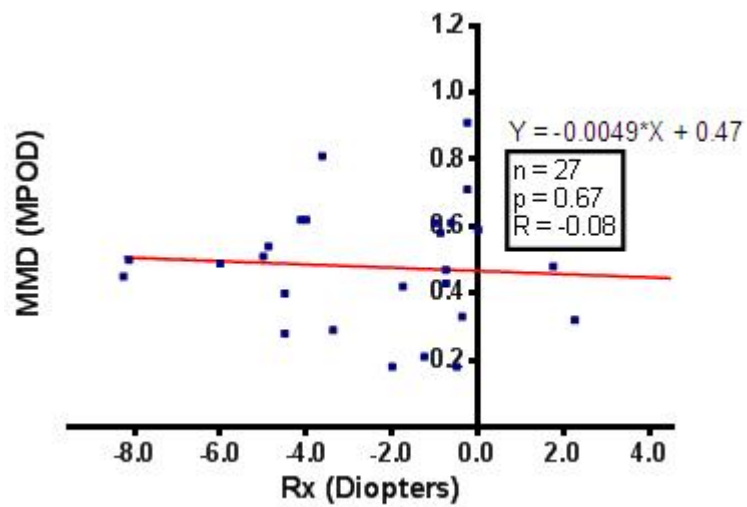
Figure 7.8.12. Relationship between MPOD and Rx in Indian subjects at 1.00° retinal eccentricity using MMD.
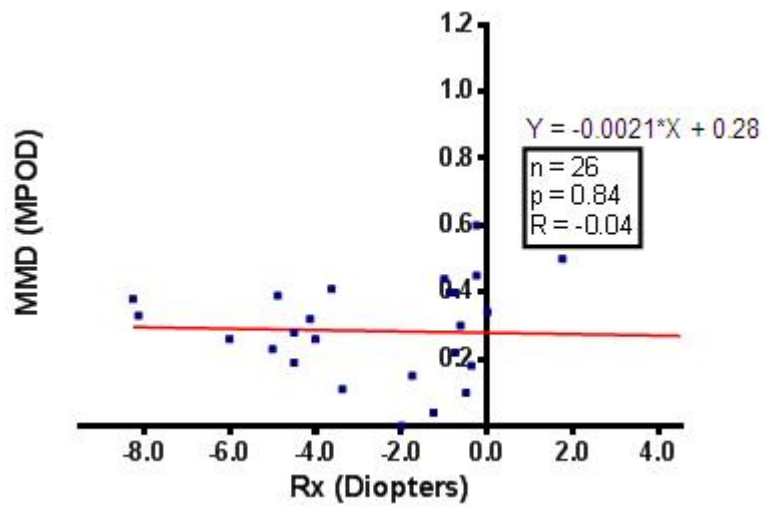
Figure 7.8.13. Relationship between MPOD and Rx in Indian subjects at 1.75° retinal eccentricity using MMD.
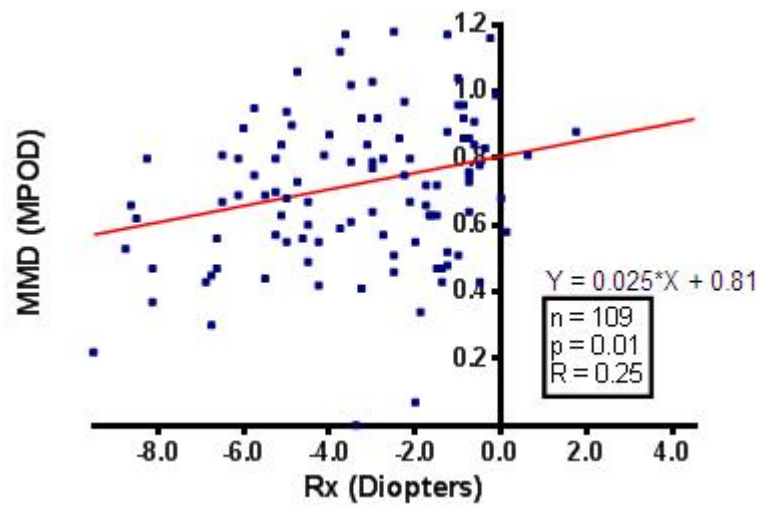
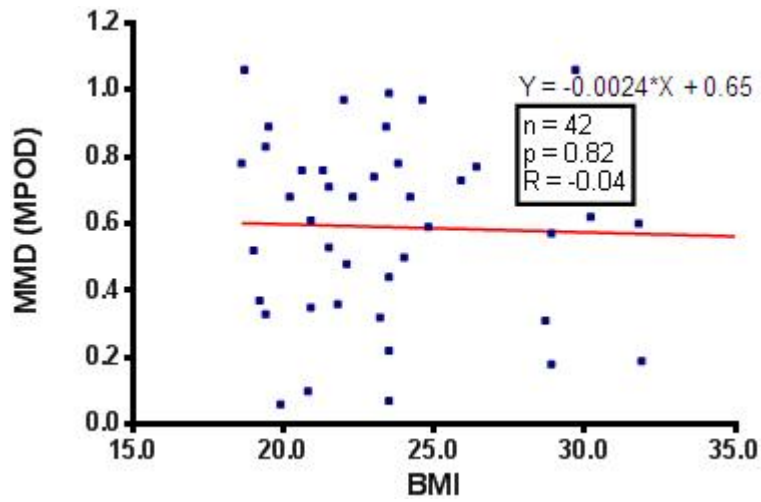*Figure 7.8.14. Relationship between MPOD and Rx in all subjects at 0.25° retinal eccentricity using MMD.*
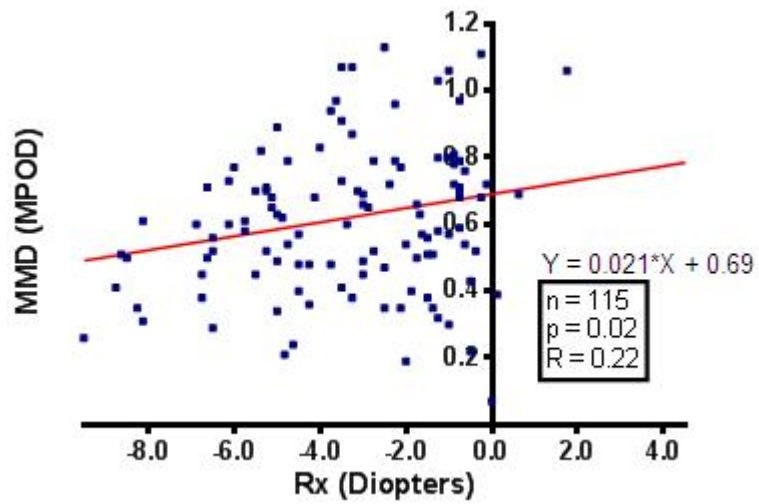
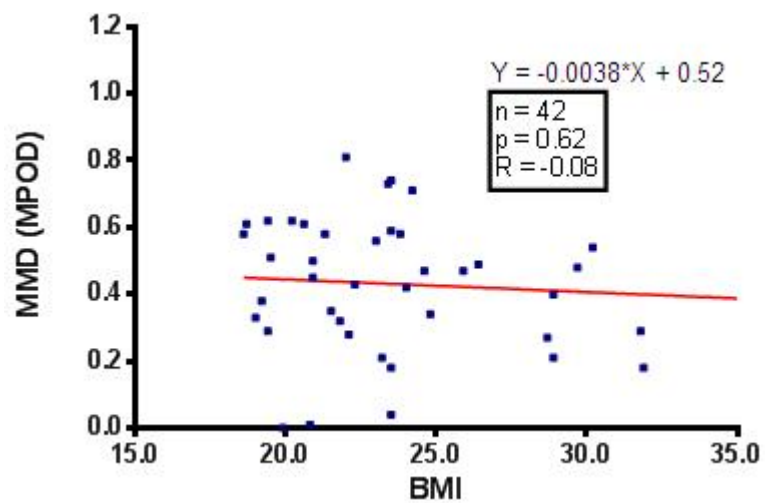*Figure 7.8.15. Relationship between MPOD and Rx in all subjects at 0.50° retinal eccentricity using MMD.*
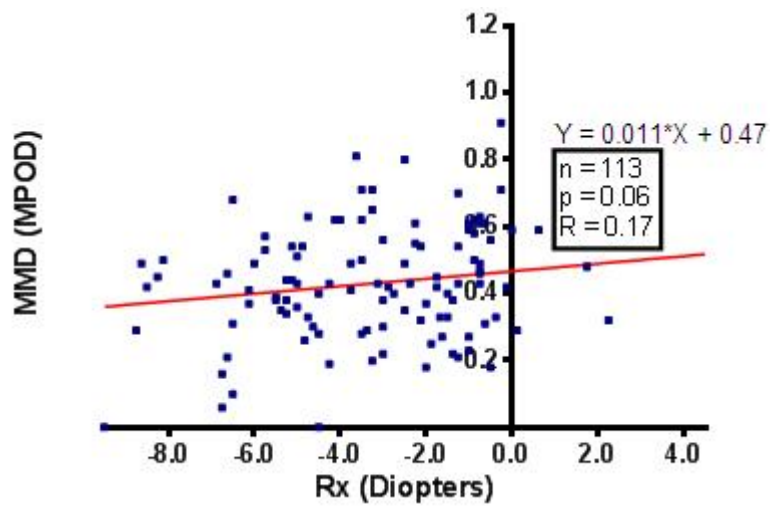
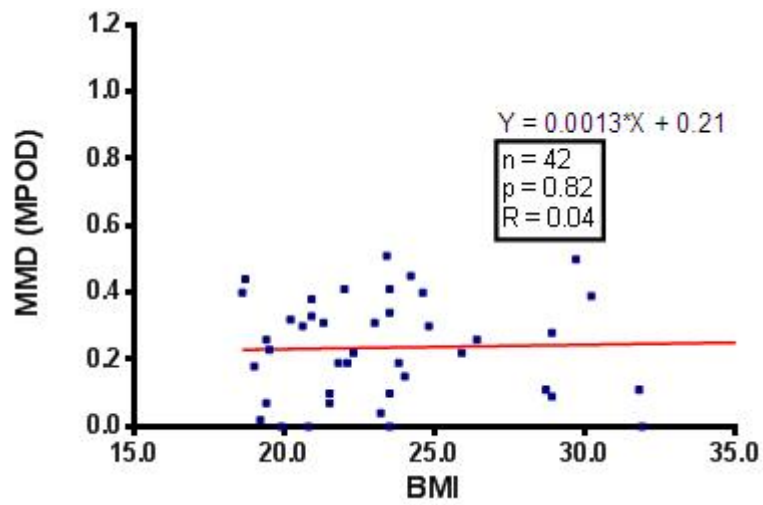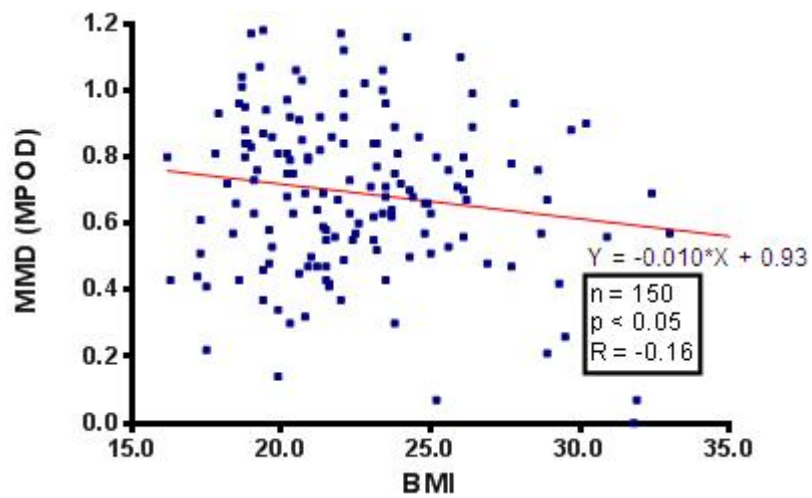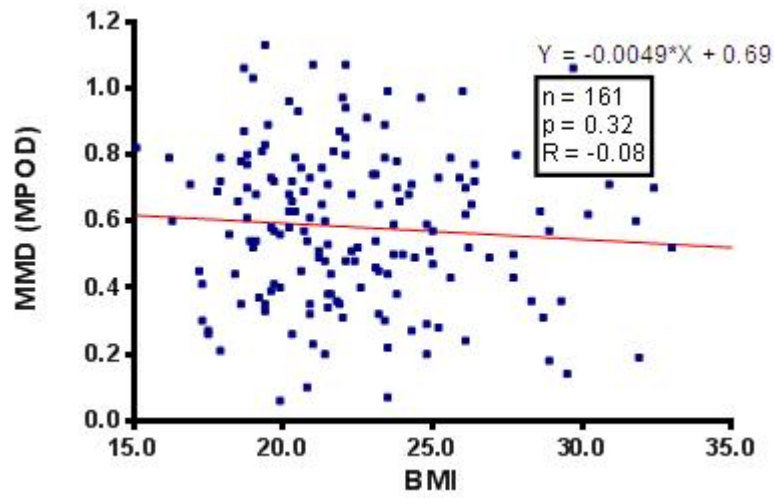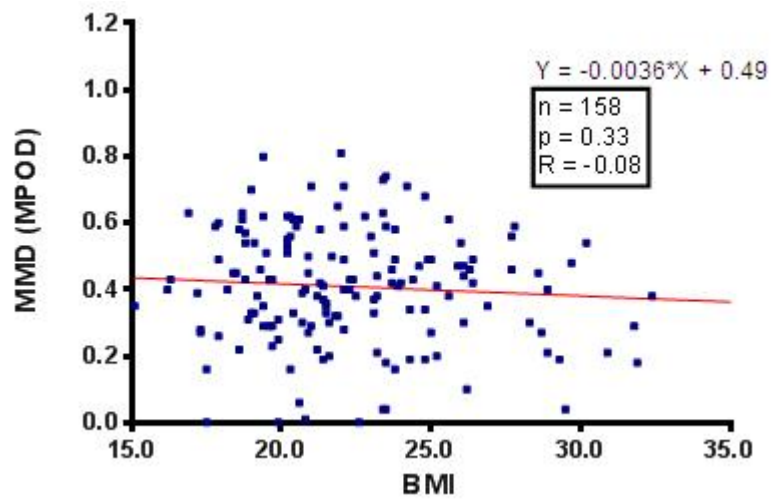*Figure 7.8.16. Relationship between MPOD and Rx in all subjects at 1.00° retinal eccentricity using MMD.*
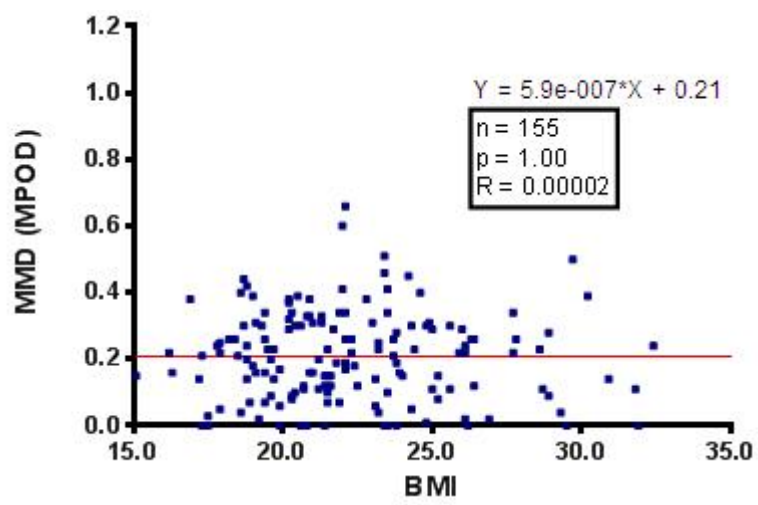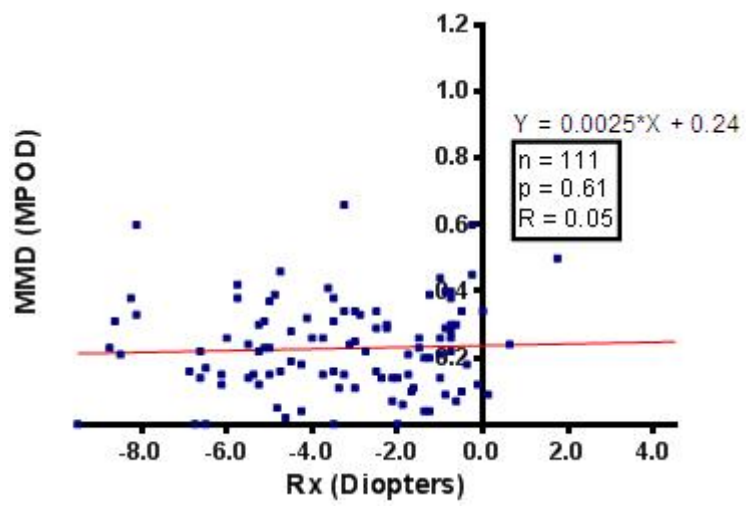
Figure 7.8.17. Relationship between MPOD and Rx in all subjects at 1.75° retinal eccentricity using MMD.

7.9 Variability of MP measurement

The variability of the subject's performance in measuring MPOD using MMD and MPS II is not related to age in all ethnic groups except Chinese. There is a significant higher variation in the Chinese subject's performance with increasing age when measuring MPOD using MMD at 1.75° retinal eccentricity ($p = 0.03$, $R = 0.27$).

SD vs Age	F0.25	F0.50	F1.00	F1.75
Chinese MPS II	NA	n = 73 p = 0.26 R = -0.13	NA	NA
Chinese MMD	n = 67 p = 0.68 R = 0.05	n = 69 p = 0.16 R = 0.17	n = 67 p = 0.25 R = 0.14	n = 66 p = 0.03* R = 0.27
Malay MMD	n = 41 p = 0.58 R = 0.09	n = 47 p > 0.05 R = -0.29	n = 46 p = 0.28 R = -0.16	n = 44 p = 0.34 R = -0.15
Indian MMD	n = 42 p = 0.85 R = -0.03	n = 42 p = 0.33 R = 0.15	n = 42 p = 0.24 R = -0.19	n = 42 p = 0.85 R = -0.03
Total MMD	n = 150 p = 0.57 R = 0.05	n = 158 p = 0.39 R = 0.07	n = 155 p = 0.98 R = -0.002	n = 152 p = 0.25 R = 0.09

Table 7.9.1. Variability of the subject's performance in measuring MPOD using MMD and MPS II with age.
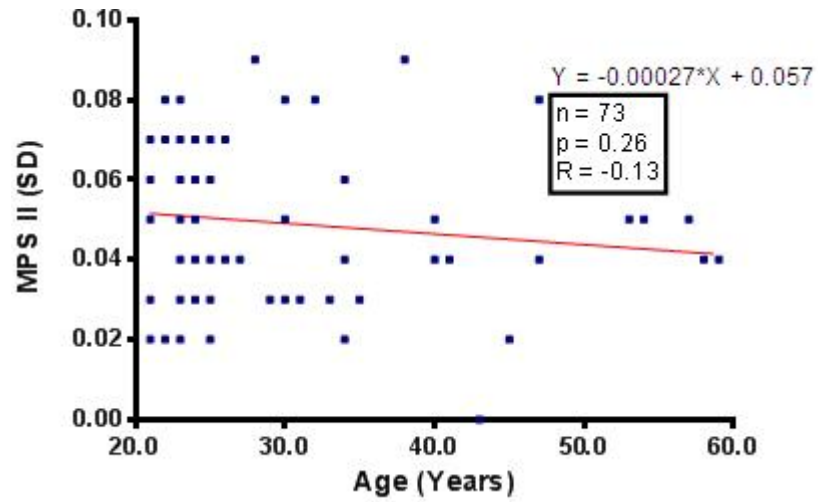
Figure 7.9.1. Relationship between variability of MP measurement and age in Chinese subjects at 0.50° retinal eccentricity using MPS II.
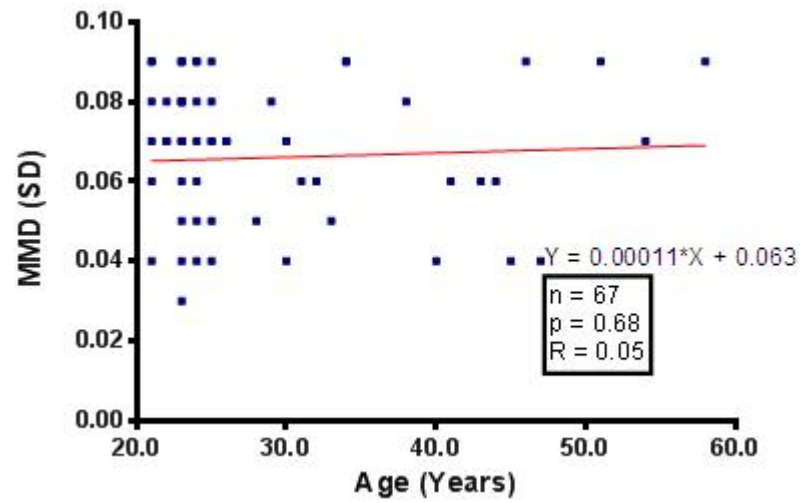
Figure 7.9.2. Relationship between variability of MP measurement and age in Chinese subjects at 0.25° retinal eccentricity using MMD.
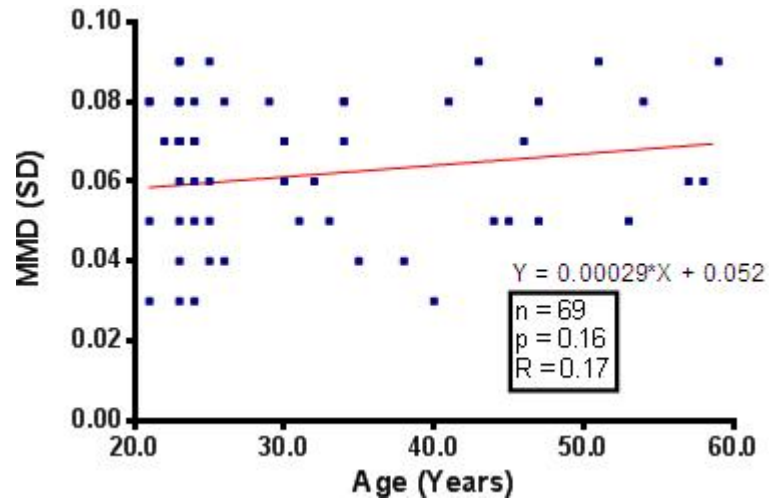
Figure 7.9.3. Relationship between variability of MP measurement and age in Chinese subjects at 0.50° retinal eccentricity using MMD.
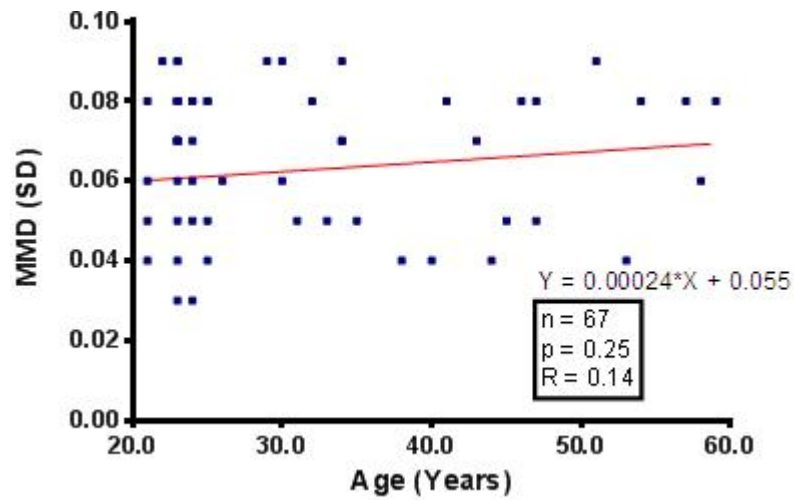
Figure 7.9.4. Relationship between variability of MP measurement and age in Chinese subjects at 1.00 ° retinal eccentricity using MMD.
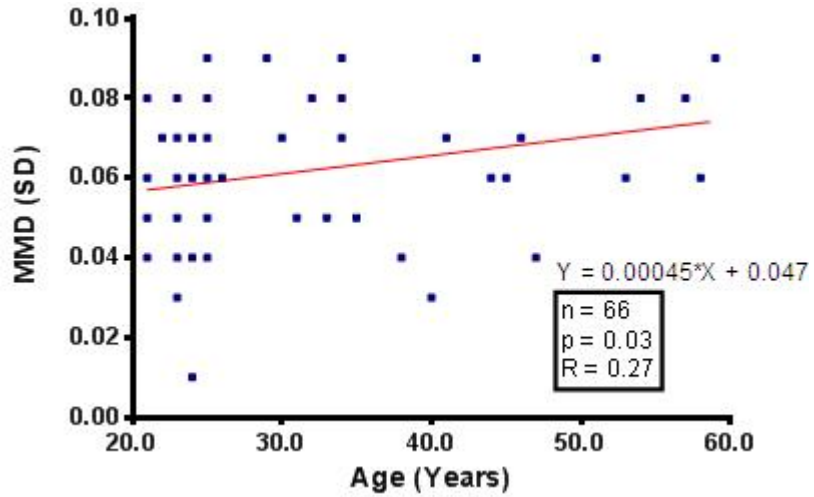
Figure 7.9.5. Relationship between variability of MP measurement and age in Chinese subjects at 1.75 ° retinal eccentricity using MMD.
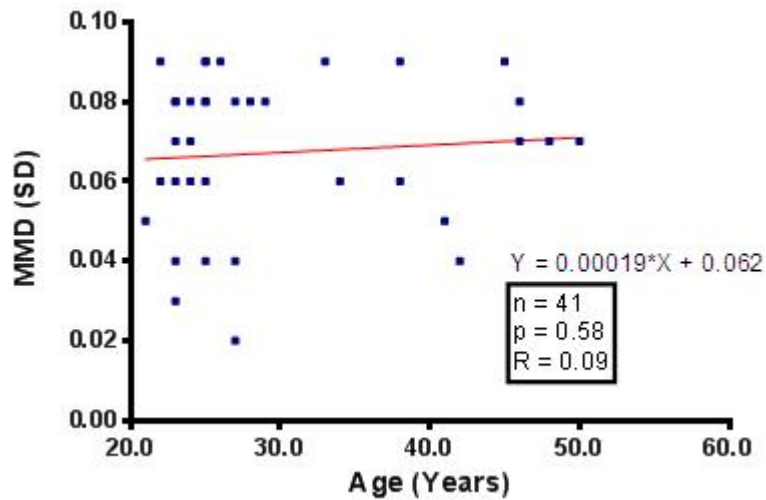
Figure 7.9.6. Relationship between variability of MP measurement and age in Malay subjects at 0.25° retinal eccentricity using MMD.
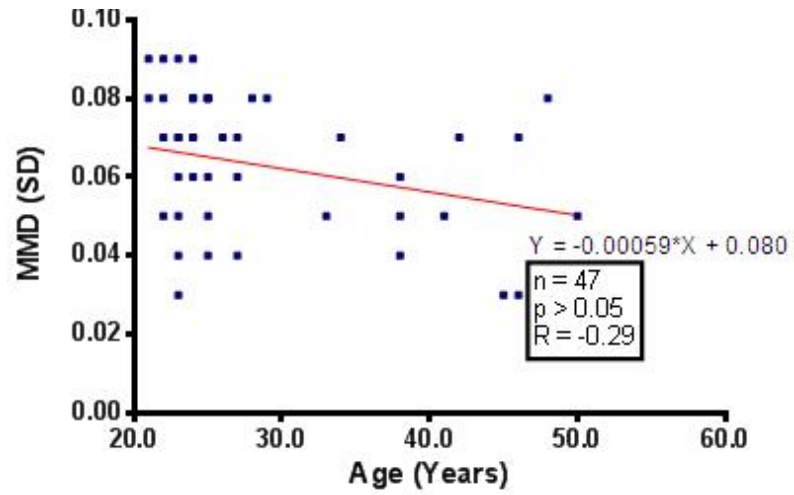
Figure 7.9.7. Relationship between variability of MP measurement and age in Malay subjects at 0.50° retinal eccentricity using MMD.
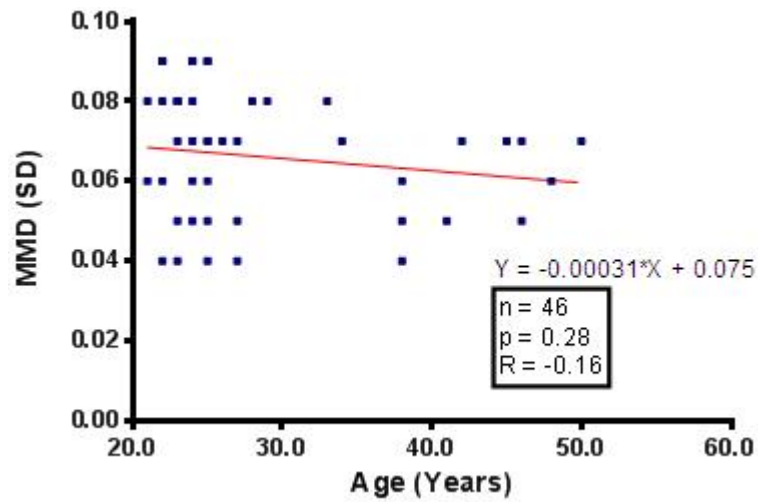
Figure 7.9.8. Relationship between variability of MP measurement and age.in Malay subjects at 1.00° retinal eccentricity using MMD.
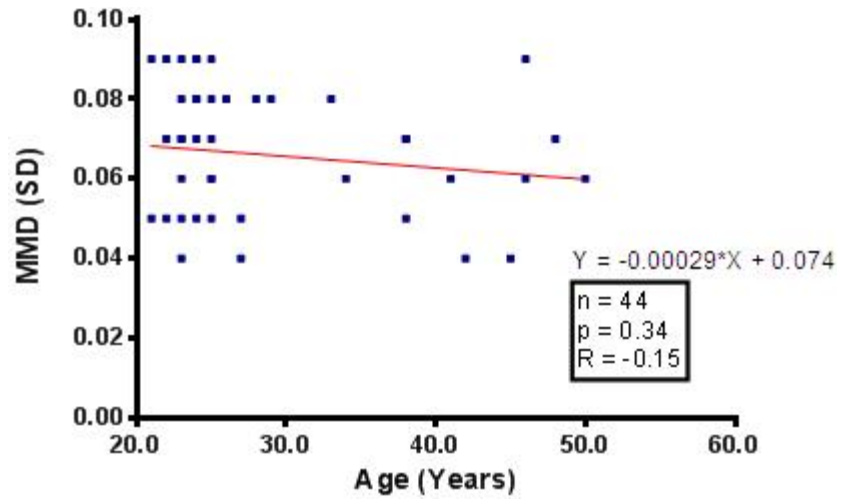
Figure 7.9.9. Relationship between variability of MP measurement and age in Malay subjects at 1.75° retinal eccentricity using MMD.
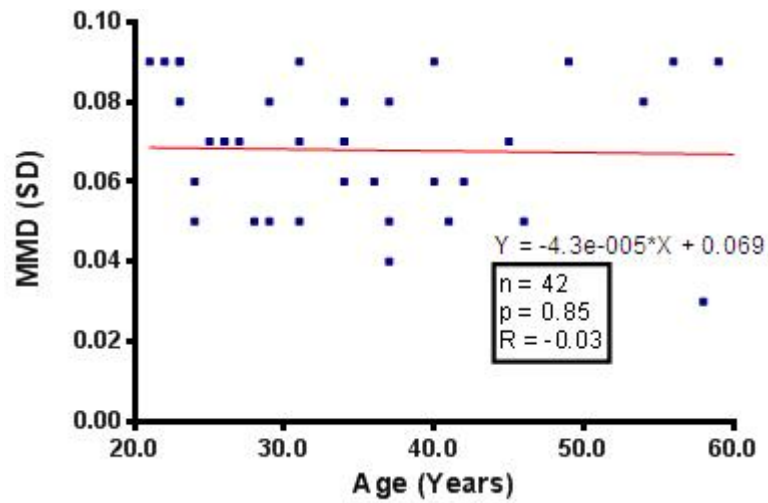
Figure 7.9.10. Relationship between variability of MP measurement and age in Indian subjects at 0.25° retinal eccentricity using MMD.
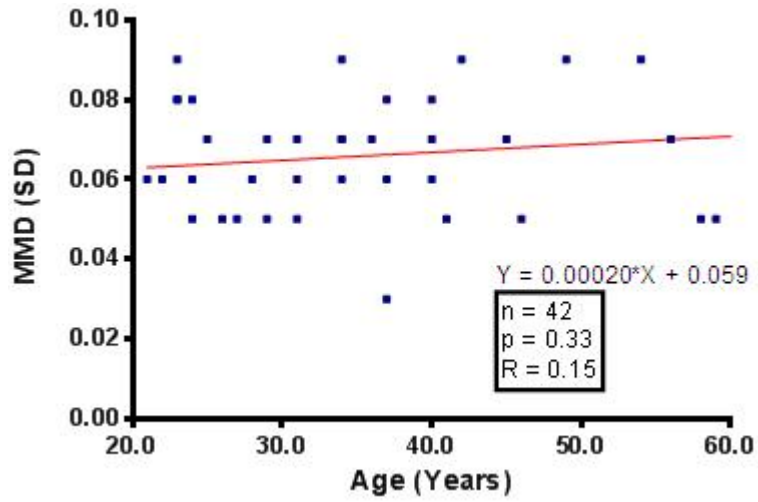
Figure 7.9.11. Relationship between variability of MP measurement and age in Indian subjects at 0.50 ° retinal eccentricity using MMD.
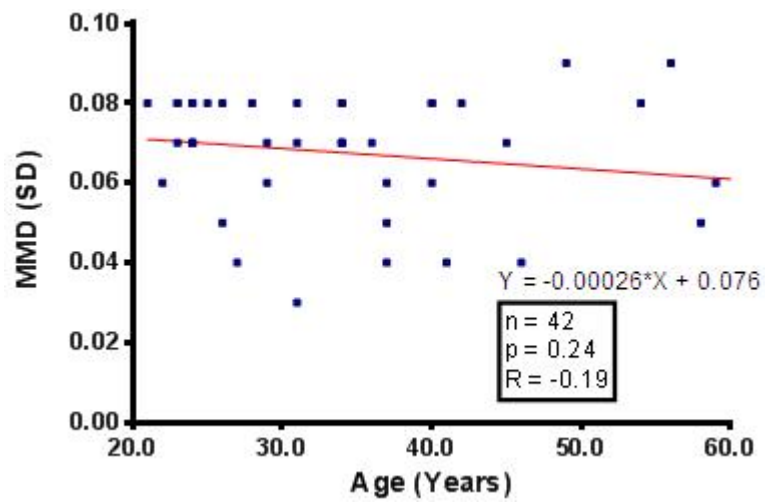
Figure 7.9.12. Relationship between variability of MP measurement and age in Indian subjects at 1.00 ° retinal eccentricity using MMD.
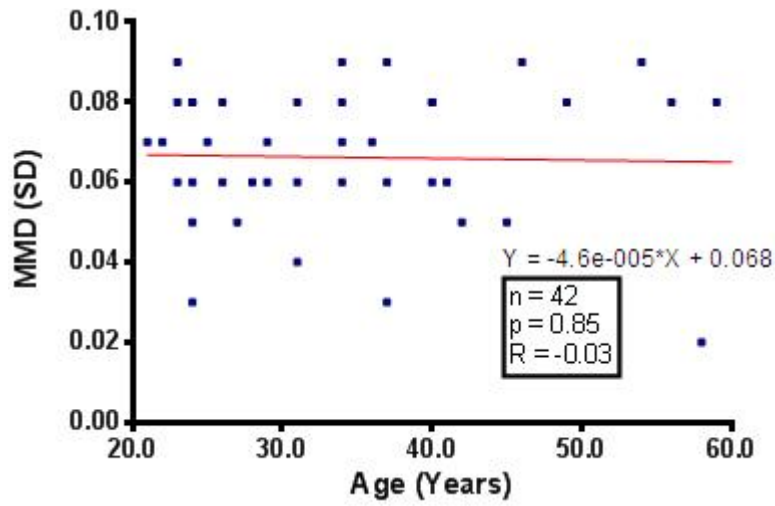
Figure 7.9.13. Relationship between variability of MP measurement and age in Indian subjects at 1.75 ° retinal eccentricity using MMD.
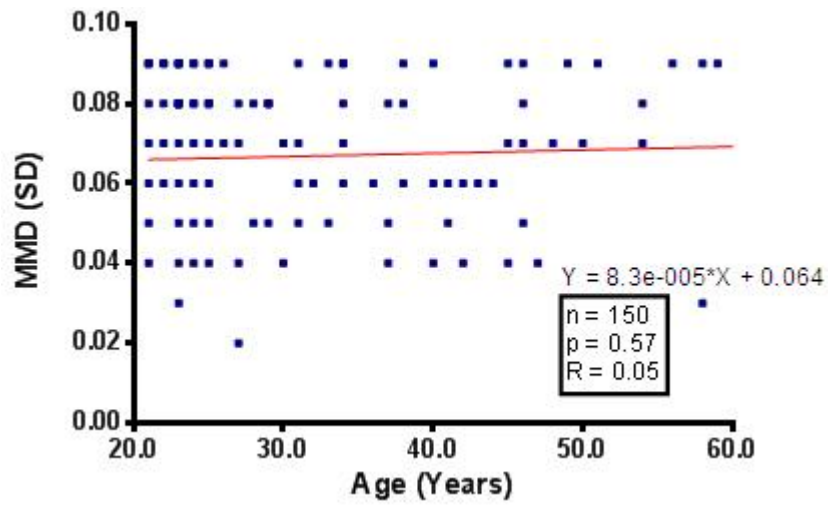
Figure 7.9.14. Relationship between variability of MP measurement and age in all subjects at 0.25 ° retinal eccentricity using MMD.
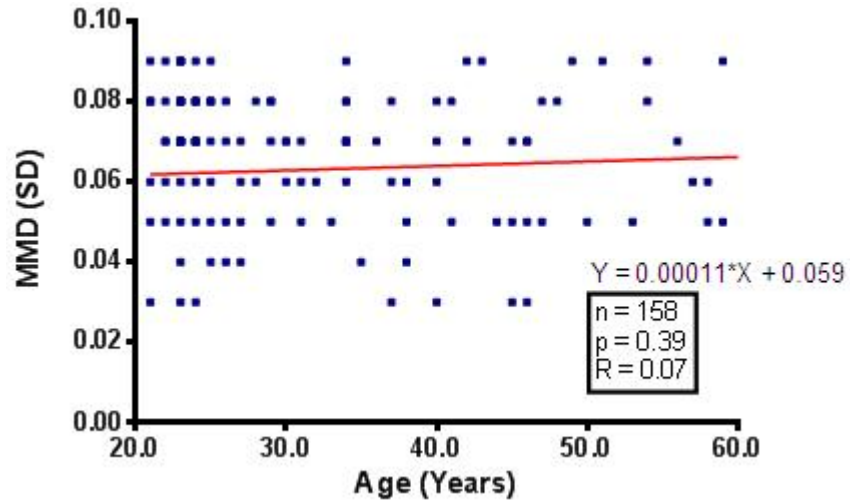
Figure 7.9.15. Relationship between variability of MP measurement and age in all subjects at 0.50 ° retinal eccentricity using MMD.
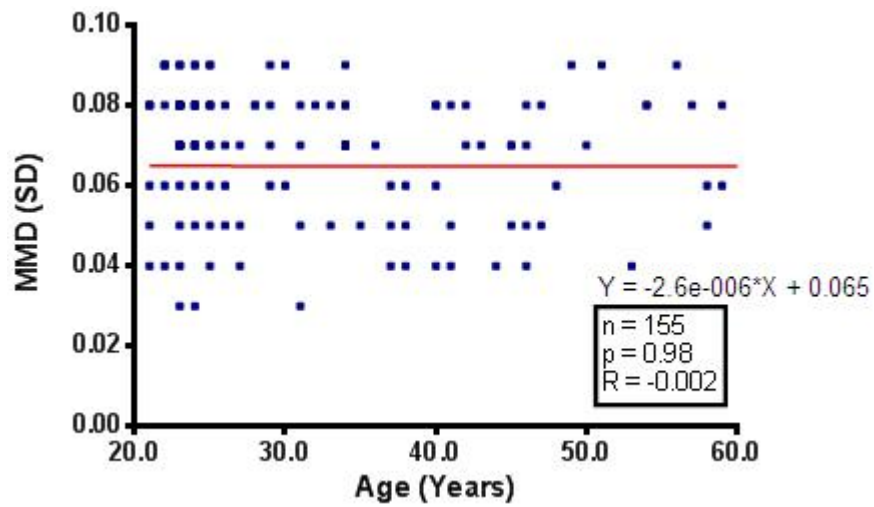
Figure 7.9.16. Relationship between variability of MP measurement and age in all subjects at 1.00 ° retinal eccentricity using MMD.
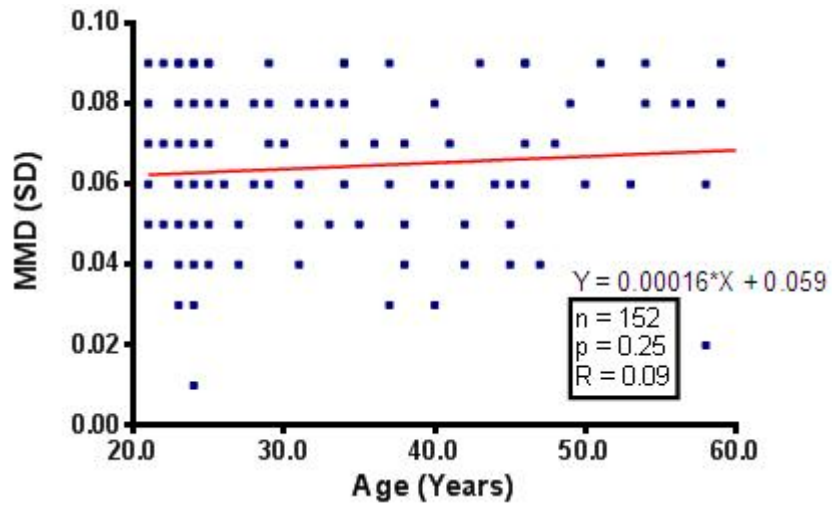
Figure 7.9.17. Relationship between variability of MP measurement and age in all subjects at 1.75 ° retinal eccentricity using MMD.

7.10 Relationship between MPOD with CFF threshold

There is no significant relationship between CFF threshold and MPOD using MMD (p = 0.57, R = -0.04) and MPS II (p = 0.52, R = 0.08).
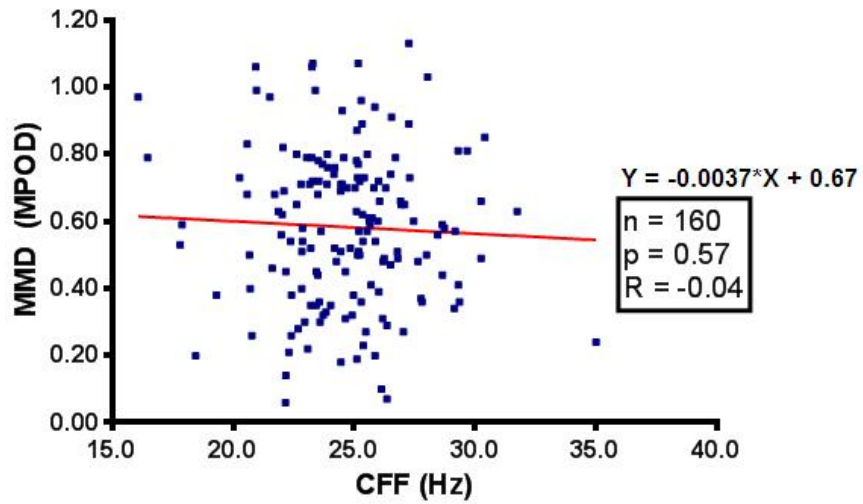
Figure 7.10.1. Relationship between CFF threshold and MPOD measured with MMD.
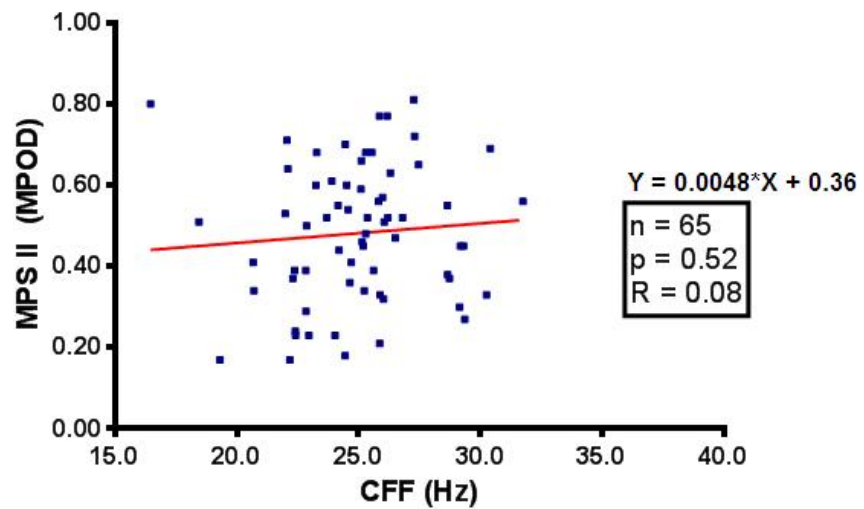
Figure 7.10.2. Relationship between CFF threshold and MPOD measured with MPS II.

7.11 Relationship between CFF threshold and age

There is a significantly lower CFF threshold with increasing age (p = 0.009, R = -0.21) with MMD.
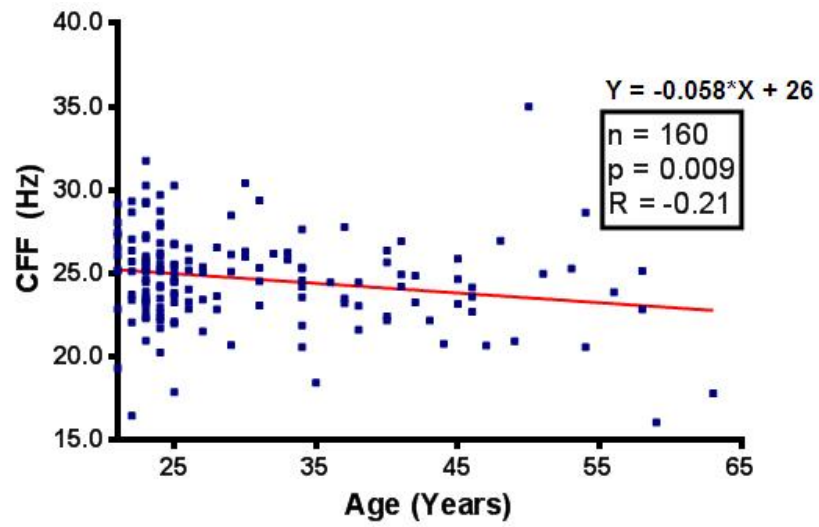
Figure 7.11.1. Relationship between age and CFF threshold measured with MMD.

Chapter 8 : Discussion

8.1 MPOD measured by MMD and MPS II

Loughman et al. (2012) found a significant lower MPOD in MPS 9000 than MMD. Technically, MPS II readings should be higher compared to MMD as the reference point is 1° further away from the foveal region. However, both Loughman et al. (2012) and our result found a higher MPOD in MMD compared to MPS 9000 and MPS II. Our study demonstrated that MMD has an average MPOD 0.09 higher than MPS II.

The consistent higher MP measurement in MPS II could be due to the difference in background colour. MMD uses blue LED to desensitize the blue cone in the macular while MPS II do not desensitize the blue cone as the background is white.

To the present day, no study has verified if the peripheral database in MPS II can be used for Asian population. In our project, we studied the relationship between expert mode and standard mode. Our results show that MPOD using expert mode is significantly correlated to standard mode ($p < 0.0001$; R = 0.97). In other words, there is no difference in peripheral reference value between the European database and in our Chinese subjects.

The ability to save peripheral results as the reference point using computer is an advantage of MPS II over MMD. As MMD need to be operated manually, peripheral point must be measured in each subject so that MPOD can be calculated. MPS II is capable of measuring MP without the reference point, thus minimising chair time and fatigue.

However, the time taken to measure MPOD was significantly longer using MPS II in subjects with higher MPOD. Time taken to measure MP using MMD is not depending on the level of MP. In MMD, subjects can adjust the blue/green light ratio freely. However, in MPS II, subjects need to wait for the stationary target to flicker as the flickering rate is controlled by the computer. Subject with very high MP will take longer measuring time, thus causing more fatigue and unreliable response towards the end of the test.

The variability of both MMD and MPS II shows no significant difference. However, the methodology of the 2 instruments and the way of analysing their variability in our study are different. In MMD, subjects adjust the ratio of blue/green until isoluminant flicker becomes minimum. Thus, the variability is calculated from the standard deviation of the blue light absorbed. On the other hand, MPS II has preset blue/green light ratio and the flickering rate of the test target varies in each presentation by the computer. In this case, the variability is calculated from the standard deviation of the 5 MP results taken.

Although MPS II is a good macular pigment screening tool, subject with MPOD close to 1.00 cannot be measured.

8.2 Ethnicity and MPOD

Different ethnic group has different culture and their lifestyle will be different. For example, Chinese believe that the dried dark red berry of Lycium barbarum (LB) Linnaeus (also known as Fructus Lycii, wolfberry, Kei Tze in Cantonese, and Gou Qi Zi in Mandarin) can nourish the eye and prevent eye diseases such as cataract, retinitis pigmentosa, age-related macular degeneration (AMD) and glaucoma (Cheng et al., 2005; Inbaraj et al., 2008). Thus, Chinese like to add this Chinese herb in their tea, soup and stew. Inbaraj et al. (2008) found 11 free carotenoids and 7 carotenoid esters from unsaponified and saponified LB extracts. There were also 11.3 to 62.8 µg/g Z monopalmitate and its two isomers. In a human supplementation trial, Cheng at el. (2005) found that plasma Z increase by 2.5 times after daily supplementation with 15 gram of LB (approximately 3 mg of Z) for 28 days. However, Malay and Indian do not have this belief and therefore, they will not take the LB as part of their diet.

Since many studies reported that serum L and Z concentration have a significant positive relationship with MPOD measured with HFP method (Neelam et al, 2005b; Nolan et al, 2007; Sandberg et al, 2010), Chinese is believed to have higher MPOD than Malay and Indian as the latter do not eat LB. Interestingly, our results showed otherwise. Malay and Indian have higher MPOD compared to Chinese, although it did not reach statistical significance. This could be due to their genetical difference, which affect the bioavailability of L and Z in the blood and retina (During et al., 2008; Borel et al., 2011). Future research need to be done to demonstrate the genetical differences and its effect to MPOD in different ethnic groups.

Table 8.1.1 summaries the MPOD measured in different ethnic groups from different countries at 0.25°, 0.50°, 1.00°, and 1.75° retinal eccentricities. All data were measured using the same instrument (MMD) for comparison. Malay seems to have a highest MPOD compared to Chinese, Indian, White and Black population.

MPOD measured by MMD in our study is comparable with the Chinese population done by Yu et al. (2012) and Neelam et al. (2014). MPOD measured by MMD in Asian population (Chinese, Malay, and Indian) from our study were found to be higher compared to White and Black population (Iannaccone et al., 2007) and White population (Nolan et al., 2008). The lower MPOD could be due to the greater transmission of short-wavelength light into lighter iris eyes (Hammond et al., 1996c) in White population compared to Non-white, Black and Asian. It may result in increased oxidative stress to the retina. Since all subjects in our study have dark irises, the iris colour effect has been controlled between ethnic groups. We found

no significant difference in MPOD among the 3 ethnic groups at all 4 measuring locations using MMD. This slight ethnic difference in MPOD could be due to their genetic influence, environmental factors, or dietary habits.

Since Chinese have a higher prevalence of myopia compared to Malay and Indian (Wu et al., 2001; Saw et al., 2006), and higher degree of myopia have longer AL and lower MPOD (Tong et al., 2013), Chinese may have lower MPOD among the 3 ethnic groups. In our study, Chinese subjects were found to have a significant higher degree of myopia, and Malay and Indian has higher MPOD (but not significant) compared to the Chinese population. This slight lower in MPOD might be due to the effect of high myopia instead of ethnic differences.

Author / Year	Ethnic group (n)	Age range	MPOD measured by MMD in locations from fovea			
			0.25°	0.50°	1.00°	1.75°
Iannaccone et al., 2007	White (148)	69 to 86	NA	0.37 ± 0.19	NA	NA
Nolan et al., 2008	White (41)	18 to 60	0.43 ± 0.17	0.34 ± 0.13	0.22 ± 0.13	0.07 ± 0.07
Iannaccone et al., 2007	Black (35)	69 to 86	NA	0.22 ± 0.23	NA	NA
Nolan et al., 2008	Non-white* (18)	18 to 60	0.64 ± 0.29	0.55 ± 0.28	0.39 ± 0.20	0.18 ± 0.13
Yu et al., 2012	Chinese (281)	17 to 85	0.56 ± 0.19	0.49 ± 0.18	0.36 ± 0.15	0.19 ± 0.12
Neelam et al., 2014	Chinese (95)	21 to 68	0.65 ± 0.19	0.55 ± 0.19	0.37 ± 0.15	0.19 ± 0.11
My result	Chinese (72)	21 to 58	0.67 ± 0.22	0.57 ± 0.21	0.39 ± 0.16	0.19 ± 0.12
Raman et al., 2011	South Indian (161)	20 to > 60	0.64 ± 0.23	0.50 ± 0.21	0.37 ± 0.19	0.21 ± 0.16
My result	Indian (44)	21 to 63	0.69 ± 0.24	0.59 ± 0.27	0.43 ± 0.20	0.24 ± 0.15
My result	Malay (47)	21 to 50	0.73 ± 0.23	0.60 ± 0.22	0.43 ± 0.16	0.21 ± 0.13

Table 8.1.1. Summary of MPOD measured by MMD in different ethnicities at 0.25°, 0.50°, 1.00°, and 1.75° from the fovea. All data were measured using the same instrument (MMD) for comparison. (*Non-white includes 5 Indian, 6 Asian, 3 Hispanic/Spanish, and 4 Black.)

8.3 Rx and MP

Neelam et al. (2005a) reported no significant relationship between MPOD and ocular biometry, such as Rx and axial length (AL), in 180 healthy subjects. However, their results were negative as most of the subjects recruited were emmetrope. Tong et al. (2013) found lower MPOD in subjects with AL longer than 26 mm, but there is no significant relationship between AL and MPOD, as described by Neelam et al. (2005a), when AL is shorter than 26 mm.

Our study agreed with Tong et al. (2013) since our Chinese subjects have a significant higher degree of myopia, compared to our Malay and Indian subjects, and a significantly lower MPOD at 0.25°, 0.50° and 1.00° retinal eccentricity. However, the correlation is weak (R = 0.28 to 0.32).

When all the subjects are analysed together, only MPOD at 0.25° and 0.50° retinal eccentricities are lower with higher degree of myopia. However, this effect is mainly contributed by the Chinese subjects. Malay and Indian subjects do not have a significant difference between MPOD and Rx as majority of them are emmetrope or have low degree of ametrope which do not affect their AL. It is unclear if the MPOD is affected by AL or Rx in the Chinese subjects since AL is not measured in our study.

8.4 MP Spatial Profile (SP)

To date, no study has been done to find out difference in MP SP among different ethnic groups. Van der Veen et al. (2009a) also showed that majority of the subjects have exponential SP with slight asymmetries between nasal and temporal retina. Similarly, majority of our subjects have exponential decline in MP with foveal eccentricity. Our results showed that Malay has most percentage of subjects with exponential MP SP compared to Chinese and Indian.

Delori et al. (2006) described the presence of atypical MP SP, and females are more prone to ring-like profile (i.e. secondary MP peak), shoulders, or plateaus with MP peaks at approximately 0.7° and 0.5° retinal eccentricities rather than at the fovea. Our study found that higher percentage of females has central dip and secondary peak compared to males in all ethnic group. However, higher percentage of our Indian males has central dip than Indian females. Indian has the highest percentage of subjects with central dip compared to Chinese and Malay.

Our study found that there are no significant difference in MPOD among the 3 ethnic groups – Chinese, Malay, and Indian. However, MP SP is significantly different among ethnicities (p = 0.05 when subjects with plateau SP is excluded). Majority of our subjects have exponential MP SP (Malay = 78%, Chinese = 73%, Indian = 62 %). Overall 22% to 38% of the subjects have atypical MP SP in form of central dip, secondary peak and plateau. Central dip, followed by secondary peak, was the most common atypical MP SP seen in our study. Both were commonly observed in Indian (Central dip: Indian = 26%, Chinese = 19%, Malay = 10%; Secondary peak: Indian = 12%, Chinese = 5%, Malay = 7%). Indian has higher percentage of people with atypical MP SP, while Malay has higher percentage of people with exponential MP SP.

This central dip in females may be associated with a higher risk of AMD compared to males (Mitchell et al., 1995; Smith et al., 1997). However, longitudinal studies is needed to determine the risk of developing AMD with atypical MP SP.

It is still unknown if low MPOD or a specific type of MP SP can be an biomarker for early diagnosis and prevention of eye, systemic, and degenerative diseases. Further research need to be done to find the normative value and MP SP type so as to find out its relationship with the related diseases.

8.5 Gender and MP

A prototype MPS is used to measure MPOD in a large sample size (age 20 to 90) from 48 optometric practices in United Kingdom. Females were found to have a slightly lower MPOD compared to males from age group 20 to 79 except the age group 80 to 90 years old (Van der Veen et al., 2009a). Similarly, Hammond et al (2002) and Neelam et al. (2014) found higher MPOD in males compared to females.

Our results also show slightly higher MPOD in males than females. Neelam et al. (2014) believed that the absorption and storage of MP may be different between genders, while Yu et al. (2012) proposed that it could be due to the more adipose tissue in female gender which affects carotenoid levels present in serum and retina.

8.6 BMI and MP

It is known that obese individual had lower MPOD than normal weight subjects (Hammond et al, 2002; Nolan et al, 2004, Neelam et al., 2014). This could be caused by two reasons. First, the adipose tissues compete with the retina for uptake of L and Z (Hammond et al, 2002). Second, obese individual tends to have lower dietary intake of L and Z (Hammond et al, 2002). However, BMI may not be the most accurate way to compare the relationship between MPOD and body fat as BMI did not differentiate muscle mass with fat.

Burke et al. proven the above hypothesis in 2005. The authors recruited 98 subjects, age 45 to 73, to measure their dietary carotenoids (beta-carotene, lycopene, L and Z) intake using food frequency questionnaire, serum carotenoids, BMI, and MPOD using MVOS at 10, 30, 60, and 120 arc minute away from fovea. Subjects with BMI more than 27 kg/m^2 (n = 41 out of 98, 41.8%) has significant lower overall dietary carotenoids, serum carotenoids, and MPOD at all 4 measuring locations compared to subjects with BMI lower than 27 kg/m^2.

Our results agree with Burke et al. (2005) study. We found a significantly lower MPOD in subjects with higher BMI in the total study population at 0.25° retinal eccentricity. Subjects with BMI more than 27 kg/m^2 (n = 17 out of 150, 11.3%) has significant lower MPOD at 0.25° retinal eccentricity when compared to subjects with BMI less than 27 kg/m^2 (p < 0.05).

8.7 Age and MP

Neelam et al. (2014) measured Chinese MP SP using MMD and found a central dip in older and higher BMI subjects. Beirne (2014) also measured Caucasian MPOD at 4 locations: 0.25°, 0.50°, 1.00°, and 1.75° away from the fovea. MPOD was found to be positively correlated with increasing age at 1.75° retinal eccentricity. However, it is not significantly correlated at 0.25°, 0.50°, and 1.00° retinal eccentricities. These proved that old subjects had a reduce MPOD, but may be at different location of the retina in different ethnicity. Older subjects had lower MP as they may have insufficient uptake or excessive depletion of the retinal carotenoids (Beatty et al., 2001), or inadequate systemic absorption of L and Z (Tucker et al., 1999). Van der Veen et al. (2009a), Neelam et al. (2014), and Beirne (2014) also found a significant lower MPOD in older subjects. Our results also shows a significantly lower MPOD at 0.50° retinal eccentricity, using both MMD and MPS II, with increasing age in Chinese subjects.

Lower MPOD in subjects with increasing age may also be related to the reduction of CFF with age. Hammond and Wooten (2005) assessed the relationship between MPOD and CFF threshold using 1.00° size stimulus in MMD on 134 subjects. The authors also found a significantly lower CFF threshold with increasing age. However, they found a significant positive relationship between CFF threshold and MPOD while there is no significant relationship in our study. This could be due to difference in age range among these studies. Hammond and Wooten (2005) recruited subjects age 17 to 92 while this study recruited subjects age 21 to 63. Since older subjects have lower CFF, and lower CFF threshold produces a lower MPOD, older subjects may have lower MPOD due to low CFF threshold.

Although our study did not find any significant relationship between CFF threshold and MPOD, there is a significantly lower CFF threshold with increasing age.

Our study also shows a significant higher variability in MPOD of the older Chinese subject at 1.75° retinal eccentricity. This means that the peripheral target may be more difficult to see for subjects with increasing age.

Chapter 9 : Conclusion

There is no statistically significant difference in MPOD at all 4 locations measured by MMD among the 3 ethnic groups. The mean MPOD is slightly higher among Malay and Indian when compared to Chinese population. Overall, we did not observe significant relationship in MPOD with age, gender and body mass, with the exception myopia in all 3 ethnic groups.

Majority of our subjects demonstrated an exponential SP and atypical SP in the form of central dip, followed by secondary peak. A significant difference is observed in MP SP among the 3 ethnic groups, with Indian ethnicity demonstrating a higher number of atypical SP in the form of secondary peaks. Female gender demonstrates significantly higher atypical SP compared to male gender in the form of central dip.

In this study, we have found a strong and positive relationship of MPOD measured by MMD and MPS II. MPS II is a good tool to screen MP among population at risk of developing AMD. However, investigators need to be aware that MP readings are lower in MPS II compared to MMD. Furthermore, MPS II also takes significantly longer duration to measure MP for subjects with high MPOD.

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Appendix

National Healthcare Group
Adding years of healthy life

6 Commonwealth Lane
Level 6 GMTI Building
Singapore 149547
Tel: 6496 8900 Fax: 64966870
www.nhg.com.sg
RCB No. 200002150H

NHG DSRB Ref: **2012/01185**

28 February 2013

Dr Kumarineelam Kumari
Department of Ophthalmology
Khoo Teck Puat Hospital

Dear Dr Neelam,

NHG DOMAIN-SPECIFIC REVIEW BOARD (DSRB) APPROVAL

STUDY TITLE: Macular pigment optical density in multi-ethnic population of Singapore

We are pleased to inform you that the NHG Domain Specific Review Board has approved the application as titled above to be conducted in **Khoo Teck Puat Hospital.**

The approval period is from **28 February 2013** to **27 February 2014**. The NHG DSRB reference number for this study is **2012/01185**. Please use this reference number for all future correspondence.

The documents reviewed are:

a)   NHG DSRB Application Form: **Version No. 1**

b)   Participant Information Sheet: **Version 1 dated 07 February 2013**

c)   Informed Consent Form: **Version 2 dated 07 February 2013**

d)   Annex 1 – Eye Screening Form: **Version dated 08 November 2012**

e)   Annex 2 - Data Collection Form: **Version dated 18 October 2012**

f) Annex 3 – Food Diary: **Version 2 dated 06 February 2013**

g) Annex 4 – Recording Sheet for Macular Pigment: **Version dated 22 November 2012**

Continued approval is conditional upon your compliance with the following requirements:

1. Only the approved Informed Consent Form should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject should be given a copy of the signed consent form.

2. No deviation from or changes of the study should be implemented without documented approval from the NHG DSRB, except where necessary to eliminate apparent immediate hazard(s) to the study subjects, or when the change(s) involves only logistical or administrative aspects of the study.

3. Any deviation from or a change to the study to eliminate an immediate hazard should be promptly reported to the NHG DSRB within <u>seven</u> calendar days.

4. Please note that for studies requiring Clinical Trial Certificate, apart from the approval from DSRB, no deviation from, or changes of the Research Protocol and Informed Consent Form should be implemented without documented approval from the Health Sciences Authority unless otherwise advised by the Health Sciences Authority.

5. Please submit the following to the NHG DSRB:

a) All Unanticipated Problems Involving Risk To Subjects Or Others (UPIRTSOs) must be reported to the NHG DSRB. All problems involving local deaths must be reported immediately within 24 hours after first knowledge by the Investigator, regardless of the causality and expectedness of the death. All other problems must be reported as soon as possible but not later than <u>seven</u> calendar days after first knowledge by the Investigator.

b) Report(s) on any new information that may adversely affect the safety of the subject or the conduct of the study.

c) NHG DSRB Study Status Report Form – this is to be submitted 4 to 6 weeks prior to expiry of the approval period. The study cannot continue beyond **27 February 2014** until approval is renewed by the NHG DSRB.

d) Study completion – this is to be submitted using the NHG DSRB Study Status Report

Form within 4 to 6 weeks of study completion.

6.   Established since May 2006, the NHG Research Quality Management (RQM) Program seeks to promote the responsible conduct of research in a research culture with high ethical standards, identify potential systemic weaknesses and make recommendations for continual improvement. Hence, this research study may be randomly selected for a review by the Research Quality Management (RQM) team. For more information, please visit www.research.nhg.com.sg.

 Yours sincerely,

A/Prof Sim Kang
Chairman
NHG Domain Specific Review Board A

 Cc: Institutional Representative, KTPH
          c/o Clinical Research Unit, KTPH
          Departmental Representative of Ophthalmology, KTPH

(This is an electronic-generated letter. No signature is required.)

NHG DSRB Ref: **2012/01185**

27 June 2013

Dr Kumarineelam Kumari
Department of Ophthalmology
Khoo Teck Puat Hospital

Dear Dr Neelam,

NHG DOMAIN SPECIFIC REVIEW BOARD (DSRB) APPROVAL OF AMENDMENT

STUDY TITLE: Macular pigment optical density in multi-ethnic population of Singapore

We are pleased to inform you that the NHG Domain Specific Review Board has reviewed and approved the amendments submitted for the application as titled above.

The documents reviewed are:

a)   NHG DSRB Study Amendment ID: **2012/01185-AMD0001**

b)   NHG DSRB Application Form: **Version No. 2**

c)   Informed Consent Form: **Version 3 dated 14 June 2013**

Yours sincerely,
Dr Yip Chee Chew
Deputy Chairman
NHG Domain Specific Review Board A

Cc: Institutional Representative, KTPH
          c/o Clinical Research Unit, KTPH
          Departmental Representative of Ophthalmology, KTPH
     (This is an electronic-generated letter. No signature is required.)

National Healthcare Group

Adding years of healthy life

NHG DSRB Ref: **2012/01185**

12 February 2014

Dr Kumarineelam Kumari
Department of Ophthalmology
Khoo Teck Puat Hospital

Dear Dr Neelam

RENEWAL OF NHG DOMAIN SPECIFIC REVIEW BOARD (DSRB) APPROVAL

STUDY TITLE: Macular pigment optical density in multi-ethnic population of Singapore

We are pleased to inform you that the NHG DSRB has renewed the approval for the application as titled above, being conducted in **Khoo Teck Puat Hospital**. The approval period is from **12 February 2014** to **11 February 2015**.

The documents reviewed are:

a)   NHG DSRB Study Status Report Form ID: **2012/01185-SRF0001**
b)   NHG DSRB Application Form: **Version No. 2**
c)   Informed Consent Form: **Version 3 dated 14 June 2013**
d)   Participant Information Sheet: **Version 1 dated 07 February 2013**
e)   Annex 1 – Eye Screening Form: **Version dated 08 November 2012**
f)   Annex 2 - Data Collection Form: **Version 1 dated 18 October 2012**
g)   Annex 3 – Food Diary: **Version 2 dated 06 February 2013**
h)   Annex 4 – Recording Sheet for Macular Pigment: **Version dated 22 November 2012**

Continued approval is conditional upon your compliance with the following requirements:

1.   Only the approved Informed Consent Form should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject should be given a copy of the signed consent form.

2.   No deviation from, or changes of the protocol should be implemented without documented approval from the NHG DSRB, except where necessary to eliminate apparent immediate hazard(s) to the study subjects.

3.   Any deviation from, or a change of, the protocol to eliminate an immediate hazard should be promptly reported to the NHG DSRB within seven calendar days.

4.   Please note that for studies requiring Clinical Trial Certificate, apart from the approval from NHG DSRB, no deviation from, or changes of the Research Protocol and Informed Consent Form should be implemented without documented approval from the Health Sciences Authority unless otherwise advised by the Health Sciences Authority.

5.   Please submit the following to the NHG DSRB:

a.   All Unanticipated Problems Involving Risk To Subjects Or Others (UPIRTSOs) must be reported to the NHG DSRB. All problems involving local deaths must be reported immediately within 24 hours after first knowledge by the Investigator, regardless of the causality and expectedness of the death. All other problems must be reported as soon as possible but not later than seven calendar days after first knowledge by the Investigator.

b.   Report(s) on any new information that may adversely affect the safety of the subject or the conduct of the study.

c.   NHG DSRB Study Status Report Form – this is to be submitted 4 to 6 weeks prior to expiry of the approval period. The study cannot continue beyond **11 February 2015** until approval is renewed by the NHG DSRB.

d.   Study completion – this is to be submitted using the NHG DSRB Study Status Report Form within 4 to 6 weeks of study completion or termination.

6.   Established since May 2006, the NHG Research Quality Management (RQM) Program seeks to promote the responsible conduct of research in a research culture with high ethical standards, identify potential systemic weaknesses and make recommendations for continual

improvement. Hence, this research study may be randomly selected for a review by the Research Quality Management (RQM) team. For more information, please visit www.research.nhg.com.sg.

Yours Sincerely

Dr Yip Chee Chew
Deputy Chairman
NHG Domain Specific Review Board A

Cc: Institutional Representative, KTPH
    c/o Clinical Research Unit, KTPH
    Departmental Representative of Ophthalmology, KTPH

(This is an electronic-generated letter. No signature is required.)

NHG DSRB Ref: **2012/01185**

18 August 2014

Dr Kumarineelam Kumari
Department of Ophthalmology
Khoo Teck Puat Hospital

Dear Dr Neelam,

NHG DOMAIN SPECIFIC REVIEW BOARD (DSRB) APPROVAL OF AMENDMENT

STUDY TITLE: Macular pigment optical density in multi-ethnic population of Singapore

We are pleased to inform you that the NHG Domain Specific Review Board has reviewed and approved the amendments submitted for the application as titled above.

The documents reviewed are:
a)   NHG DSRB Study Amendment ID: **2012/01185-AMD0002**
b)   NHG DSRB Application Form: **Version No. 3**
c)   Informed Consent Form: Version 4 dated 18 August 2014

The NHG DSRB acknowledges the receipt of the following documents:

a)   Informed Consent Form English with [Chinese] Short Consent Form: Version 1 dated 18 August 2014
b)   Informed Consent Form English with [Malay] Short Consent Form: Version 1 dated 18 August 2014
c)   Informed Consent Form English with [Tamil] Short Consent Form: Version 1 dated 18 August 2014

Yours sincerely,

A/Prof Sim Kang
Chairman
NHG Domain Specific Review Board A

Cc: Institutional Representative, KTPH
       c/o Clinical Research Unit, KTPH
       Departmental Representative of Ophthalmology, KTPH

(This is an electronic-generated letter. No signature is required.)

Adding years of healthy life

NHG DSRB Ref: **2012/01185**

12 February 2015

Dr Kumarineelam Kumari
Department of Ophthalmology
Khoo Teck Puat Hospital

Dear Dr Neelam

RENEWAL OF NHG DOMAIN SPECIFIC REVIEW BOARD (DSRB) APPROVAL

STUDY TITLE: Macular pigment optical density in multi-ethnic population of Singapore

We are pleased to inform you that the NHG DSRB has renewed the approval for the application as titled above, being conducted in **Khoo Teck Puat Hospital**. The approval period is from **12 February 2015** to **11 February 2016**.

The documents reviewed are:

a)   NHG DSRB Study Status Report Form ID: **2012/01185-SRF0002**
b)   NHG DSRB Application Form: **Version No. 3**
c)   Informed Consent Form: **Version 4 dated 18 August 2014**
d)   Participant Information Sheet: **Version 1 dated 07 February 2013**
e)   Annex 1 - Eye Screening Form: **Version dated 08 November 2012**
f)   Annex 2 - Data Collection Form: **Version 1 dated 18 October 2012**
g)   Annex 3 - Food Diary: **Version 2 dated 06 February 2013**
h)   Annex 4 - Recording Sheet for Macular Pigment: **Version dated 22 November 2012**

The documents acknowledged are:

a)   Informed Consent Form English with [Chinese] Short Consent Form: **Version 1 dated**

18 August 2014

b) Informed Consent Form English with [Malay] Short Consent Form: **Version 1 dated 18 August 2014**

c) Informed Consent Form English with [Tamil] Short Consent Form: **Version 1 dated 18 August 2014**

Continued approval is conditional upon your compliance with the following requirements:

1. Only the approved Informed Consent Form should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject should be given a copy of the signed consent form.

2. No deviation from, or changes of the protocol should be implemented without documented approval from the NHG DSRB, except where necessary to eliminate apparent immediate hazard(s) to the study subjects.

3. Any deviation from, or a change of, the protocol to eliminate an immediate hazard should be promptly reported to the NHG DSRB within seven calendar days.

4. Please note that for studies requiring Clinical Trial Certificate, apart from the approval from NHG DSRB, no deviation from, or changes of the Research Protocol and Informed Consent Form should be implemented without documented approval from the Health Sciences Authority unless otherwise advised by the Health Sciences Authority.

5. Please submit the following to the NHG DSRB:

a. All Unanticipated Problems Involving Risk To Subjects Or Others (UPIRTSOs) must be reported to the NHG DSRB. All problems involving local deaths must be reported immediately within 24 hours after first knowledge by the Investigator, regardless of the causality and expectedness of the death. All other problems must be reported as soon as possible but not later than seven calendar days after first knowledge by the Investigator.

b. Report(s) on any new information that may adversely affect the safety of the subject or the conduct of the study.

c. NHG DSRB Study Status Report Form – this is to be submitted 4 to 6 weeks prior to expiry of the approval period. The study cannot continue beyond **11 February 2016** until approval is renewed by the NHG DSRB.

d. Study completion – this is to be submitted using the NHG DSRB Study Status Report

Form within 4 to 6 weeks of study completion or termination.

6.   Established since May 2006, the NHG Research Quality Management (RQM) Program seeks to promote the responsible conduct of research in a research culture with high ethical standards, identify potential systemic weaknesses and make recommendations for continual improvement. Hence, this research study may be randomly selected for a review by the Research Quality Management (RQM) team. For more information, please visit www.research.nhg.com.sg.

Yours Sincerely

A/Prof Sim Kang
Chairman
NHG Domain Specific Review Board A

Cc: Institutional Representative, KTPH
      c/o Clinical Research Unit, KTPH
      Departmental Representative of Ophthalmology, KTPH

(This is an electronic-generated letter. No signature is required.)

INFORMED CONSENT FORM

You have been invited to participate in this research study. Prior to your participation, it is important to us that you first take time to read through and understand the information provided in this consent form sheet. Nevertheless, before you take part in this research study, our interviewers will explain the study to you and you will be given the chance to ask questions. Once you are properly satisfied that you understand this study, and that you wish to take part in the study, you must sign this informed consent form. You will be given a copy of this consent form to take home with you.

1. Study Information

Protocol Title: Macular pigment optical density in multi-ethnic population of Singapore

Principal Investigator & Contact Details:

Dr Kumari Neelam

Department of Ophthalmology and Visual Sciences

Khoo Teck Puat Hospital

90 Yishun Central

Yishun, Singapore

Ph No: 96187670, Email: kumari.neelam@alexandrahealth.com.sg

2. Purpose of the Research Study

The yellow spot at the macula is due to the accumulation of macular pigment. The macula is the central part of light-sensitive layer (retina) that is responsible for sharp and clear vision. Macular pigment (MP) function as a blue filter to improve visual resolution and may protect the macula from age-related macular degeneration (AMD, an aging disease of the macula). Studies shown that higher MP had lower risk of AMD.

You have been invited to participate in this research study as a healthy control because you have good eyesight and absence of eye diseases. This study's aim is to measure density of the yellow pigment within the retinal layers in 3 ethnic groups (ie. Chinese, Malay, and Indian). The study will recruit 300 Singaporean Chinese, Malay, and Indian subjects in the Department of Ophthalmology and Visual Sciences over a 2-year period in the Khoo Teck Puat Hospital (KTPH).

3. What Procedures will be Followed in This Study

On the day of your clinic visit, you will be asked to sign a consent form after having discussed the details of the study with the study investigator, and having satisfied yourself with regards to any queries that you may have about the study. The following procedures will be conducted in each study participant: eye screening, socio-demographic, lifestyle, medical, standard medication and family history using a questionnaire; height and weight measurement; autofluorescence image of the eye (special imaging technique to make the pigmented layer of the eye more visible); measurement of density of the yellow pigment within the retinal layers; OCT of the macula; 7 day food diary; blood sample collection (10ml equivalent to 2 teaspoons). Your participation in this study will last approximately 90 - 120 minutes, depending on your performance.

4. Your Responsibilities in This Study

If you agree to participate in this study, you should follow the advice given to you by the study investigator. You should be prepared to visit the hospital once and undergo all the procedures that are outlined above.

5. What Is Not Standard Care or Experimental in This Study

Sociodemographic, lifestyle, medical and drug history taking

Macular pigment measurement

Seven days food diary

Height and weight measurement

Blood sample collection

Autofluorescence image of the eye

Imaging of the macula using Optical Coherence Tomography (OCT)

6. Blood Sample for Future Research

A proportion of blood sample (5 ml equivalent to 1 teaspoon) will be stored for future studies in the field of antioxidants and eye diseases. The samples will be coded and stored in the Clinical Research Unit at Khoo Teck Puat Hospital for a period of 5 years. The samples will not be provided to other researchers, institutions and commercial biomedical companies. The investigators will be happy to provide pertinent information to the participating subjects whenever appropriate.

The blood samples collected for the study will be deemed to be gifted to KTPH and will not be returned to you. However, you retain your right to ask the Principal Investigator to discard or destroy any remaining samples if they have not been anonymised.

7. Possible Risks and Side Effects

There are no major anticipated risks and side effects associated with this research study. Obtaining blood may cause pain, bleeding, bruising, or swelling at the site of needle stick. Fainting sometime occurs and infection rarely occurs. Light exposure level from the machines to the eye are within safety standards.

8. Possible Benefits from Participating in the Study

By participating in this study, you can help clinicians better understand if ethnicity affects level of macular pigment and allow further work to be done to evaluate the protective role of the macular pigment in age-related macular degeneration.

9. Alternatives to Participation

This study will not interfere the relationship with your attending doctor should you choose not to participate. You will receive medical treatment as per disease management.

10. Voluntary Participation

Your participation in this study is voluntary. If you decide to stop taking part in this study, you should tell the Principal Investigator. Your decision not to take part in this study or to stop your participation will not affect your medical care or any benefits to which you are entitled.

Your doctor and/or the Investigator of this study may stop your participation in the study at any time if they decide that it is in your best interests. They may also do this if you do not follow instructions required to complete the study adequately. If you have other medical problems or side effects, the doctor and/or nurse will decide if you may continue in the research study.

In the event of any new information becoming available that may be relevant to your willingness to continue in this study, you will be informed in a timely manner by the Principal Investigator or his/her representative.

11. Costs & Payments if Participating in the Study

You will not have to pay for the tests conducted solely for the purpose of this research study. The following tests conducted for the purpose of the study will not be charged:
- Sociodemographic, lifestyle, medical and drug history taking
- Macular pigment measurement
- Seven days food diary
- Height and weight measurement
- Autofluorescence image of the eye
- OCT macula
- Blood sample collection

However, you will have to pay for the consultation visits, tests and treatment that the attending doctor prescribes as clinically necessary.

A reimbursement of SGD 40 will be provided to each study participant upon completion of the study.

12. Confidentiality of Study and Medical Records

Information collected for this study will be kept confidential. Your records and responses, to the extent of the applicable laws and regulations, will not be made publicly available.

However, the Sponsoring company (Alexandra Health Pvt Ltd, Singapore) and NHG Domain-Specific Review Board and Ministry of Health will be granted direct access to your original medical records to check study procedures and data, without making any of your information public. By signing the Informed Consent Form attached, you (or your legally acceptable representative, if relevant) are authorizing such access to your study and medical records.

Data collected and entered into the Case Report Forms are the property of Alexandra Health Pvt Ltd., Singapore. In the event of any publication regarding this study, your identity will remain confidential.

13. Compensation for Injury

If you follow the directions of the doctors in charge of this study and you are physically injured due to the trial substance or procedure given under the plan for this study, KTPH will pay the medical expenses for the treatment of that injury.

Payment for management of the normally expected consequences of your treatment will not be provided by KTPH.

KTPH without legal commitment will compensate you for the injuries arising from your participation in the study without you having to prove KTPH at fault. There are however conditions and limitations to the extent of compensation provided. You may wish to discuss this with your Principal Investigator.

By signing this consent form, you will not waive any of your legal rights or release the parties involved in this study from liability for negligence.

14. Who To Contact if You Have Questions

If you have questions about this research study, you may contact the Principal Investigator.

Dr Kumari Neelam

Department of Ophthalmology and Visual Sciences

Khoo Teck Puat Hospital

90 Yishun Central

Yishun, Singapore

Ph No: 96187670, Email: kumari.neelam@alexandrahealth.com.sg

The study has been reviewed by the NHG Domain Specific Review Board (the central ethics committee) for ethics approval.

If you want an independent opinion of your rights as a research subject you may contact the NHG Domain Specific Review Board Secretariat at 6471-3266.

If you have any complaints about this research study, you may contact the Principal Investigator or the NHG Domain Specific Review Board Secretariat.

CONSENT FORM

Protocol Title:
Macular pigment optical density in Multi-ethnic population of Singapore

Principal Investigator & Contact Details:
Dr Kumari Neelam
Department of Ophthalmology and Visual Sciences
Khoo Teck Puat Hospital
90 Yishun Central
Yishun, Singapore
Ph No: 96187670, Email: kumari.neelam@alexandrahealth.com.sg

☐   I voluntarily consent to take part in this research study. I have fully discussed and understood the purpose and procedures of this study. This study has been explained to me in a language that I understand. I have been given enough time to ask any questions that I have about the study, and all my questions have been answered to my satisfaction.

☐   I agree to donate my specimen for future research studies in the field of antioxidants and eye diseases. Investigators may use my specimen for future research as long as the research.

☐   I do not agree to donate my specimen for future research studies in the field of antioxidants and eye diseases.

_____   _____   _____
Name of Participant   Signature   Date

Translator Information
The study has been explained to the participant / legally acceptable representative in
_____ by _____

Witness Statement
I, the undersigned, certify to the best of my knowledge that the participant signing this informed consent form had the study fully explained in a language understood by him / her and clearly understands the nature, risks and benefits of his / her participation in the study.

_____   _____   _____
Name of Witness   Signature   Date

Investigator Statement
I, the undersigned, certify that I explained the study to the participant and to the best of my knowledge the participant signing this informed consent form clearly understands the nature, risks and benefits of participation in the study.

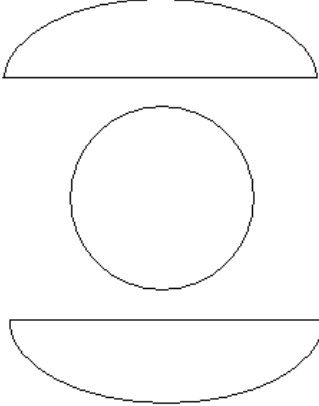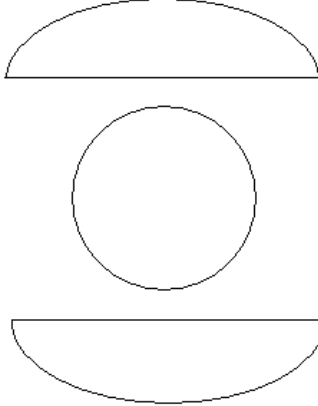_____   _____   _____
Name of Investigator /   Signature   Date
Person administering consent

<u>Eye Screening Form</u>

Study ID / Initial : _____

Date: _____

	RE	**LE**
Visual acuity	6/	6/
Anterior Segment	☐ Healthy ☐ Abnormal finding Specify:_____	☐ Healthy ☐ Abnormal finding Specify:_____
Posterior Segment	☐ Healthy ☐ Abnormal finding Specify:_____	☐ Healthy ☐ Abnormal finding Specify:_____

Is the subject eligible to participate in this study?

☐ Yes

☐ No

Data Collection Form

Study ID / Initial: _____

Date: _____

A. Personal Systemic Health
☐ None
☐ Hypertension, onset _____ year(s) ago/years old
☐ Diabetes mellitus, onset _____ year(s) ago/years old
☐ High cholesterol, onset _____ year(s) ago/years old
☐ Heart problem, onset _____ year(s) ago/years old
☐ Rheumatoid arthritis, onset _____ year(s) ago/years old
☐ Asthma, onset _____ year(s) ago/years old
☐ Others (Please specify: _____ , onset _____ year(s) ago/years old)

B. Personal Medical History
☐ None
☐ If yes, please specify
_____, dose/time per day _____, onset_____ year(s)/month(s)/day(s)ago
_____, dose/time per day _____, onset_____ year(s)/month(s)/day(s)ago
_____, dose/time per day _____, onset_____ year(s)/month(s)/day(s)ago

C. Personal Refractive Status
Do you wear any glasses?
☐ None
☐ If yes, please specify
RE _____
LE _____

D. Family Ocular History
☐ None
☐ Age-related macular degeneration, relationship _____
☐ Others (Please specify: _____ , onset _____ years ago/years old)

E. Lifestyle Factor
D1. Smoking
☐ Never
☐ Past, duration _____year(s)/month(s), quitted_____year(s)/month(s)
☐ Current, duration_____year(s)/month(s)
average _____ stick(s) per day, maximum _____stick(s) per day

D2. Alcohol comsumption
☐ Never
☐ Past , duration _____year(s)/month(s)
☐ Current, duration_____year(s)/month(s)

D3. Physical activity / Sports
_____, _____times per week/month, _____minute(s)/hour(s)_____,
_____, _____times per week/month, _____minute(s)/hour(s)_____,
_____, _____times per week/month, _____minute(s)/hour(s)_____.

F. <u>Light Exposure</u>
Do you use the following UV protection(s) when you are outdoor?
☐ None
☐ Hat / Cap
☐ Umbrella
☐ Sunglasses

G. <u>Women health</u>
G1. How old were you when you had your <u>first menstrual period</u>?
_____ years old
G2. Do you still have periods?
☐ Yes
☐ No (Please specify: Last period _____ year(s)/month(s) ago)
G2i) Did your period stop naturally or because of a hysterectomy?
☐ Naturally
☐ Hysterectomy, _____ year(s)/month(s) ago
G2ii) Did you take hormone replacement therapy after your periods stopped?
☐ None
☐ If yes, please specify: Estrogen only, _____year(s)/month(s) ago
Both estrogen and progesterone,
_____year(s)/month(s) ago
Others _____,
_____year(s)/month(s) ago
G2iii) Are you still taking hormone replacement therapy?
☐ Yes
☐ No, stopped _____ year(s)/month(s) ago
G3. Have you been pregnant before?
☐ No
☐ Yes, _____ times

Pregnancy outcome	Number of Time(s)	Onset
Live birth		Year(s)/Month(s) ago
Abortion		Year(s)/Month(s) ago
Stillbirth		Year(s)/Month(s) ago
Miscarriage		Year(s)/Month(s) ago
Being pregnant at present		Year(s)/Month(s) ago

G4. Have you ever breast feed before?
☐ No
☐ Yes, _____times, _____ month(s)/year(s)

Recording sheet for macular pigment measurement

Date	
Study ID / Initial	

Densitometer
Time Start: _____
Time End:_____
MPS II
Time Start: _____
Time End: _____

Densitometer

Stimulus 2, CFF: _____
_____
_____
Mean : _____
x (mean ÷ 2) : _____

Stimulus no.	Optimal Flicker Frequency
1 (x-1)	
2 (x)	
3 (x+1.5)	
4 (x+1.5)	
5 (x-4)	

Trial	Readout value from Radiance (R / L eye)				
	$F_{0.25}$	$F_{0.5}$	$F_{1.0}$	$F_{1.75}$	$P_{7.0}$
1					
2					
3					
4					
5					

MPS II

Trial	MPOD reading (R / L eye)
1	
2	
3	
4	
5	

Method	Eccentricity (degree)	MPOD	SD
Densitometer	0.50		
MPS II	0.50		

Remarks:

<u>Densitometer calibration sheet</u>

Date: _____

Time: _____

Machine switch on at	
Calibration done at	

Master Control Unit: Yoked, Fix LED, Blue

Subject Unit: LIN

Main Unit: Manual, stimulus no. 5

Main Switch	**Adjustment**	**Photometer reading**	**Radiance reading**	**Remark**
Main unit	N/A	N/A	N/A	☐ Stimulus no. 5 present visually
Run	Subject Unit knob	N/A	(1760±10)	N/A
Off	Dark current: clockwise	(0±1)	N/A	Gain-setting: up
Background	Background freq knob	(362±5)	N/A	N/A
Off	Dark current: anticlockwise	(0±2)	N/A	Gain-setting: down
Green	Green (mAMP)	(72±3)	N/A	N/A
Blue	Blue (mAMP)	(972±6)	N/A	N/A

Macular Matrics Densitometer (MMD) operating procedure

Introduction

MMD consist of different "units" (in Figure 1). They are described in the following:

- The main unit is the optical system that the subject looks into.
- The subject unit is the control box with a single knob turned by the subject and a silver toggle switch on the rear.
- The master control unit is the control box with numerous digital displays abd switches.
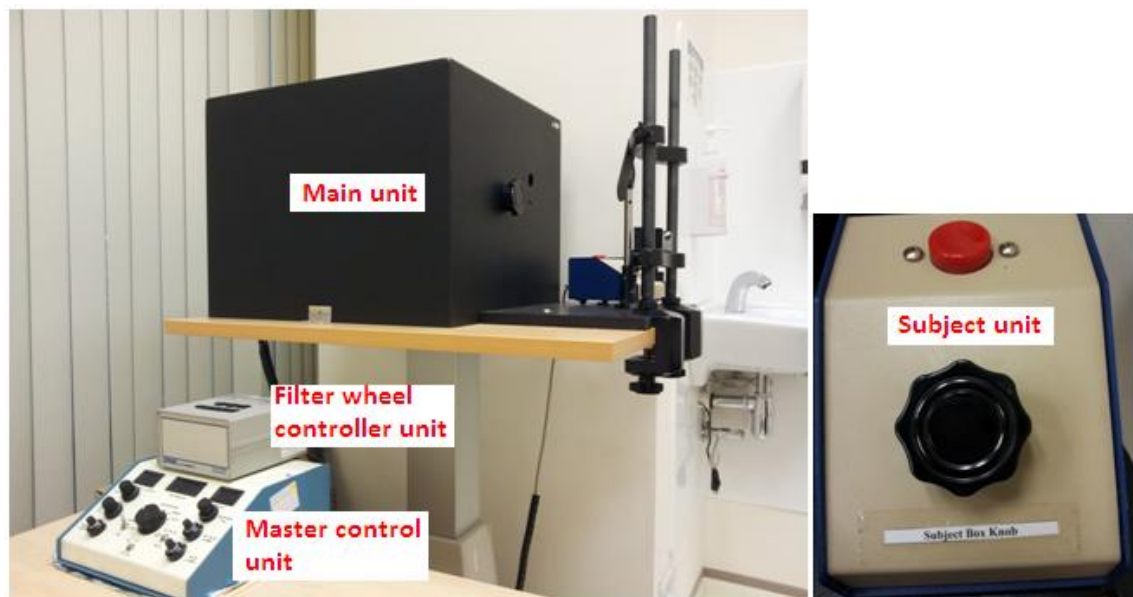- The filter wheel controller unit has buttons including "manual" and filter 1 to 5.

Figure 1. Physical appearance of Macular Metrics Densitometer (Left); Subject response knob (Right)

Calibration

1. All cables are visually inspected to make sure they are connected in correct position and MMD is calibrated daily at the start of the day.

2. Turn on all power switches including the one on the back of the master control unit and filter wheel controller unit.

3. MMD is allowed to warm up for 10 minutes before calibration.

4. Ensure that the following switches are in correct position:

    a) Master control unit: Bottom 3 sliver toggle switches point towards YOKED, FIX LED, and BLUE.

    b) Subject unit: Silver toggle switch on the back point towards "Lin".

5. Open the viewing aperture in front of the main unit.

6. Press the button "manual" and stimulus number 5 on the top of the filter wheel controller unit.

7. Check visually that stimulus number 5 is seen and then close the viewing aperture in front of the main unit.

8. Switch off the room lights.

9. Set the center knob on the master control unit in "run" position.

10. Turn the knob on the subject unit till the radiance readout is 1760 ± 10.

Checking the background radiance

1. Set the center knob on master control unit in the "off" position.

2. Place the gain-setting switch at the back of the main unit in the up position.

3. Adjust the dark current for the photometer using clockwise rotation of the blue knob (located next to the gain setting switch) until the photometer readout is 0 ± 1.

4. Set the center knob on the master control unit in the "background" position.

5. Adjust the background (freq) knob till the photometer readout is 362 ± 5.

Checking the green LED current

1. Set the center knob on the master control unit in the "off" position.

2. Place the gain setting switch at the back of the main unit in the down position.

3. Adjust the dark current for the photometer using anticlockwise rotation of the blue knob (located next to the gain setting switch) until the photometer readout is 0 ± 2.

4. Set the center knob on the master control unit in the "green" position.

5. Adjust the green (mAMP) knob on the master control unit until the photometer readout is 72 ± 3.

Checking the green LED current

1. Set the center knob on the master control unit in the "blue" position.

2. Adjust the blue (mAMP) knob on the master control unit until the photometer readout is 972 ± 6.

The calibration is recorded in Annex 6. Before measuring MP, instruction is given to the subject (Annex 8 (English version) and 9 (Chinese version)). Subject sits comfortably in front of the MMD with a chin rest after they have understood the instruction.

Estimation of CFF threshold

1. Subject is informed that before the actual measurement, the speed of the flickering light will be estimated.

2. Stimulus number 2 is used to estimate the subject's CFF threshold.

3. Set the silver toggle on the master control unit (corresponding to alt blue/off/blue) to the "off" position.

4. Turn the knob on the subject unit till the radiance readout is 1500.

5. Instruct the subject to look at the flickering disc situated in the center if the larger blue field, and fixate on the center of the disc.

6. Turn the low frequency flicker adjust knob on the master control unit in clockwise direction until the subject reports that the flicker has just stop.

7. Repeat step 6 three times and calculate the mean value. The, divide this value by 2.

8. This resulting value (x) is the optimal flicker frequency to be used for the stimulus number 2. Determine the flicker frequency for the remaining stimuli (Figure 2) as follows:

   - Stimulus number 1: x – 1
   - Stimulus number 2: x
   - Stimulus number 3: x + 1.5
   - Stimulus number 4: x + 1.5
   - Stimulus number 5: x – 4
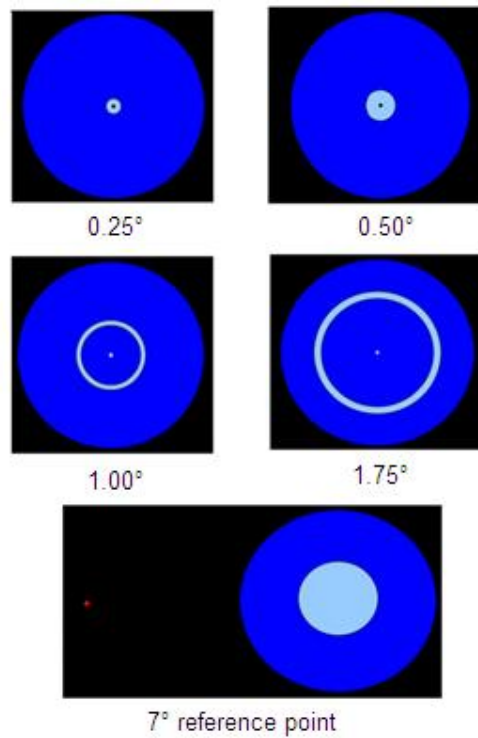
Figure 2. Five stimuli in MMD. Subject is asked to fixate at the centre dot of the flickering target when measuring MPOD at 0.25° (stimulus number 1), 0.50° (stimulus number 2), 1.00° (stimulus number 3), and 1.75 (stimulus number 4)° retinal eccentricities. At the peripheral 7° (stimulus number 5) test, subject fixates at the red dot on the left side.

MP measurement

1. Set the silver toggle on the master control unit (corresponding to alt blue/off/blue) to the "blue" position.

2. Start measurement of MP at stimulus number 1.

3. Press the button for stimulus number 1 on the filter wheel controller unit.

4. Set the low frequency flicker to the predetermined value for stimulus number 1.

5. Turn the knob on the subject unit to near one extreme.

6. Request subject to turn the knob clockwise and anticlockwise until he/she is in the middle of the "no flicker" zone. (Detailed instruction is given using Annex 6 (English version) and 7 (Chinese version).)

7. Remind the subject to fixate at the central dot and blink normally.

8. Once the subject has reached the middle of the "no flicker" zone, request him/her to let go the dial on the subject unit.

9. Record the radiance value that appears on the master control unit.

10. If the subject always sees flicker, or the "no flicker" zone is too wide, make fine adjustment to the flicker values to optimize the operating conditions.

11. Repeat step 8 for four more times or more until 5 consistent readings (standard deviation is not more than 0.1). If the values obtained are not within the acceptable range, adjust the flicker rate and record 5 additional values.

12. Instruct subject to lean back and close eyes while settings are adjusted for the next stimulus.

13. For measurement of MP at stimulus number 2, 3, 4, and 5, repeat steps 5 to 12 after adjusting to the low frequency flicker to the predetermined flicker frequencies for each of the corresponding stimuli.

14. Use both the near extremes of the knob on subject unit so that the subject is not always turning knob in the same direction.

15. The radiance value is keyed into the MPOD analysis excel worksheet. The software allows automatic calculation of the mean MP at each of the stimuli and generation of SP of MP.

16. Adjust the knob in the master control unit in "off" position if the instrument is not in use. Turn off all power switches at the end of the day.

Instruction on Macular Matrics Densitometer (MMD)

MMD is a machine that measures the amount of yellow pigment.

Your optometrist will help you to determine the suitable target. You will need to look at the flashing target while your optometrist increases the flashing speed until there is no flicker. Once the light stop flashing, report to your optometrist.
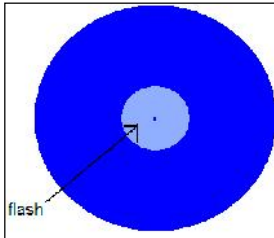

Figure 1 shows the appearance of the flashing light. You need to look at the center dot of the flash throughout the whole experiment.
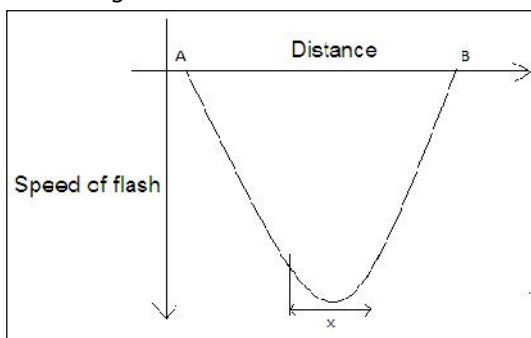
Figure 1

Figure 2

The next part of the test is to adjust the knob to determine the no/minimum flicker zone. At extreme point A/B you will see the blue/green target gradually turns green/blue as you turn the knob towards other end while the speed of flash decreases until light become stable or almost no flash is seen. If you see the flashing light has stopped or minimized, continue to turn the knob in the same direction until you see the flash starts or increases in speed again. When you see this happens, reverse the direction of the knob and repeat a few times, if necessary. Finally, put the knob at the center of the minimum / no flashing zone (x).
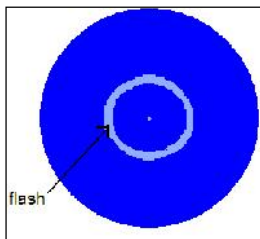
Figure 3 shows a ring target. You need to look at the center dot while observing the ring at the side.

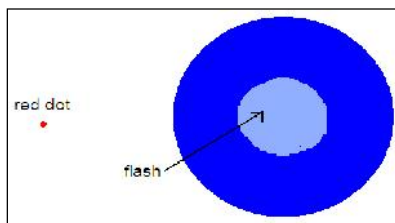*Figure 3*

Figure 4 shows a round patch of flicker at the center. The fixation target is now the red dot at the left side of the flashing light.

Figure 4

Sometimes the flicker will fade away after a while. This can be easily mistaken as no flicker. An easy remedy to solve this problem is to blink a couple of times every few seconds.

Five sets of reading will be done for each target in your right eye. This will take around 30 minutes to complete.

使用 **Macular Matrics Densitometer (MMD)** 的方法

MMD 是一台测量黄斑色素之**密度**(MPOD)的**机器**。

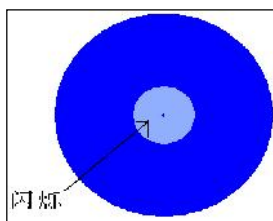您的视光师会帮助您确定合适的闪烁速度。首先，您的视光师会让您看到闪烁。然后，帮您加快闪烁的速度直到您看不到闪烁。请眨眼几次。一旦您没有看到闪烁，请通知您的视光师。

图 1

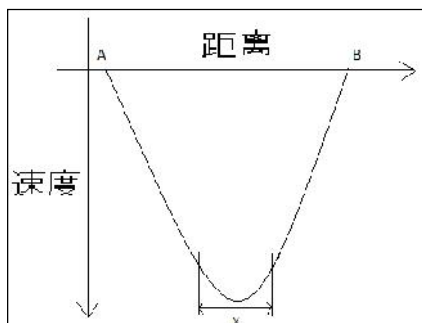图 1 显示了第一个闪烁目标。您的任务是看着闪烁的中心，然后观察闪烁。

图 2

测试的下一部分需要您自己调整旋钮到无或**最低**闪烁的中心区。当您转动旋钮到另一个极端(A 到 B)的时候，蓝/青色的闪烁速度将会渐渐变慢到无或**最低**闪烁，而且会慢慢变青/蓝色。如果你开始看到闪烁已停止或减少，请继续在同一个方向旋转，直到您看到闪烁再次增加速度。请重复做这一点直到您可以却定无或**最低**闪烁的中心区 (X)。
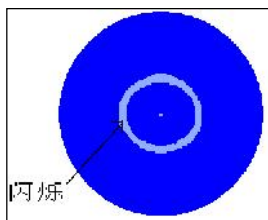

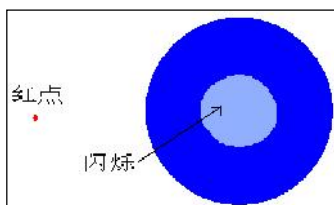
图 3

图 3 显示了第二个闪烁目标。您的任务是看着闪烁的中心，然后观察中心边的闪烁与调整旋钮。

图 4

4 显示了第三个闪烁目标。您的任务是看着一旁的红点，然后观圆型的闪烁与调整旋钮。

有时闪烁到一段时间之后会慢慢淡去。这可以很容易被误认为无闪烁。一个简单的解决办法是每隔**几秒**钟眨一次眼睛。

您需要重复这个测验在您的右眼五次。 这将会花大越 30 分钟。

Macular Pigment Screener II (MPS II) operating procedure

Starting up the instrument

1. Switch on the laptop and MPS II (Figure 1).

Figure 1. Physical appearance of Macular Pigment Screener II.

2. Connect the MPS II to the computer using the USB cable.

3. Run MPS II software under Microsoft Office Access 2007.

4. Click "Add" button and enter patient's name, date of birth and gender, then click the "save" button. (Figure 2)

Figure 2. Screenshot of MPS II window: Patient data.

5. Start measuring macular pigment optical density (MPOD) by clicking the "Test/View" button.

6. Enter examiner's name and click "OK" button (Figure 3).

Figure 3. Screenshot of MPS II window: Operators name.

If software prompt "USB port is not connected" (Figure 4), please do the following.

Figure 4. Screenshot of MPS II window: USB connection.

7. Click "Cancel" button.

8. Close the MPS II software.

9. Unplug and plug in the MPS II's USB cable.

10. Run MPS II software again under Microsoft Office Access 2007.

11. Find patient's name, then click "Test/View" button.
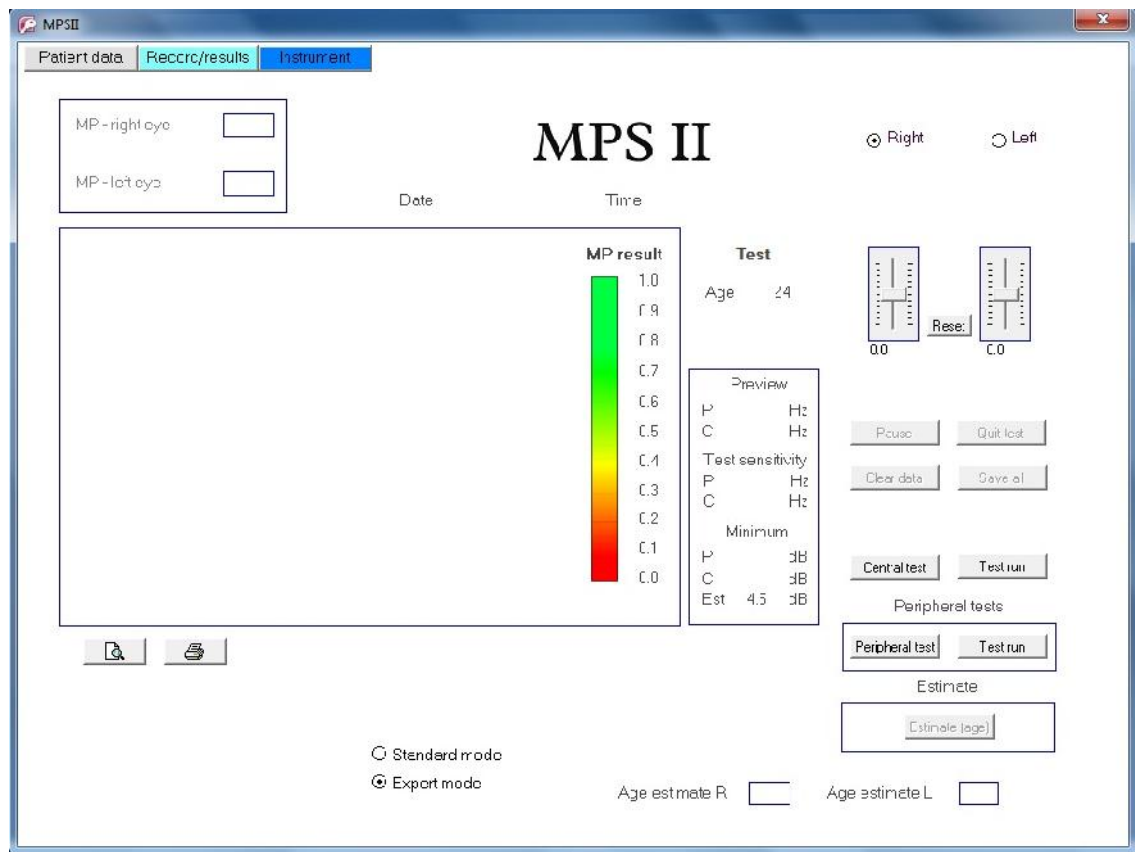
MP measurement

Figure 5. Screenshot of MPS II window: Record/results.

1. Detailed instruction given to subjects was attached in Annex 11 (English version) and 12 (Chinese version) before measurement.

2. Click "Expert mode" button (Figure 5) to start measurement.

3. Start measuring MPOD in the right eye by clicking on "central test" button.

4. Instruct subject to press the response button when the stationary target appears to be flickering (Figure 6).

5. After the central test is completed, start peripheral test by clicking on "peripheral test" button (Figure 6).
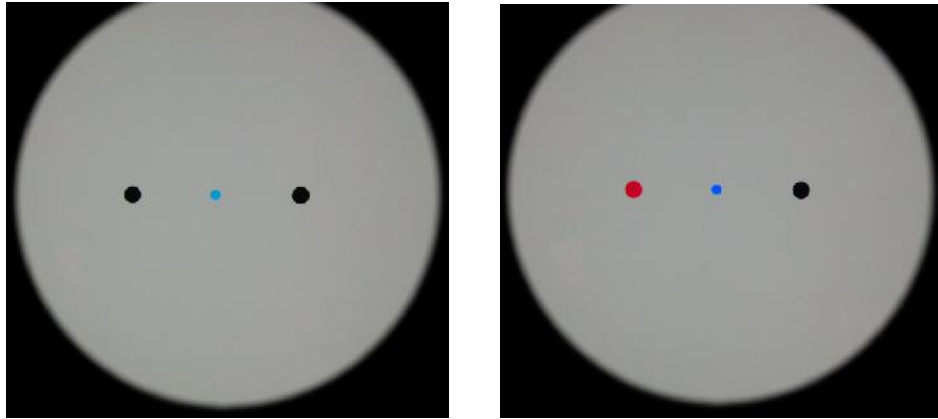

Figure 6. MPS II stimulus and fixation target. Central test (Left). Subject fixates at the centre dot and press the response button when it starts flickering. Periphery test (Right). Subject fixates at the red dot while observing the centre target flickers.

6. Upon completion of both central and peripheral test, proceed to save result by clicking "save all" button.

If patient could not perform peripheral test reliably, measure MPOD using the "centre-only" method by clicking the "standard mode" button.

Instruction on Macular Pigment Screener II (MPS II)

MPS II (Figure 1) is a machine that measures the amount of yellow pigment inside your eye.

Figure 1

When performing the central test, you will need to look at the center blue dot (Figure 2) and press the response button once you see the steady center blue dot start to flicker. When performing the peripheral test, you will need to look at the red dot (Figure 3), and press the response button when you see the steady central dot flickers. Sometimes the target will fade away after a while. An easy remedy to solve this problem is to blink a few times every few seconds after the response button is pressed.
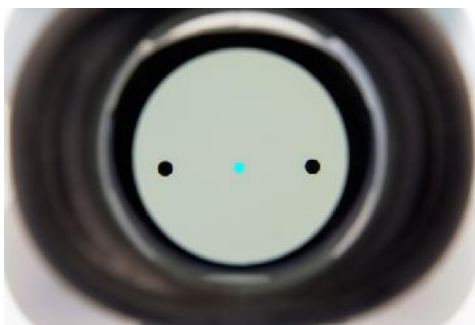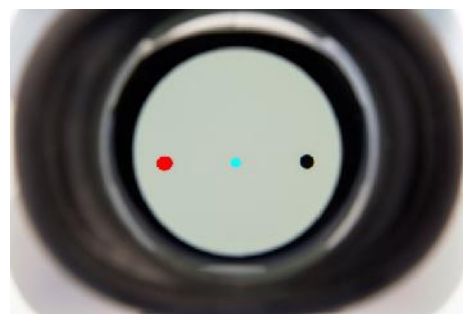

Figure 2

Figure 3

Five sets of reading will be done in your right eye. This will take around 30 minutes to complete.

使用 **Macular Pigment Screener II (MPS II)** 的方法

MPS II **是一台**测量黄斑色素之**密度**(MPOD)**的机器**（图 1）。

图 1

首先，您的任务是看着中心的蓝点。当您看到闪烁时（图 2 ），请按钮。您的第二个任务是看着红点 (图 3)。当您看到旁边的闪烁时，请按钮 。
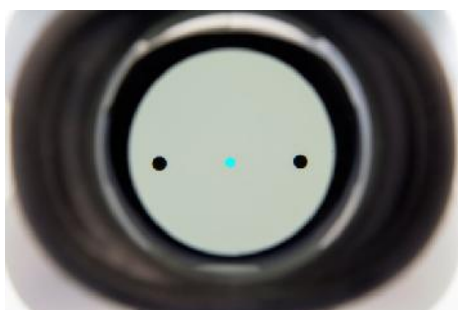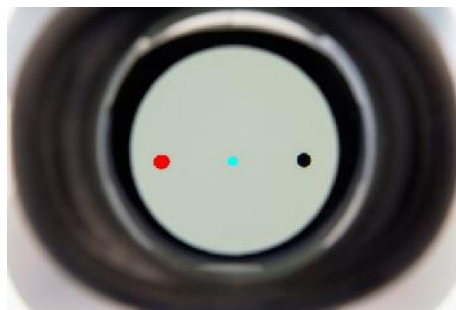

图 2   图 3

有时闪烁在一段时间之后会渐渐消失。一个简单的解决办法是在您按了钮之后，眨一次眼睛。

您的视光师会重复这个测验在您的右眼五次。 这将会花大越 30 分钟。