# OPHTHALMIC DOCTORATE

# Cataract, macular characteristics and assessing lens opacities

Martin R. Smith

2014

Aston University

# Cataract, macular characteristics and assessing lens opacities

MARTIN RICHARD SMITH

Doctor of Optometry

ASTON UNIVERSITY

November 2013

©Martin Richard Smith, 2013

Martin Richard Smith asserts his moral right to be identified as the author of this thesis

Aston University
Cataract, macular characteristics and assessing lens opacities
Martin Richard Smith
Doctor of Optometry
2013

**Summary:**

Age-related macular degeneration and cataract are very common causes of visual impairment in the elderly. Macular pigment optical density is known to be a factor affecting the risk of developing age-related macular degeneration but its behaviour due to light exposure to the retina and the effect of macular physiology on this measurement are not fully understood.

Cataract is difficult to grade in a way which reflects accurately the visual status of the patient. A new technology, optical coherence tomography, which allows a cross sectional slice of the crystalline lens to be imaged has the potential to be able to provide objective measurements of cataract which could be used for grading purposes.

This thesis set out to investigate the effect of cataract removal on macular pigment optical density, the relationship between macular pigment optical density and macular thickness and the relationship between cortical cataract density as measured by optical coherence tomography and other measures of cataract severity.

These investigations found:

1) Macular pigment optical density in a pseudophakic eye is reduced when compared to a fellow eye with age related cataract, probably due to differences in light exposure between the eyes.

2) Lower macular pigment optical density is correlated with thinning of the entire macular area, but not with thinning of the fovea or central macula.

3) Central macular thickness decreases with age.

4) Spectral domain optical coherence tomography can be used to successfully acquire images of the anterior lens cortex which relate well to slit lamp lens sections.

5) Grading of cortical cataract with spectral domain optical coherence tomography instruments using a wavelength of 840nm is not well correlated with other established metrics of cataract severity and is therefore not useful as presented as a grading method for this type of cataract.

**Key words: Cortical cataract; MPOD; Macular pigment; Macular thickness; OCT**

Dedication

To Hazel, Isaac and Arthur.

Acknowledgements

I would like to thank:

My wife Hazel for supporting me throughout this endeavour and for putting up with me being busy and unavailable for all the other things I should have been doing for the last 5 years;

My children Isaac and Arthur for being a constant source of entertainment and distraction;

My supervisor Professor James Wolffsohn for his advice and encouragement via email at all times of the day, however unsociable;

Dr Bartlett, Dr Eperjesi, Dr Howells, Prof. Dunne, Dr Naroo, Dr Davies and all who have helped me through correspondence during this course;

The subjects who agreed to give up their time to participate in the study, without whom it would not have happened;

Finally I would like to thank all my friends, family and various acquaintances who had the good grace to look interested when they made the mistake of asking what I was studying.

**Contents**

List of Tables:

List of Figures:

List of abbreviations:

| | |
|---|---|
| AMD | Age-related Macular Degeneration |
| AREDS | Age Related Eye Disease Study |
| ARM | Age Related Maculopathy |
| AS-OCT | Anterior Segment Optical Coherence Tomography |
| AVT | Average macular Thickness |
| C | Cortical Opacity |
| D | Dioptres |
| FAZ | Foveal Avascular Zone |
| FT | Foveal Thickness |
| HFP | Heterochromatic Flicker Photometry |
| IOL | Intraocular Lens |
| IOP | Intraocular Pressure |
| IR | Infrared |
| L | Lutein |
| LED | Light-Emitting Diode |
| LOCS III | Lens Opacity Classification System Three |

| | |
|---|---|
| MP | Macular pigment |
| MPOD | Macular Pigment Optical Density |
| MT | Macular Thickness |
| MZ | Meso-Zeaxanthin |
| NC | Nuclear Colour |
| NHS | National Health Service |
| NO | Nuclear Opacity |
| OCT | Optical Coherence Tomography |
| PEDF | Pigment Epithelium-Derived Factor |
| PP | Pseudo-Phakic |
| RNFL | Retinal Nerve Fibre Layer |
| ROS | Reactive Oxygen Species |
| RPE | Retinal Pigment Epithelium |
| UV | Ultraviolet |
| VA | Visual Acuity |
| VEGF | Vascular Endothelial Growth Factor |
| Z | Zeaxanthin |

Chapter One

Introduction: Age related diseases of the lens and macula

Cataract and macular degeneration are common age related causes of vision loss which between them are detectable in most people to some extent in later life (Klein et al., 1992, 2007). Age-related macular degeneration affects the ability of the central retina to detect light and cataract affects the ability of the eye to form an image on the retina. This research concerns aspects of the crystalline lens and macula.

It will cover the effect of cataract removal and the subsequent presumed increase in macular light exposure on levels of macular pigment optical density (MPOD), the relationship between MPOD and central macula thickness and assessment of cortical cataract development using optical coherence tomography.

This first chapter will cover the background knowledge regarding the macula, macular pigment (MP) and age-related macular degeneration. It will then cover the structure of the macula and its relationship to macular pigment and the development of cataract with regard to its potential measurement using optical coherence tomography.

1.1: Age-related disease of the macula and macular pigment

The macula is a region of the retina responsible for the highest resolution vision which gives us the ability to read print, discern details of faces and perform visually intensive tasks such as driving. It is defined as the central 3mm of the retina or approximately 2 optic disc diameters, with the macula lutea, or fovea, at the centre (Bird et al., 1995a) (Figure 1.1; Li et al., 2010). This area is largely responsible for our ability to perceive colour, with the majority of the cone receptors located there, the periphery being populated by the more light sensitive rods(Jonas et al., 1992).

Age-related macular degeneration (AMD) is the second most common cause of vision loss in the world after cataract, the leading cause of vision loss in the developed world (Congdon, 2003) and the most common cause of registered blindness in the UK (Rahi et al., 2002). It is caused by age-related dysfunction and ultimately atrophy of the outermost layer of the sensory retina, the retinal pigment epithelium (RPE). The photoreceptors are embedded into the anterior surface of the RPE and loss of this layer leads to cell death of the photoreceptors and consequent loss of vision.

Figure 1.1: The normal macula (white dashed line) and the structure of the three components of macular pigment: from Li et al (2010).

AMD is currently irreversible but as its name suggests generally only affects the macula, leaving the peripheral retina intact (Sunness et al., 1985) (figure 1.2). It leads to severe difficulty with many common tasks such as reading. Some RPE dysfunction is identifiable in most elderly people (Bressler et al., 1989; Mitchell et al., 1995), but only a minority of those will experience vision loss, the critical factors being the area and location of the damage . Even minor damage to the fovea can lead to serious vision loss whereas atrophy not involving the fovea can be asymptomatic, as can significant loss of vision in only one eye (Jager et al., 2008).
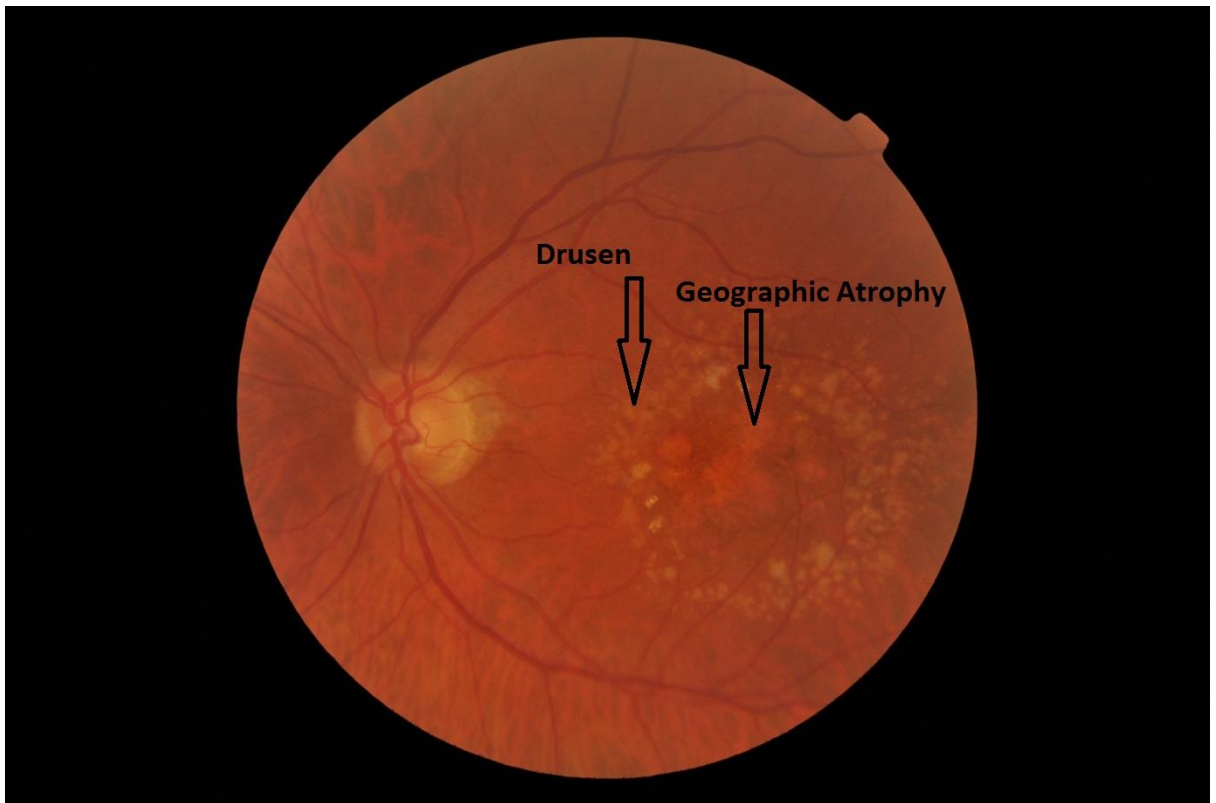
Figure 1.2: Macular degeneration with geographic atrophy at the centre surrounded by soft drusen.

Macular pigment is the name given to a collection of pigments which are selectively accumulated in the macular region of the eye. They are yellow in colour due to their blue light absorbing properties and this gives the region its name, the macula lutea. Macular pigment optical density represents the measurable absorption of light by the MP in the retina.

The yellow appearance of the fovea has been documented since 1792 and the existence of MP was first postulated some 150 years ago as described by Whitehead (2006) but the two principal components only specifically identified in the last 30 years (Bone et al., 1985).

The existence of MP is thought to be an evolutionary adaptation to improve glare control and reduce chromatic aberration. It is thought to do this by reducing light scatter by short wavelength blue light which would otherwise degrade the retinal image quality by passing unfocused scattered light through the photoreceptors (Wooten and Hammond, 2002).

Located primarily in the axons of the cone photoreceptors where it is able to filter light before it reaches the photoreceptor pigments, MP is found in primates and other animals such as bovines, frogs and birds (Khachik et al., 2002; Li et al., 2010). It has been demonstrated that macular pigment improves contrast sensitivity (Nolan et al., 2011), improves retinal image quality through reduced chromatic aberration (Thibos, 1987), increases tolerance to light levels, delaying the onset of photophobia (Wenzel et al., 2006) and improves the recovery from high levels of light exposure (Sasaki et al., 2012). Older subjects with high MPOD have been shown to retain their visual sensitivity better than those with low MPOD (Hammond et al., 1998).

MP also appears to have an antioxidant role and a barrier protection against oxidative damage caused by blue light, the highest energy light encountered by the retina, to the RPE and choriocapillaris (Kijlstra et al., 2012). This protection is thought to help delay the onset of AMD and there have been several studies which have shown a correlation between MPOD and AMD risk (Bone et al., 2001; Delcourt et al., 2006; Raman et al., 2012) but not all studies show this effect (Berrow et al., 2011; Mares-Perlman et al., 1996, 1995) (see chapter 2 for a more comprehensive list of relevant studies).

As AMD in humans only occurs in those largely beyond reproductive age the protective role may not be the evolutionary selection pressure which has led to its selective uptake. However, Malinow and colleagues (1980) showed that window defects and drusen like

abnormalities occurred in monkeys deprived of xanthophylls for just seven years. Monkeys with normal levels of MP have been demonstrated to develop macular degeneration at just 19-28 years of age, unlike humans (Gouras et al., 2008). Therefore the antioxidant effect may be for other animals important in survival and reproduction in a way which may not apply to humans.

Oxidative damage occurs to retinal tissues from both light exposure and normal metabolic processes which both tend to generate reactive oxygen species, with the RPE being the most metabolically active tissue in the body (Feigl, 2009). It has been proven that supplementation with antioxidants slows the progression of some forms of AMD and reduces the likelihood of neovascular AMD (Chew et al., 2013). Macular pigment is thought to have a role in controlling oxidative damage to tissues (Barker et al., 2011; Bhosale and Bernstein, 2005; Chucair et al., 2007; Kernt et al., 2012) and oxidation products of the macular pigments Lutein and Zeaxanthin have been found in the retina confirming their antioxidant action (Khachik et al., 1997). The continuous phagocytosis of the pigment discs of the photoreceptors (figure 1.3) and high levels of unsaturated lipids are also responsible for the high levels of oxidative stress in this region (Sun et al., 2006).



Aston University

Illustration removed for copyright restrictions

figure 1.3: Schematic drawing showing the arrangement of the photoreceptors and the phagocytosis of the receptor tips by the RPE. ROS = rod outer segments From Schraermeyer and Heimann (1999)

The pigments which make up MP are the structural isomers lutein (L), zeaxanthin (Z) and meso-zeaxanthin (MZ). MZ is a stereoisomer of zeaxanthin. L Z and MZ are all xanthophyll carotenoids and the only carotenoids to be found in the retina (Figure 1.1). Being isomers, all have the same chemical formula $C_{40}H_{56}O_2$. These pigments have several double bonds which act as free radical scavengers due to the high availability of free electrons (Bone et al., 1993).

The concentration of these pigments in the retina varies between individuals and the peak absorption can be measured, producing a result known as the macular pigment optical density, or MPOD. The distribution of these pigments is not even across the macular region (Bone et al., 1997) (figure 1.5) and the distribution of the pigments can also be measured through various methods described subsequently. The peak absorbance of MP is around 460nm (figure 1.4) and it has been estimated that an average level of MP in humans absorbs between 20-40% of light at this wavelength, whereas those with a high level of MP may absorb up to 98% (Hammond and Caruso–Avery, 2000; Landrum and Bone, 2001).



Aston University

Illustration removed for copyright restrictions

Figure 1.4: Absorption spectra of the lens, macular pigment, oxygenated and deoxygenated blood, and melanin. In this graph lens density is 0.54 at 420 nm, MPOD is 0.5 at 460 nm and the melanin density is 0.8 at 500 nm. From Berendschot et al. (2003).

Macular pigment is accumulated in high concentrations in the Henle fibres and inner plexiform layers in primate retinas and is highest in concentration at the fovea, from which it declines to uniform, constant density from approximately 1mm eccentricity and is

detectable throughout the retina using high performance liquid chromatography (Snodderly et al., 1984).

There may be slightly different roles for L and Z in the retina linked to their differing orientations which have been observed experimentally (Sujak et al., 2000). L is proposed to be involved in absorbing short wavelength radiation penetrating retina membranes and Z protecting the lipid phase against oxidative damage. The ratio of L and Z also varies across the retina with Z being dominant in the fovea and the ratio being biased towards L with increasing eccentricity (Bone et al., 1997). MZ is only present at the central fovea and is the most potent antioxidant of the three (Bhosale and Bernstein, 2005).



Aston University

Illustration removed for copyright restrictions

Figure 1.5: The distribution of yellow macular carotenoids in the fovea of a monkey. The fovea is generally slightly smaller in humans. From Kijlstra et al. (2012).

There are over 600 carotenoids in nature, of which 30-50 are contained in a typical human diet and there is uptake of 10-15 of these into the serum. Only L, Z and MZ are present in the human retina and the central retina has the highest concentration of carotenoids anywhere in the body (Ermakov et al., 2001). The sole source of L and Z is from the diet as, in common with all carotenoids, the human body is unable to synthesise them. MZ is produced in the retina from dietary L, not being found in serum (Bone et al., 1993) although supplementation with MZ leads to detectable increases in serum and retinal concentrations

and MPOD (Bone et al., 2007; Connolly et al., 2010). It is debated whether the sole source of MZ is from conversion from L within the retina or not (Nolan et al., 2013).

It has been shown that concentrations of MP in the retina are associated with serum concentrations of the specific carotenoids and that supplementation with high levels of L and Z can increase the concentrations of pigment in the retina (Berendschot et al., 2000) There is a time lag between both introduction and withdrawal of supplements and MPOD changes (Landrum et al., 1997) although it is debated how long this is with responses to supplementation in as little as two weeks reported (Connolly et al., 2010).

In dietary terms, L and Z are high in concentration in green leafy plants, egg yolk, yellow foods such as corn and other sources such as spices and pistachio nuts (Sommerburg et al., 1998) (see table 1.1) with eggs being a particularly good source due to the fat content which assists bioavailability (Chung et al., 2004). Chickens fed on corn or L/Z enriched feed produce eggs high in L and Z which give them their characteristic colour desired by food producers and consumers. Free range chickens produce eggs with a higher lutein content than caged chickens (Schlatterer and Breithaupt, 2006) and commercially the macular pigments are often extracted from marigolds (Bosma et al., 2003). However these carotenoids are not popular as a food colouring due to its poor stability when exposed to light (Mortensen and Skibsted, 1997), a key property of interest in this study (Haila et al., 1996). Some eye targeted or AREDS based supplement manufacturers instead of listing L or Z label their products as containing marigold extract to appeal to the popular naturalistic health fallacy whereby products are assumed to be beneficial purely by virtue of being natural.

Table 1.1: Major carotenoids in fruits and vegetables in mole %, sorted by lutein/zeaxanthin combined content. From (Sommerburg et al., 1998)



Aston University

Illustration removed for copyright restrictions

The uptake of L and Z from the diet is through the small intestine whereupon it is transported by lipoproteins in the bloodstream (figure 1.6). The pigments are transported in the blood bound to HDL cholesterol as they are hydrophilic and travel to the choriocapillaris to be taken up by the RPE. There is a 10000 fold higher concentration of L and Z in the retina compared to the blood which indicates that there is a regulated active transport mechanism in place to accumulate the pigment, probably by xanthophyll binding proteins, of which there are several candidates (Bhosale et al., 2004; Li et al., 2010).

Retinal tubulin has been found to have a high affinity for carotenoids and likely acts as a binding site for L and Z taken up by the binding proteins in the Henle fibres where, like L and Z, it is highly concentrated. It is unlikely to be responsible for uptake itself due to its non-selective affinity for other carotenoids and the absence of those carotenoids in the retinal tissue. It has been speculated that some of the greater risk of AMD seen in those with low MPOD may be due to low levels of basement membrane bound transport proteins (Li et al., 2010). This may be an explanation for the existence of people for whom dietary supplementation does not increase the MPOD in the retina (Tanito et al., 2012).

 Dietary supplementation of L Z and MZ appears to be safe, with no significant health effects detected (Alves-Rodrigues and Shao, 2004; Bartlett and Eperjesi, 2005; Connolly et al., 2011). There is some recent evidence of a relationship between MP and glaucoma (Igras et al., 2013) which indicates a possible neuroprotective role for MP or that glaucomatous eyes either have a lower ability to transport or deposit MP or increased oxidation destroys MP.

Figure 1.6: Possible pathway for MP carotenoid uptake, transport, and accumulation in the human retina. Choroicapillaris (CH); Bruch's membrane (BM); retinal pigment epithelium (RPE); inner segments (IS); outer plexiform layer (OPL); inner plexiform layer (IPL); selective binding proteins (StARD and GSTP1); HDL receptors (SR-BI) .From Li et al (2010).

Serum concentrations of L and Z are relatively poor indicators of MPOD and about half of the variability in pigment can be attributed to differences in diet and only a third to differences in serum concentrations (Bone et al., 2000). The remaining difference may be due to differences in uptake; differences in decay rates of the MP due to light exposure or other oxidative stress which degrades the MP; differences in transport protein levels or differences in macular architecture.

There are some gender differences in MPOD which are thought to be due to differences in the uptake mechanism from serum into the RPE. Males have MPOD levels which are on average higher than females and there is a stronger correlation between serum levels and MPOD in males. This may partly account for the higher rate of AMD in females (Beatty et al., 1999).

There appears to be some racial differences in MPOD with Blacks having lower levels of MPOD than Whites (Iannaccone et al., 2007). This is despite African Americans having almost double the dietary intake of L than White and Hispanic Americans and having higher serum concentrations of these pigments as a result (Mares-Perlman et al., 2001).

AMD risk and melanin levels have been found in some studies to be generally inversely correlated (Weiter et al., 1985). Racial differences correlate poorly with dietary intake indicating there may be differences in MP uptake rates. Melanin may provide a protective role (Hammond et al., 1996), however, the absorption profile of melanin correlates poorly with the wavelengths implicated in macular degeneration, indicating that the primary protective role of melanin might be as a 'sink' for Reactive Oxygen Species (ROS) rather than purely photoprotective.

These differences in MPOD observed between races are likely due to genetic differences, possibly influenced by geographical differences in light levels encountered through evolutionary history (Friedman et al., 1999) although the recent finding that MPOD has also recently been found to be higher in South Asians than Whites (Howells et al., 2013a) contradicts this hypothesis.

In general, those with darker irises in a single geographical area tend to have greater levels of macular pigment, which could be linked to lower light exposure to the retina due to greater iris pigmentation (Hammond and Caruso–Avery, 2000).

1.2: Methods of MPOD measurement

It is desirable, given the importance of MP and its supposed role in protecting the eye from several factors leading to the development of macular degeneration, to be able to measure MPOD *in vivo* for both research and clinical purposes. In a clinical setting it can be helpful to identify which patients are at increased risk of macular degeneration and to be able to assess the effect or otherwise of any supplementation or protective measures such as light filtration. Many practitioners, with reference to recent research in the area, recommend their patients to take supplements which contain L and Z, often combined with an antioxidant formulation based on the AREDS and AREDS2 research (Bartlett and Eperjesi, 2004a; Kassoff et al., 2001) and many patients with AMD are aware of the research surrounding this area and take supplements (Bartlett and Eperjesi, 2004b). These supplements are expensive at a recommended retail price of between £9-£15/month and being able to confirm that the supplementation levels is having the desired effect on MPOD and being able to differentiate which patients are likely to benefit from them is a useful tool.

From a research perspective, there are many gaps in the current knowledge of the exact role MP has in the development of AMD. There are reliable invasive methods of measuring MP in the retina such as chromatography, but these are not of use in determining the status of living tissue where MP levels cannot be directly measured and must therefore be inferred by measuring MPOD. The technique used in this study is heterochromatic flicker photometry and this will be described. As other studies referenced have used different techniques to measure MPOD, a brief description of alternative techniques for measuring MPOD and reasons for the selection of this technique over others will be provided. Measurements of

MPOD can be principally categorised into psychophysical and optical methods. A detailed review of the various methods has recently been produced by Howells et al. (2011).

## 1.2.1: Resonance Raman measurement:

This optical method illuminates the macula with a low-power argon laser spot and measures Raman backscattered light using a spectrograph. The Raman signal intensity at 1525 $cm^{-1}$ generated by the carbon–carbon double-bond vibrations of lutein and zeaxanthin can be used to quantify the levels of these carotenoids in the retina (figure 1.7). One study using this technique failed to demonstrate a difference between levels of MP in those who had undergone cataract surgery although it is acknowledged that there is a drop in signal in those with age related lens yellowing which may confound results, (Ermakov et al., 2001). There are no commercially available instruments. This technique requires a correction to compensate for ocular media absorption which makes it unsuitable for use in this particular study (Delori et al., 2001). A more recent study discussed in more detail later which used results pre and post operatively to calibrate the correction required to compensate for lens yellowing has demonstrated a drop in MPOD 1-2 years after surgery (Obana et al., 2011).

The technique is controversial, with questions over its repeatability raised (Howells et al., 2012)

Figure 1.7: A map of MPOD produced by resonance Raman spectroscopy. From (Sharifzadeh et al., 2008)



Aston University

Illustration removed for copyright restrictions

1.2.2: Reflectometry:

An established technique which has been used for 25 years, reflectometry analyses the spectral reflectance of the retina to provide estimates of MP (Kilbride et al., 1989). There are several variations on the method which use both subjective colour matching and objective analysis of light reflected from different points on the fundus which will differ as the light reflected from areas with high levels of MP will have certain wavelengths attenuated by absorption of them by the MP. There are commercial instruments available such as the macular pigment reflectometer (MPR, Maastricht instruments, Maastricht, Netherlands) and it provides a reliable measurement of MP which is comparable to other methods (Berendschot et al., 2000; Delori et al., 2001). There is limited information on the reliability

of this method in the presence of cataract and the instruments make assumptions regarding the homogeneity of the ocular media which it is reasonable to suppose might be violated in cases of cortical cataract in particular and the measurements are known to be impaired by light scatter. The technique usually requires pupil dilation and due to the complexity of the optics involved the instruments tend to be rather expensive (Howells et al., 2011). It requires bleaching of the photoreceptors before measurements are taken to prevent absorption of light by the photoreceptor pigments confounding the results, which requires levels of light patients may find unpleasant.

1.2.3: Fundus autofluorescence:

This optical technique uses the attenuation of lipofuscin fluorescence originating from the retinal pigment epithelium to estimate MPOD. The retina is illuminated using two sources of monochromatic light, one of which stimulates fluorescence from MP and lipofuscin together and the other which stimulates lipofuscin alone. The spectrum of light reflected from the retina can then be used to infer MP levels (figure 1.8). First described in 2001 (Delori et al., 2001) it has been used in research settings but no commercial instruments are available. A similar technique using only one wavelength of light is used by the Visucam device (Zeiss Meditec, Jena, Germany) however this makes the assumption that the fluorophores are evenly distributed over the area analysed, which may not be the case and is not therefore as reliable a method as the two wavelength technique (Dennison et al., 2013; Trieschmann et al., 2006). Recent research casts doubt on its ability to reliably measure MP in the presence of cataract (Sasamoto et al., 2011)

Aston University

Illustration removed for copyright restrictions

Figure 1.8: Fundus autofluorescence measurement of MPOD in two different eyes. The yellow line is the MPOD, the blue and green the aoutoflurescence signal at 488 and 514nm. From (Waldstein et al., 2012)

1.2.4: Heterochromatic flicker photometry:

The most popular technique for measuring MPOD in practice and widely used in research, heterochromatic flicker photometry (HFP) is a psychophysical subjective technique which uses the absorption properties of MP and its effect on the visibility of different wavelengths of light. This technique has been shown to be reliable in older patients such as those likely to be eligible for this research (Snodderly et al., 2004). Two alternating stimuli are presented, the wavelength of one stimuli being absorbed by and therefore having reduced visibility in

36

the presence of MP and the other stimulus not being affected by the presence of MP. Where the perceived luminance is unequal, the stimulus will appear to flicker and where the perceived luminance is equal, the stimuli will appear to be static. The point at which the stimulus appears to be steady will depend on the MPOD in the region of the retina being stimulated by the test and a measure of MPOD can be inferred from this point. This point will be affected by outside factors such as the absorption spectra of the crystalline lens which will vary between subjects; therefore a single measurement at the fovea will not produce a particularly reliable result. To correct for this typically a measurement is taken from the fovea and compared to a measurement taken in the peripheral macula where MPOD is known to be low, and the ratio of the two measurements can be used to calculate MPOD.

One potential source of error in patients supplementing with high doses of L, Z and MZ is that peripheral deposition of the MP where it is assumed by the instrument to be close to zero may affect the validity of the results (Howells et al., 2011). An estimate of the effect of lens yellowing based on age can also be used and although this is prone to error as that assumption may not be correct it can still give useful results in patients who are unable to complete the peripheral test. It may be adequate in patients where a change in MPOD is the outcome to be measured, rather than the absolute value. In subjects with a clear IOL the software in the instrument can account for this and assume lens yellowing to be zero.

There are several methods used to present the stimulus to the patient, the most common being a method where the subject adjusts the ratio of the two stimuli until the perception of flicker is minimised, and another where the two stimulus are automatically alternated at progressively lower frequency until the perception of flicker appears. There are commercial

instruments available for both methods, the Macuscope and the MPS II. A recent comparison of the two instruments in a clinical setting similar to the conditions used in this research found that the MPS II gave a more reliable result of the two (Bartlett et al., 2010a, 2010b) and it is this instrument which has been chosen.

The MPS 9000, also known as the M|POD, Quantifeye and most recently MPS II, instrument (Topcon Great Britain Ltd. Kennet Side, Bone Lane, Newbury Berkshire United Kingdom RG14 5PX) is described in detail by Van Der Veen et al., (2009) it will hereafter be referred to as the MPS (figure 1.9). The repeatability of the instrument has been measured to be between 0.33 (Bartlett et al., 2010b) and 0.08 (Howells et al., 2013b) by the same research group with others falling inside this range (de Kinkelder et al., 2011; Loughman et al., 2012; Makridaki et al., 2009; Van Der Veen et al., 2009) The improvements in repeatability made by Howells et al were largely made by either removing unreliable data from analysis altogether and repeating the test or by subtle adjustment of data to reduce ambiguous results where there are two or three possible minima close together as well as the recommendation that for best results the two curves should both be taken twice

Figure 1.9: The MPS instrument. From www.topcon-medical.co.uk



Aston University

Illustration removed for copyright restrictions

The instrument has been shown to give consistent results when confronted with simulated age related lens yellowing (Makridaki et al., 2009) which is important for this study which assumes the instrument will produce the same reading in phakic and pseudophakic subjects. The technique of flicker photometry has been used in two studies which tested subjects before and after removal of their cataract, in timescales in which MPOD is unlikely to have significantly altered. It was found in both that MPOD values before and after cataract removal were not significantly different (Ciulla et al., 2001; Nolan et al., 2009). These results indicate that in both a clinical and research setting, this technique and specifically this instrument can produce results in untrained observers which are reliable enough to achieve the aims of this study.

1.3: Description of the disease process leading to age-related macular degeneration

AMD is already the most common cause of blindness in the developed world and is likely to increase in prevalence as populations age (Congdon NG, 2003). It is a progressive but ultimately self-limiting condition which affects the macula region of the eye. These changes do not affect the peripheral retina probably due to differences in metabolism between the retinal regions (Holz, 1994) although in certain situations widespread haemorrhaging and scarring could conceivably cause complete loss of vision to the affected eye.

The precise aetiology of AMD is not fully understood but appears to multifactorial (Ambati et al., 2003). AMD is characterised by dysfunction of the RPE which usually takes the form of pigmentary changes or atrophy in the RPE and lipid rich deposits called drusen (figure 1.10) which appear between Bruch's membrane and the RPE and within Bruch's membrane.

Drusen are preceded by basal laminar and basal linear deposits within bruch's membrane (Loffler and Lee, 1986) and do not form randomly - they are more likely to form above the 'pillars of choriocapillaris'. Though the significance of this finding is currently uncertain it may be due to the reduced clearing abilities of the retina of debris in these regions (Lengyel et al., 2004). Focal hyperpigmentation of the RPE also often accompanies drusen and is associated with a greater risk of vision loss (Beatty et al., 2001; Wang , 2003).
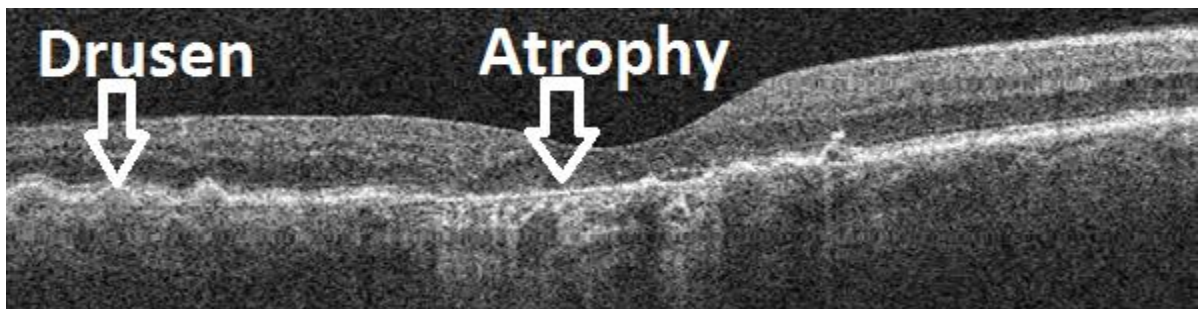


Figure 1.10: dry macular degeneration with drusen and central RPE atrophy

The mechanism leading to the formation of drusen is thought to involve oxidative damage and ischemia to the RPE and dysfunction of the normal mechanisms which remove cellular waste and debris. Metabolic changes lead to incomplete phagocytosis of the photoreceptor outer segments as well as the build-up of material which the RPE is unable to remove. The drusen and primary aging changes in Bruch's membrane (Pauleikhoff et al., 1990) are thought to interfere with the transfer of oxygen and nutrients between the RPE and the choroid, along with other factors such as vitreoretinal adhesion (Stefánsson et al., 2011; Weber-Krause and Eckardt, 1996). This often progresses to atrophy of the RPE (figure 1.10) and consequent destruction of the photoreceptors which have their outer segments embedded into it. Oxidative stress, hypoxia, light exposure and inflammatory processes all

thought to contribute to the level and rate of deterioration (Algvere et al., 2006; Bressler, 2009; Feigl, 2009).

The RPE cells are either incapable of mitosis (Fleming et al., 1996) or divide very slowly (Al-Hussaini et al., 2008) and therefore do not significantly regenerate so any damage is permanent. Drusen can be dynamic - large drusen when monitored with OCT can sometimes be seen to spontaneously regress, although these areas which initially appear to show improvement often, but not always, subsequently undergo atrophic changes (Dietzel et al., 2013; Yehoshua et al., 2011) (figures 1.11, 1.12).

The visual outcome of dry AMD is dependent on both the location and extent of the damage as a small area of foveal atrophy can create more difficulty with everyday tasks than widespread geographic atrophy with foveal sparing, although this creates difficulties of its own, particularly as the nature of the vision loss is not always apparent to the patient and magnification can be unhelpful (Sunness et al., 2008; Wolffsohn and Eperjesi, 2005).

Early age related maculopathy (ARM) is defined by Bird et al as "soft drusen ≥63µm, hyperpigmentation and/or hypopigmentation of the retinal pigment epithelium (RPE), RPE and associated neurosensory detachment, (peri)retinal haemorrhages, geographic atrophy of the RPE, or (peri)retinal fibrous scarring in the absence of other retinal (vascular) disorders." (Bird et al., 1995b). When more advanced, these changes are classified as late ARM and this includes AMD. Dry ARM can sometimes, particularly with 'soft' large, ill-defined drusen, lead to neovascularisation of the retina or choroid from the choroidal blood vessels (Freund et al., 2008).

Figure 1.11: Spontaneous drusen regression in a 64 year old woman over 6 months without progression to advanced AMD. From (Yehoshua et al., 2011)



Figure 1.12: Drusen progressing to geographic atrophy involving the fovea over 6 months: from (Yehoshua et al., 2011)

Drusen number, size and confluence are significant risk factors for progression to advanced AMD (Pauleikhoff et al., 1990; Sarks, 1980). Soft drusen are also associated with a higher risk of advanced dry disease (Pollack et al., 1996; Wang, 2003). It is thought that in the disease

42

process leading to neovascular 'wet' AMD the damaged RPE alters the balance of the hormones it produces, specifically vascular endothelial growth factor (VEGF), which encourages vascular proliferation, and pigment epithelium-derived factor (PEDF), which suppresses it (Bhutto et al., 2006).

Increased VEGF and decreased PEDF lead to vascular budding and proliferation of vessels which penetrate Bruch's membrane and typically leak fluid into the retina (figure 1.13), causing a characteristic symptom of distortion of the central vision. The vessels if untreated usually then haemorrhage and cause widespread scarring and rapid loss of central vision although sometimes neovascular membranes spontaneously regress (Freund et al., 2008). Fortunately, new drugs known as anti-VEGF agents such as Lucentis (Ranibizumab) and Avastin (Bevacizumab) when injected into the eye can supress and reverse this development.
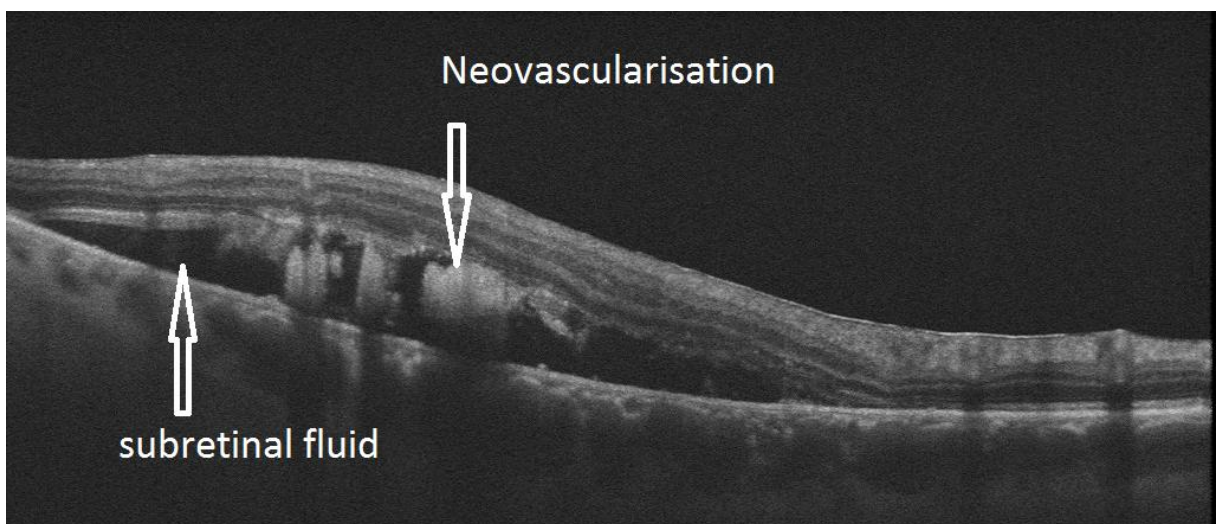


Figure 1.13: Neovascular ('wet') AMD

After treatment, vision often improves to the levels allowed by existing dry changes (Loughman, 2013) which will usually continue to progress, albeit often slowly. It is thought

that in the absence of excess VEGF, PEDF causes regression of the neovascular membrane (Mori et al., 2002). These treatments, unlike laser and photodynamic therapy, are useful for lesions involving the fovea which were previously difficult to treat (Bakri and Kaiser, 2004).

Dry macular changes and the geographic atrophy that they tend to lead to given sufficient time are currently incurable. There are several suggested treatment modes involving regeneration of the RPE through stem cell injection; gentle laser treatment of drusen (Ho et al., 1999); transplantation of healthy RPE from the periphery to the macula (Peyman et al., 1991); translocation of the retina (Lai et al., 2002) and implantation of photosensitive CCD chips into the retina to replace the damaged tissue (Ahuja and Behrend, 2013). As yet none of these methods are sufficiently developed to restore any significant sight to the eye with advanced AMD. Current interest therefore is focussed not only on treatment but also on the cause of AMD and possible mitigation of those causes

There are several known modifiable risk factors for AMD, the principal ones being age, diet, sunlight exposure, alcohol use, vascular health, exercise and smoking (Thornton et al., 2005). These all contribute to the ischemia and oxidative damage experienced by the RPE due to the high levels of metabolism and the free radicals produced by exposure to light, particularly blue light in the presence of high oxygen concentrations such as those found at the macula (Ruffolo Jr. et al., 1984), the increased production of oxygen free radicals caused by incomplete metabolism in chronic ischemia and in the presence of lipofuscin (Dorey et al., 1989).

Lipofuscin is electron rich autofluorescent membrane bound cellular waste composed of free radical damaged protein and fat. It results from the incomplete breakdown of waste such as used photoreceptor segments which cells cannot remove. In the case of non-dividing cells

such as the RPE lipofuscin accumulates with age (figure 1.14). It has been implicated in a wide range of neurological disorders as well as AMD (Family et al., 2011; Schraermeyer and Heimann, 1999). It is not yet clear whether lipofuscin accumulates continuously over an individual's lifetime or whether it only starts to accumulate as the RPE ages. Lipofuscin is photoreactive at the same wavelengths which cause maximum damage to the RPE (Margrain et al., 2004). This implies that reducing the exposure of the retina to light and improving the ability of the retina to deal with the by-products of metabolism may help preserve the health of the RPE and delay the onset of AMD.

The defence mechanisms of the retina against oxidative damage are known to decline with age (Boulton et al., 2001). However, the precise mechanism of damage is not well understood and it is unclear under what timescale modifying the risk factors is likely to be most effective. For example, much of the light exposure from the blue and UV end of the spectrum that the retina receives is in the first few years of life implying that childhood control of light exposure may delay AMD development. Conversely, due to the action of increasing lipofuscin with age the effect that blue light has on the retina actually increases with age, implying that reducing light exposure has a greater effect with advancing age (Margrain et al., 2004; Simons, 1993). Unfortunately, partly due to the weakness of epidemiological studies in determining light exposure over lifetimes through subjective questionnaires, it is not clear which of these aspects is the more important (Klein et al., 2004).

This lipofuscin mediated damage may explain why antioxidants delay AMD progression (Kassoff et al., 2001). Lipofuscin produces reactive oxygen species (ROS) when exposed to light as well as physically affecting the internal workings of the RPE cells through congestion.

Further evidence for the theory of lipofuscin mediated damage comes from the fact that lipofuscin and drusen are spatially correlated (Delori et al., 2000). Racial differences in lipofuscin mirror AMD risk, with RPE lipofuscin accumulation being greater in whites. Although the RPE melanin content is similar between racial groups, the choroidal melanin content is greater in dark skinned individuals (Weiter et al., 1986).



Aston University

Illustration removed for copyright restrictions

Figure 1.14: Lipofuscin (L) granules in the RPE cell of a 70 year old woman. Marked with an arrow are melano-lipofuscin granules, which contain a core of melanin. From (Schraermeyer and Heimann, 1999)

As the crystalline lens ages, it gradually reduces the intensity of light exposure to the retina, particularly blue wavelengths (Figure 1.15), probably protecting the retina from earlier damage. Early removal of cataracts, as is now becoming more widespread through the use of clear lens extraction as a procedure to correct refractive error, presbyopia (Alfonso et al.,

2010) or angle closure (Rathi et al., 2011) and also as a consequence of the procedure to remove them being less risky, changing the risk/benefit ratio, could potentially increase the risk of AMD in these individuals. However, it should be borne in mind that left untreated cataract causes greater levels of vision loss than AMD (Cook and Stulting, 1995).



Aston University

Illustration removed for copyright restrictions

Figure 1.15: The transmission of different colours through the human lens as it ages. From (Kessel et al., 2010)

There is evidence that AMD progresses faster in eyes which have had the cataracts removed (Pollack et al., 1996; Schaft et al., 1994) although the evidence is not conclusive (Chew et al., 2009; West, 1989) the Beaver dam study showed an association between cataract removal and late, but not early AMD (Klein et al., 2012). A recent meta-analysis concluded that cataract surgery is a strong risk factor for AMD comparable to smoking (Chakravarthy et al., 2010). There is evidence of several non-modifiable risk factors apart from age with studies

showing that the predisposition to AMD has both genetic as well as environmental aspects (Meyers, 1994; Silvestri et al., 1994).

## 1.4: Implications of MPOD for macular degeneration

MP is thought to have a dual protective role against the development of AMD with both barrier and antioxidant action. It is therefore thought that pigment levels may influence the risk of AMD, and there is growing evidence that this is the case (Sabour-Pickett et al., 2012). A recent meta-analysis found that those with increased intake of L and Z had a lower risk of late AMD, although the association between early AMD and L/Z intake was not statistically significant (Ma et al., 2012). The xanthophylls may also protect against cataract (Delcourt et al., 2006) although preliminary results of the AREDS2 study did not find any effect (The Age-Related Eye Disease Study 2 (AREDS2) Research Group, 2013).

Low MPOD has been found to correlate with a higher risk of AMD in several studies (Sabour-Pickett et al., 2012) although the association appears to be complex, as many risk factors for AMD are associated with lower MPOD (Nolan et al., 2010, 2007) complicating the cause/effect relationship between the two variables. There is some evidence that MP and antioxidant supplementation increases VA in patients with AMD (Richer et al., 2004; Weigert et al., 2011). However the effect is mild and requires further study with other studies not finding an effect (Bartlett and Eperjesi, 2007). It is unclear whether the effect these studies have found is due to an improvement in function or a reduction of light scatter as has been demonstrated in normal eyes (Weigert et al., 2011). Improvements in contrast sensitivity have also been reported in normal subjects who supplement with L and Z (Nolan et al., 2011).

A recent study in mice showed that those supplemented with lutein had significantly lower levels of light induced photoreceptor apoptosis and DNA damage than those fed a normal diet (Sasaki et al., 2012) although caution should be applied when extrapolating the results of animal experiments to humans.

Provisional results of the AREDS2 study suggest that L and Z supplementation provides significantly greater benefit than the original AREDS supplement in individuals who have a low dietary intake of carotenoids. The same results also imply that replacing beta carotene with L and Z increases the protective effect mildly (The Age-Related Eye Disease Study 2 (AREDS2) Research Group, 2013) whilst making the supplement safe for smokers, who are at a significantly increased risk of AMD. Beta-carotene significantly increases the risk of lung cancer in smokers and ex-smokers (Tanvetyanon and Bepler, 2008).

It appears therefore that factors which reduce MPOD also increase the risk of AMD. This is thought to occur through a lack of barrier protection from blue light and the increased oxidative damage associated with both an increase in light levels generating free radical species and impaired neutralisation of those free radicals due to the absence of the antioxidant properties of MP (Kijlstra et al., 2012). ROS generation through light exposure is lower in the retinas of mice fed lutein than those that were not (Sasaki et al., 2012). It is thought that light exposure to the retina, particularly blue light, increases the risk of AMD through these effects. Certainly acute light exposure can damage the retina (Michels and Sternberg Jr., 1990) but remains to be proven whether chronic exposure to sunlight causes AMD (Sui et al., 2013).

Blue light has been shown to have significant impacts on photoreceptor and RPE health (Algvere et al., 2006). Light of shorter wavelengths than 300nm are absorbed in adults by the

cornea and between 300-400 by the lens (Margrain et al., 2004) which makes it unlikely that these wavelengths are responsible for significant retinal damage.  Increasing MPOD and reducing visible light exposure, particularly at the blue end of the spectrum, may therefore reduce the risk of  AMD and the most significant factor determining blue light exposure to the retina in the elderly is probably cataract due to the absorbance of blue light by the lens (Charman, 2003).

1.5: The natural course of cataract and the effect on retinal light exposure

Cataract is the gradual increase in opacity in the crystalline lens which sits behind the iris, suspended from the cilliary muscle by fibres called zonules (Michael and Bron, 2011). It is a gradual process which occurs in all people from birth although usually only becomes visually significant in the elderly with the exception of congenital and secondary cataracts.

The crystalline lens is made up of millions of fibres which are arranged with small articulating joints in between which gives the lens flexibility. Its transparency is due to regular arrangement and structure to the fibres which decreases with age, causing back scatter and decreasing transmittance (Trokel, 1962). Cataract is primarily classified as nuclear, involving the centre of the lens, and cortical, involving the outer layers. It is nuclear cataract which is largely responsible for the gradual changes in overall light transmittance and the changing spectrum of transmitted light through the lens with age (Lerman et al., 1978).

 The development of nuclear cataract is also linked to oxidation (Truscott, 2005). The earliest sign of the aging changes in the lens that most people notice is the onset of presbyopia, in which the loss of structural uniformity in the lens affects its ability to change shape and

focus (Glasser and Campbell, 1998) and this may be considered a stage of nuclear cataract development (McGinty and Truscott, 2006). Around the age of 45, this usually affects the ability of the eye to focus to the point where it becomes necessary for most to wear reading spectacles, although as with the transparency of the lens this process has been occurring gradually since childhood with a gradual loss of accommodative ability. The optical density of the lens increases with age (Hammond et al., 1997) but the transparency of the lens does not affect all wavelengths uniformly. By the late teens, almost all UV is blocked before it reaches the retina (Lerman and Borkman, 1976), and by the age of 60 only 20% of blue light incident on the eye reaches the retina largely due to loss of lens transparency (Mellerio, 1987). This change is due to the deposition of pigments known as chromophores into the lens which block short wavelength light (Dillon et al., 1990).

Senile miosis also reduces the level of light reaching the retina but the effect is minor compared to that of the lens (Sample et al., 1988). The rapid development of dense cataract can be distinct from the gradual aging changes, often accompanied with a rapid myopic shift as the refractive index of the nucleus increases, in contrast to the slow hypermetropic shift experienced with age by most individuals as the refractive index of the lens decreases (Brown and Hill, 1987; Dubbelman and Van der Heijde, 2001) . This myopic shift is not associated with cortical or posterior subcapsular cataract (Brown and Hill, 1987).

Cortical cataract is associated with UVB exposure (Taylor, 1999) and it may be that age related changes in the lens permit further oxidation of the nucleus through sensitisation of the lens proteins to UV related to ageing (Linetsky et al., 1996) causing an acceleration of the changes which lead to cataract. There appears to be a positive feedback process whereby

aging changes in the lens inhibit the diffusion of antioxidants into the lens nucleus, further damaging the lens (McGinty and Truscott, 2006).

Most Intra-ocular lens (IOL) implants are transparent to all visible light, leading many cataract patients to notice changes in their colour perception - the artist Monet is a well-known example of both the effect that cataract and the changes in colour perception upon their subsequent removal have on the patient (Ravin, 1985). These changes eventually renormalize, but not fully (Delahunt et al., 2004).

There is some disagreement as to whether IOLs should be blue blocking or not (Mainster, 2005) due to the potential effects on night vision due to the reduction in light transmission compared to a clear IOL and the trade off with the theoretical protection against AMD (Cuthbertson et al., 2009). Other proposed disadvantages to blue blocking IOLs include disturbance of the circadian rhythm and a reduction in scotopic vision but due to the transmission properties of blue blocking IOLs compared to a normal crystalline lens (figure 1.16), these concerns are probably unfounded (Blackmore-Wright and Eperjesi, 2012; Davison et al., 2011).

Yellow filters improve contrast but also mildly affect colour vision (Wolffsohn et al., 2000) although obviously cataract also has detrimental effects on colour perception in the people being considered. A recent study found no difference in colour vision between the eyes of people implanted with a blue blocking lens in one eye and a clear lens in the other (Kara-Junior et al., 2011). They do not appear to be in routine use in the NHS, probably due to cost. Only three patients have blue blocking IOLs in the practice from which the subjects for this study were selected. These are noticeable under slit lamp examination due to the yellow tint (figure 1.17)

Figure 1.16: comparison of the transmission spectrum of natural and artificial lenses: Blue light filtering models are represented by the red and green lines and clear IOLs by the yellow and orange. From (Romano et al., 2011)
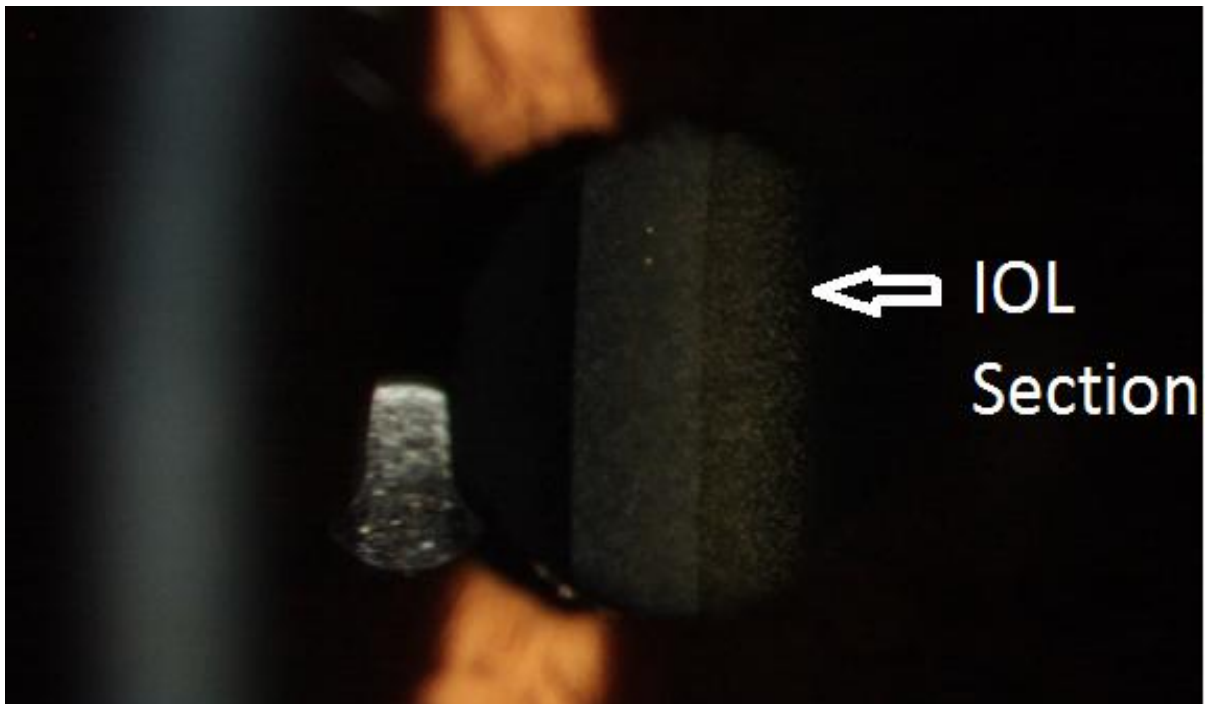


Figure 1.17: Blue blocking IOL viewed with slit lamp showing visible yellow tint.

It has long been known that sunlight damages cells, particularly those of the skin and RPE (Wang, 1975) and blue light has been found to be 30 times more efficient at inducing damage to the RPE than yellow light (van Best et al., 1997). Light induces cell apoptosis (often referred to as programmed cell death). It is thought that the increased exposure that results from cataract removal may, by increasing the light exposure to the retina, cause increased rates of cell death and AMD severity and that increased MPOD may reduce this level of damage.

## 1.6: MPOD and the effect of light exposure

Lutein and zeaxanthin are known to photobleach, that is to degrade in the presence of light (Mortensen and Skibsted, 1997), although more slowly than some other carotenoids which may be an explanation for their selective uptake into the retina (Siems et al., 1999). It has been shown that blue light filters slow cell apoptosis in *vitro* both without (Kernt et al., 2012; Rezai et al., 2008) and with (Sparrow et al., 2004) the presence of the light sensitive oxidisers lipofuscin and N-retinylidene-N-retinylethanolamine (A2E), a lipofuscin fluorophore.

Of interest to this study is the effect that light exposure has on the MPOD *in vivo*. Some studies have shown no association (Khan et al., 2006) although studies which attempt to evaluate historical light exposure between individuals are fraught with confounding factors which cast doubt on the validity of the results. It has, as would be expected, been shown by experimentation that MP is degraded *in vitro* and requires constant replacement (Lakshminarayana et al., 2008) and it is likely that light exposure plays a part in this process *in vivo*, as has been proven to occur *in vitro*

It has been shown in one study that MPOD increases after implantation of blue blocking IOLs (Nolan et al., 2009). This result is not what would be expected if the MP levels are determined by a simple equilibrium between oxidation and photobleaching versus uptake. As usually the cataract would be already acting as a blue filter and the lenses used have a transmission spectrum similar to the average 50 year old human lens (Obana et al., 2011), the light exposure to the retina following implantation of a blue blocking IOL would almost certainly be greater than the exposure before the operation.

This may suggest that the blue blocking IOL is a more effective filter than the cataract which was removed, which would seem unlikely; that there is a more complex relationship between light exposure and MPOD than previously thought; or that there was some degree of bias not controlled for such as differences in light aversion behaviour between the groups. This same study did not show that in the short term (three months) there was any drop in MPOD in the group implanted with clear IOLs.

Similarly, it has been found that no difference in MPOD was found after one month of being implanted with a clear IOL (Ciulla et al., 2001) and one week after (Nolan et al., 2009). These studies used flicker photometry to evaluate MPOD and lend confidence to the consistency of results found before and after cataract removal using HFP.

Another longitudinal study using Raman spectroscopy found that MPOD decreased in all subjects given both blue blocking and clear IOLs, but that change was greater in the clear IOL group (Obana et al., 2011). The disparity between the two groups did not appear until after 6 months had passed since the operation which removes the issues described earlier regarding the use of a conversion factor to compensate for lens colour with Raman spectroscopy. The fact that all eyes displayed reduced MPOD would indicate that blue

blocking IOLs do not eliminate the effect of light exposure on MPOD entirely and contradicts the absolute, but not the relative effect the earlier Nolan et al 2009 study found which showed augmentation of MP. It also does not allow for differences which may occur between the two groups in behaviour regarding sunlight exposure as a result of the different IOL, for example if the group with blue blocking IOLs were more likely to stay indoors or wear sunglasses, this may affect the results, a potential confounding factor.

These studies indicate that MPOD is affected by cataract removal, and by implication light exposure *in vivo*, but only after several months to a year. Further study is required to confirm this fact, preferably of alternative design. These studies do not resolve the debate on there being any significant active regulation of MPOD levels via a feedback mechanism such as that proposed by Nolan et al (2009) whereby reduced MPOD or increased light exposure increases uptake of L and Z from the bloodstream. The study by Nolan et al suggests that such a feedback mechanism exists and is strong enough to match any photobleaching caused by the increase in light exposure induced by implantation of a clear lens and causes an increase when a blue blocking lens is implanted. The studies by Obana et al (2011) and Demirel et al (2013) suggest that either no mechanism exists or it is too slow or too weak to overcome the increase in light exposure the retina experiences with either blue blocking or clear lenses at least in the duration the studies investigated. The relative effect is similar in all the studies.

## 1.7: The structure of the macula and relationship with MPOD

The normal fovea has a pit like structure when viewed in cross section (figure 1.18)



Figure 1.18: OCT scan of the normal human fovea: from (Marmor, 2008)

In this area, the transmission of the signal from photoreceptor to ganglion cell is simpler than in the rest of the retina, with fewer intermediate neurones between the photoreceptors and ganglion cells (Curcio and Allen, 1990). The area is avascular and much thinner than the surrounding retina due to the less complex circuitry above. This leads to less optical scattering of the light above the most tightly packed photoreceptors, improving the vision (Gorrand, 1979). This is an evolutionary adaptation brought about by the organisation of the retina with the photoreceptors underneath the neural tissue (Lamb et al., 2007).

In other creatures where the eye has evolved separately, such as cephalopods, the photoreceptors are on the inner surface of the retina (figure 1.19). This organisation brings advantages in resolution and the lack of a blind spot but could lead to greater vulnerability of the photoreceptors to light damage. The advantages of the 'correct' design with the

photoreceptors on the inner surface would also eliminate the need for a central avascular zone which may be partly responsible for the vulnerability of this region to damage (Novella, 2008). Only primates, and not all primates, possess a fovea. It is thought to be linked to the formation of the foveal avascular zone (FAZ) (Dubis et al. 2012) and other animals have a minimally vascularised area centralis, which is a region of more specialised cells at the centre of the retina, or a visual streak, a band of higher resolution receptors (Provis et al., 2013).



Figure 1.19: Anatomical differences between cephalopod and mammalian eyes: From (Novella, 2008)

The simple transmission of the signals at the fovea would appear to serve two functions – the elimination of most of the retinal layers leads to less scattering of light and the low convergence of photoreceptor to ganglion cell decreases the signal compression compared to the rest of the retina in the outer retinal layers, leading to better spatial resolution in this area (Curcio and Allen, 1990).

This basic model however does vary between individuals, from those with a very deep foveal pit to those where it is almost absent (Dubis et al. 2012). Figure 1.20 gives examples of foveal pits of differing depth and width
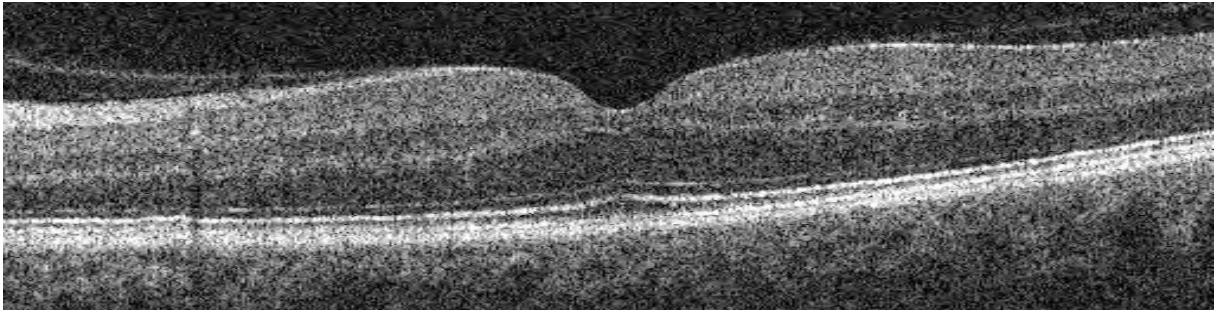
1.20a: thicker than average fovea
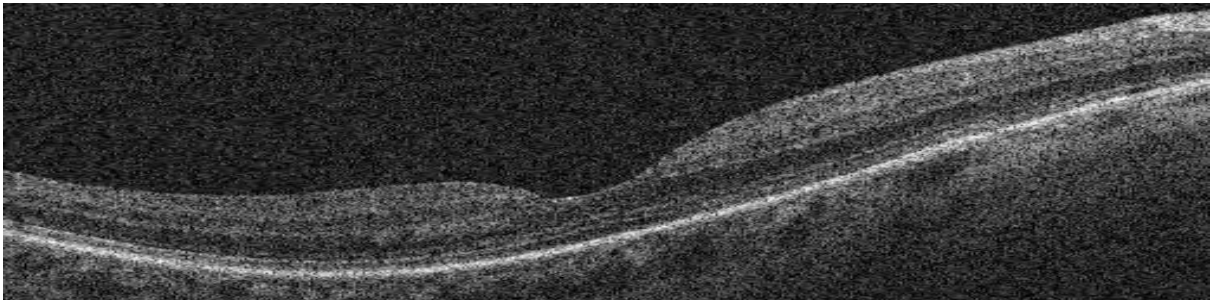

1.20b: a thinner than average fovea
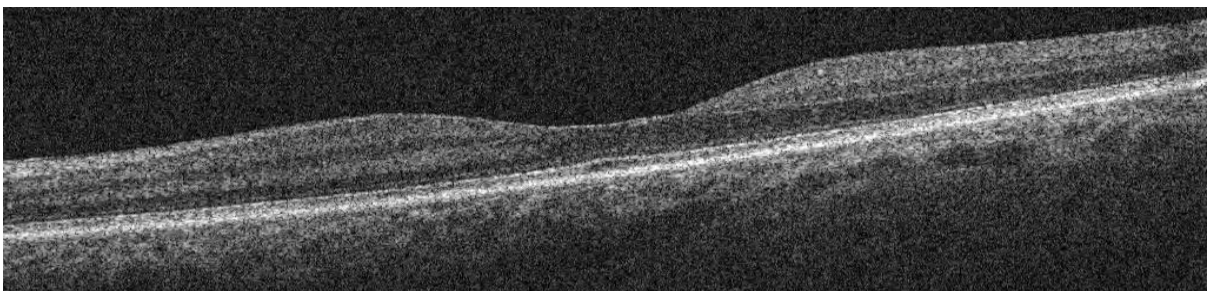

1.20c: a wider than average fovea

Figure 1.20: Anatomical differences in normal foveal pits from subjects used in this research.

In extreme cases, foveal hypoplasia is the almost complete or complete absence of the foveal pit, thought to arise from the lack of a FAZ. These cases however can vary greatly in visual acuity and are often as a result of conditions which independently impair vision, leading to doubt as to how great an effect the lack of the foveal pit itself has on the visual acuity (Marmor, 2008). It is likely that the pit itself has only a minor effect on central visual function, with the increased cone density being largely responsible for the high central acuity (Provis et al., 2013).

Macular pigment deposits across the fovea to about four degrees eccentricity in particularly high concentrations in the Henle fibres and inner plexiform layers (figure 1.21). The pigment is deposited at the fovea in the Henle fibres (figure 1.22) and parafoveally in the axons of the inner and outer plexiform layers (Trieschmann et al., 2007).



Aston University

Illustration removed for copyright restrictions

Figure 1.21: Two examples for the divergent spatial distribution of the macular pigment in the human fovea. The foveal section (a) and parafoveal section with 200 $\mu$m distance to the centre (b) of specimen 1 .The MP with an extension about 600 $\mu$m is well visible in both photographs. In contrast in the case of MP extension of 300 $\mu$m the MP is visible only in the foveal section (c), but not in the parafoveal section in 200 $\mu$m distance to the centre (d). Picture and legend from (Trieschmann et al., 2007)

The factors which lead to the density of the deposition of MP are not fully understood. It is thought that serum carotenoid levels and differences in membrane transport proteins influence the rate of uptake into the retina (Li et al., 2010; Mares-Perlman et al., 2001), but it is less clear whether anatomical differences between the retinas of individuals play a part in the quantity of MP deposited.  It is also unclear whether early disease processes, such as aging changes in the retina, lead to changes in the deposition of MP.



Aston University

Illustration removed for copyright restrictions

Figure 1.22: Section of the retina showing the Henle fibres, one location where MP is deposited (between red arrows) from (Drasdo et al., 2007)

## 1.8: The relationship between MPOD and macular thickness:

There are several reasons to suppose there might be a relationship between MPOD and central foveal thickness. The architecture of the fovea may have an impact on the uptake and binding of MP and these structural variations may also manifest themselves as thickness differences. The structure of the fovea is organised with a central pit from which many of the layers of neurons are absent to allow maximum light transmission and minimal scatter to the densely packed cones below. It may be that a shallower foveal pit is capable of retaining greater levels of MP due to the greater thickness of the outer plexiform and Henle fibre layers where L and Z are bound.

Another possibility is that there may be structural changes caused by early age related structural damage to the retina which predispose to low levels of MP and simultaneously reduce foveal thickness. It has been found that that the retina becomes thinner with AMD (Richer et al., 2012). Some, but not all, studies have found a general relationship between central retinal thickness and age (Chan, 2006; Eriksson and Alm, 2009) which could be due to either a general aging process or an increase in the number of individuals with eye diseases such as ARM which are known to cause macular thinning.

Age related thinning may reduce the capacity of the retina to retain MP, thus exposing the retina to further damage as part of an on-going disease process. Alternatively, primary loss of MP may lead to oxidative damage which causes retinal thinning through apoptosis of neurons overlying the photoreceptors in response to oxidative damage or loss of RPE. It is known that in patients with macular disease, both MT and MPOD are reduced which could be due to a causal relationship either way or comorbidity depending on the disease (Aleman et al., 2001; Duncan et al., 2002).

Understanding the relationship between MPOD and macular thickness may be important for our understanding of the factors which contribute to increased risk of AMD and uptake of MP into the retina. Higher resolution OCT should improve the ability to detect subtle differences in MT over earlier studies using time domain OCT. The knowledge may also allow optometrists who use OCT in practice to identify which patients to screen for low MPOD, or identify foveal thinning as a risk factor for AMD before visible changes to the RPE occur.

<u>1.9: OCT and cataract</u>

Optical Coherence Tomography (OCT) is an imaging technology first described in 1991 (Huang et al., 1991). It is a method of imaging semi-transparent tissues using low coherence interferometry to visualise layers of different reflectance. The lens, cornea and retina are excellent tissues to image in this way as they are constructed of layers which can easily be identified by their differential reflectiveness (Swanson et al., 1993).

There are two main types of OCT – the older, time domain OCT which uses a moving mirror to scan the beam and the newer, spectral domain instruments which use Fourier transformation to analyse the interference pattern created between the reference and imaging beam without the need for the mirror. This greatly reduces the scan time and increases the resolution of the instruments, reducing motion artefacts and the effects of media opacities such as cataract (Ho et al., 2009). One of the advantages of OCT in primary practice is that it can be used to image the retina in patients with media opacities but in whom it is not possible to exclude retinal pathology using conventional methods due to a poor view of the fundus (figure 1.23).

The first use of OCT was to image the posterior segment (Swanson et al., 1993). The central retina can be imaged to give novel views of pathology such as macular degeneration, epiretinal membranes, macular oedema, macular holes and other retinopathies. It is useful to differentiate between conditions which are not always readily distinguishable or even visible on retinal photography or slit lamp examination such as vitreo-macular traction and mild oedema (Drexler W et al., 2003). The optic nerve can also be imaged to provide information about the Retinal Nerve Fibre Layer (RNFL) which is the most sensitive way of detecting glaucoma after assessment of stereo photographs by a panel of expert

ophthalmologists and more sensitive than the Heidelberg retinal tomograph or GDx laser polarimiter instruments (Azuara-Blanco et al., 2003; Lee et al., 2013; Mwanza et al., 2011).
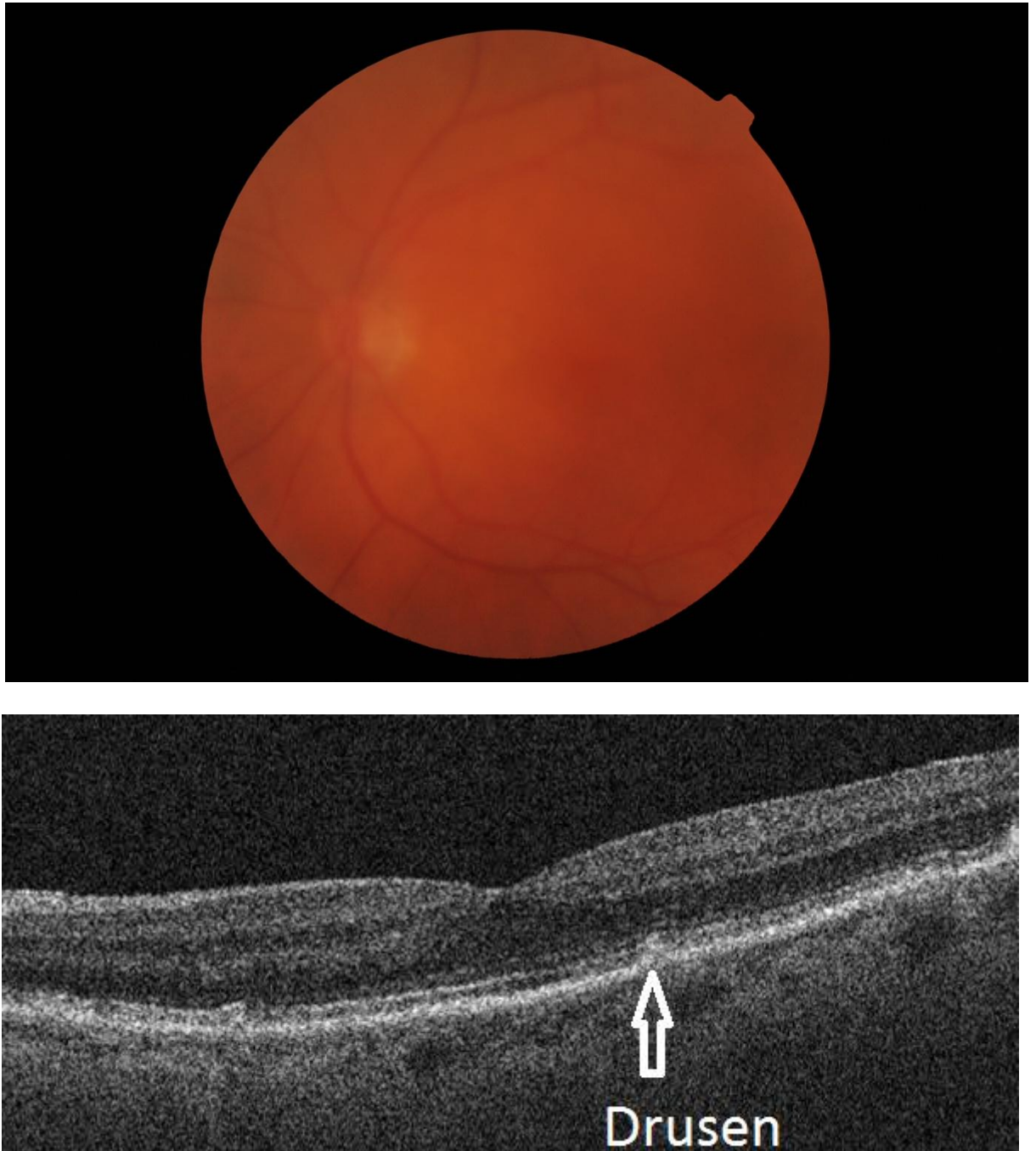


Figure 1.23: Poor retinal image using fundus photography (due to posterior capsular opacification) and below the same eye imaged with OCT showing drusen not visible on photograph.

It can be used to detect pathology through cataract or other media opacities although imaging through corneal dystrophies can be difficult due to the high reflectivity of the cornea in these conditions. Imaging is possible where these are clear areas of the cornea in between the opacities but this requires a co-operative patient who can keep very still.

A more recent development of OCT technology is its use for anterior segment imaging (Izatt et al., 1994). Instruments for this purpose are either dedicated AS-OCT instruments such as the Zeiss Visante (Zeiss Meditec, Jana, Germany) or adaptations of instruments primarily developed for posterior segment imaging. Dedicated instruments are different in a couple of important ways from multi-function OCT instruments – in the wavelength of light used and in the field of view which can be obtained. The Zeiss Visante is a time domain instrument which uses a 1300μm wavelength as opposed to the 840μm used by the Cirrus. This increases the tissue penetration and allows imaging of structures through the sclera such as the ciliary body which are not readily visible in most individuals due to the high reflectivity of the sclera to the 840μm wavelength (Hoerauf et al., 1999). This penetration comes at the expense of resolution. Instruments which have variable or wide spectrum light sources are likely to overcome the limitations of current instruments to some extent.

## 1.9.1: Using OCT to assess cataract types and similar measurements

Of interest to this study is the development of cortical cataract, as the principle of using OCT to assess nuclear cataract has already been demonstrated, albeit using a different instrument, the Zeiss Visante (Wong et al., 2009). Cortical cataract affects the outer layers of the lens (the cortex) and tends to progress gradually towards the visual axis from the

equator of the lens, although sometimes early cortical defects can occur in isolation along the visual axis, commonly to the anterior surface of the lens which receives greater light exposure, not being protected by absorption from the nucleus. Several studies have associated cortical cataract with light exposure, particularly UV exposure (Bochow, 1989; Delcourt C, 2000; Hr et al., 1988) .

In principle, the use of OCT to provide images of cataract is valid as the OCT gives images with easily extractable data on reflectance. It is possible in practice to visualise with OCT sections through human lenses – for example, figure 1.20a shows a section through an IOL, figure1.20b shows cortical opacities that can be related to lens sections imaged with a slit lamp, figure 1.20c shows a lens with mild cortical opacities, 1.20d shows a young lens cortex and 1.2e shows the visibly denser cortex of a typical 55 year old.

Whether this data can be used to reliably grade cortical cataract in a way which gives clinically useful results is unknown. The strength of OCT scanning of the lens over slit lamp images is the easy access to objective data in contrast to subjective visualisation of cataract with a slit lamp. If this data can be shown to accurately reflect the severity of a cataract it has many advantages over slit lamp grading as both a research and clinical tool.

Figure 1.24: examples of lenses imaged with OCT; 3mm OCT scan through an artificial IOL.
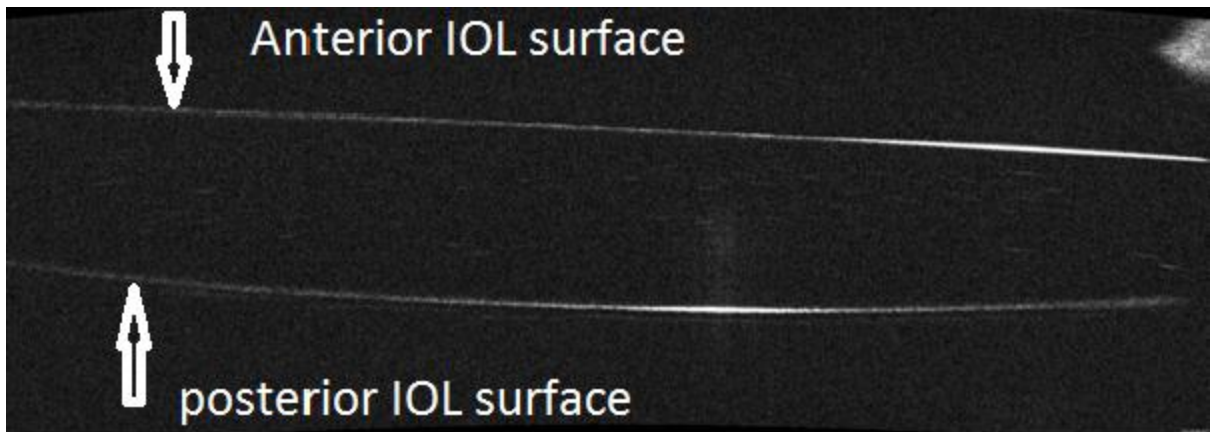


3mm OCT scan through a lens with dense cortical opacities:
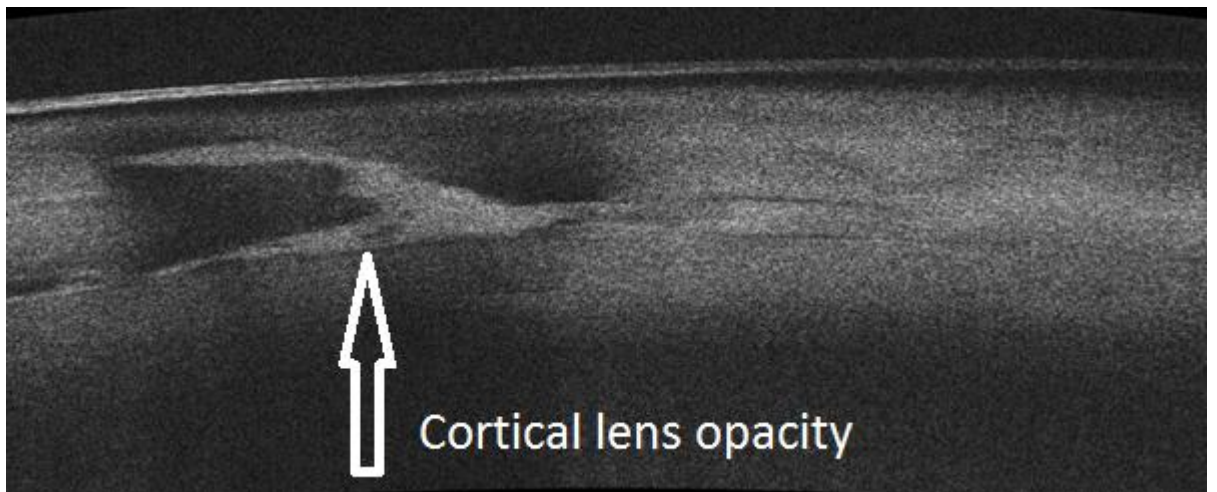


Lens cross section through an undilated 2.5mm diameter pupil with mild cortical opacities
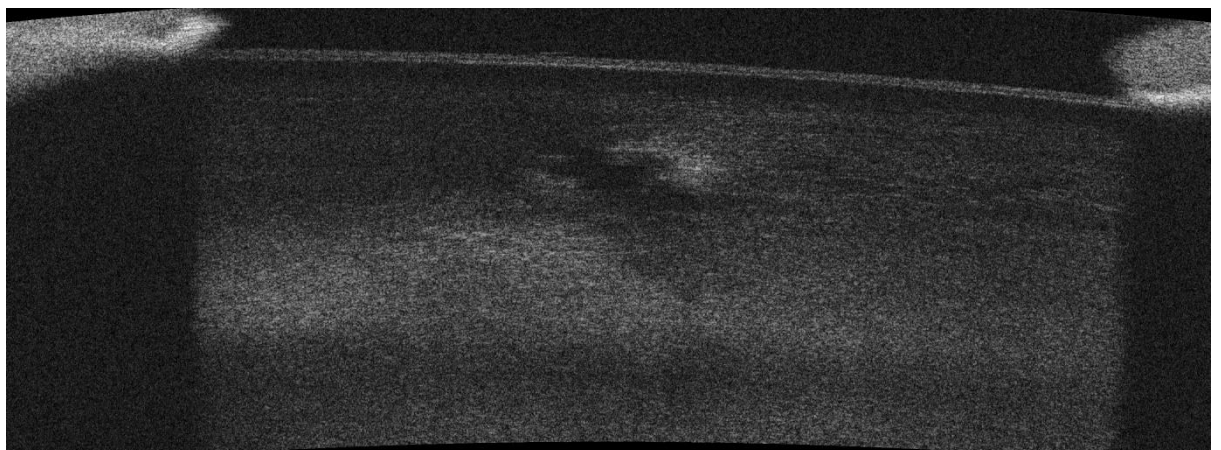
The lens cortex of a 27 year old.
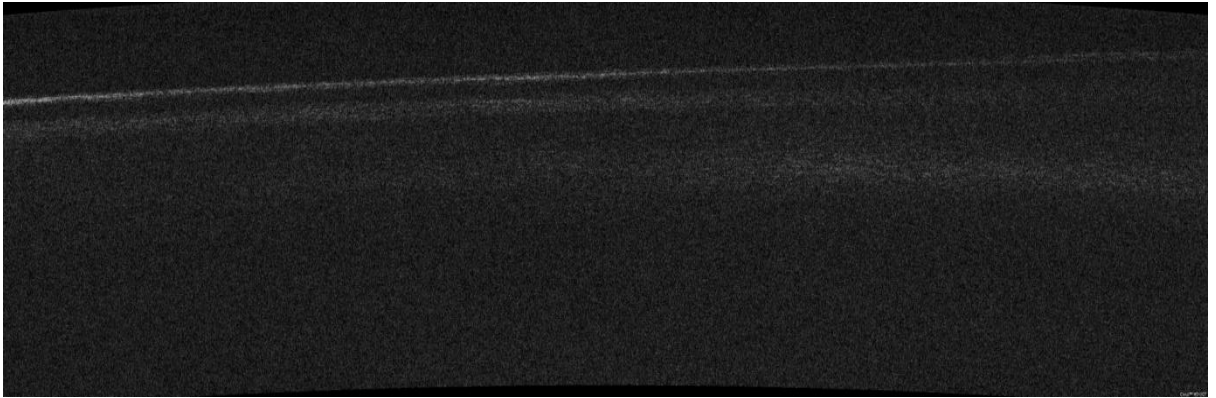


The lens cortex of a 55 year old
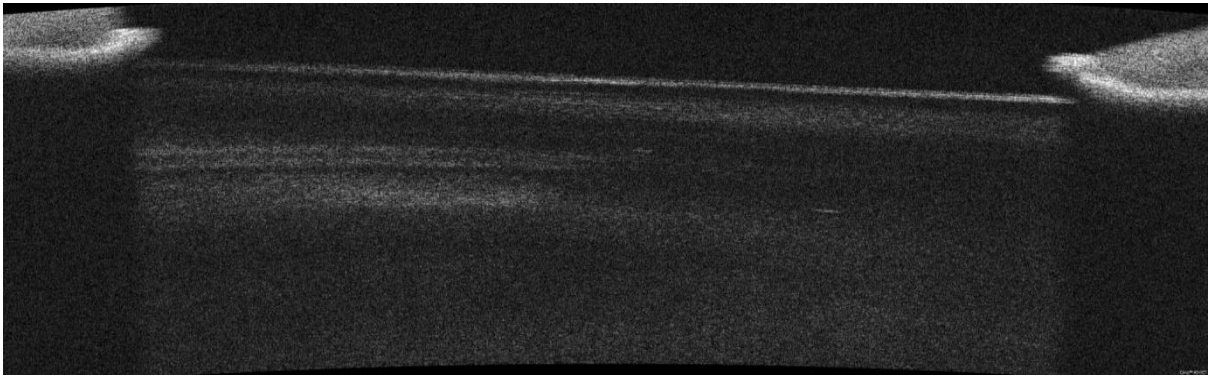


It would be an advantage to primary care practitioners to be able to quantitatively assess cataract through undilated pupils with a quick, non-invasive technique, using an instrument which is widely available. The current referral criteria widely used for NHS referrals are based on visual acuity, which although a good measure of whether for example a patient meets the required standard for driving, is a poor predictor of cataract density or symptoms (Elliott and Situ, 1998).

There have been attempts to produce vision tests, usually involving lower contrast letters than standard logMAR or Snellen charts which more accurately reflect the visual status of the patient, with some success (Ni et al., 2012) but none have gained widespread use.

The ability of OCT to give images through lens opacities which seriously degrade the quality of a fundus photograph or ophthalmoscopy image means that many of these patients will already be being examined on these instruments in practices where they are available. A protocol to quickly and objectively record the severity of cataract and perhaps track it over time would be an advantage in determining when referral is appropriate.

The LOCS III grading system is widely used to assess cataract severity for research purposes and is based on photographic grading scales of nuclear density, nuclear colour, cortical cataract extent and posterior capsular opacification (Chylack et al., 1993) (figure 1.25). It requires pupil dilation and careful slit lamp set up to achieve repeatable results and is thus inherently time consuming and, with the use of dilating drops, invasive. It is not in common usage in routine practice in UK optometric practice or ophthalmology departments to inform decisions on whether to refer or operate, with criteria being based on symptoms, VA and degree of anisometropia. A more reliable measure of cataract assessment than VA measurements could help identify those who most require treatment but do not fall within referral criteria.

Figure 1.25: The LOCS III grading scale. From (Chylack et al., 1993)

Despite the potential advantages and clearly applicable principle of using OCT to grade cataract little research on the subject seems to exist. There are many papers which mention the potential for artefacts to occur when scanning through a cataract to the posterior chamber, but only a handful which use the OCT to image the lens, perhaps due to the anterior segment capabilities of OCT only recently becoming commonplace. Another limiting factor on earlier research may be movement induced artefacts with time domain OCT which anterior segment OCT is particularly prone to given the proximity of the anterior chamber to the lens through which the scanning beam is focussed (Li et al., 2006). It is almost impossible to produce a motion artefact with posterior segment fourier domain OCT, but motion artefacts on anterior segment images do still occur (figure 1.26).
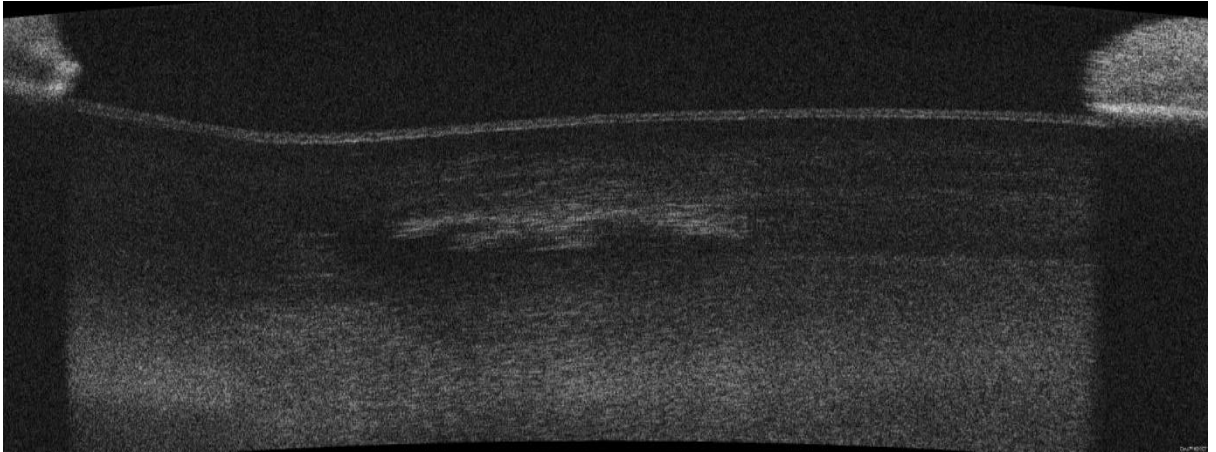
Figure 1.26: A motion artefact whist scanning the lens cortex.

OCT images have been compared with histological sections from Rhesus monkeys showing faithful tissue imaging, although limited by the technology of the time (DiCarlo et al., 1999) and one study has used OCT to compare to LOCS III grades for nuclear cataract (Wong et al., 2009). This used Visante OCT which uses a wavelength of 1310nm, whereas the instrument used in this study, the Cirrus, uses a wavelength of 840nm (figure 1.27).



Aston University

Illustration removed for copyright restrictions

Figure 1.27: Zeiss Cirrus 4000 OCT (Zeiss Meditec, Jana, Germany)

The grading of cortical cataract can present a problem when attempting to correlate symptom severity with objective measurements due to its nature. A minor cortical opacity that intersects the visual axis can have a much greater effect on vision and symptoms than a dense peripheral cortical cataract of large extent that can, particularly in those with senile miosis, be hidden behind the pupil and have little if any effect on vision (Chua et al., 2004).

A measurement of cortical opacity along the visual axis and within the pupil aperture may give a more reliable indicator of symptoms than a measure of the extent of an opacity with a dilated pupil which lies largely hidden, as with the LOCS III grading scales or other measures using retro illumination – with an undilated pupil however any attempt to gain a slit lamp image immediately constricts the pupil to an artificially small area. OCT scanning uses light outside the visible range hence it can record an image which accurately represents the maximum lens area exposed under normal dark conditions such as those encountered whilst driving at night, a task with which those with cortical cataract affecting the visual axis tend to find difficulty due to the glare of oncoming headlights. The OCT scanning beam does not tend to constrict the pupil significantly under these conditions, particularly when the fixation target is carefully focussed for the distance.

1.20: Conclusion

There are gaps in our current understanding of the *in vitro* behaviour of MP when exposed to light, our understanding of how MPOD relates to the physiology of the macular region and our ability to objectively grade cortical cataract in a way which accurately reflects the visual status or symptoms of the patient. This study will attempt to narrow those gaps by applying new techniques and technology to the assessment of MP, macular thickness and the density of the lens cortex as detailed in the following chapters.

The next chapter is a literature review of the evidence pertinent to the experimental chapters where previous research will be presented to demonstrate the evidence base for the experiments.

Chapter Two


Literature review

In the previous introductory chapter the background to AMD, MP, MPOD, the structure of the macula and its relationship to MPOD and the possible use of OCT to quantitatively measure cataract density has been presented. This chapter with cover the research on which the experimental chapters are founded, followed by the rationale for each experiment based on the previous research and the gaps in current knowledge.

<u>2.1: Research investigating relationships between light exposure, pseudophakia, MPOD and AMD</u>

Several studies have attempted to associate light exposure and AMD risk and/or MPOD. These studies are summarised in detail in table 2.1. They can be divided into *in vitro* studies, animal studies and human studies and all have specific benefits and drawbacks.

*In vitro* studies have demonstrated in principle that there are adverse implications for light exposure on MPOD and the health of the RPE and photoreceptors; for example Lakshminarayana et al. ( 2008); Siems et al. (1999); Sparrow et al. (2004). These studies by definition however create an artificial environment and there is always an inherent degree of uncertainty as to how applicable the results are to humans in real life situations as complex interactions between variables in a living organism may behave differently to single tissues are isolated in culture.

Animal studies are more realistic as an entire living organism is being studied rather than cells in culture. Many animal studies have also shown that light exposure has an adverse effect on the retina and on MPOD and that increased MPOD can mitigate the effects of light exposure, for example Gouras et al. ( 2008); Malinow et al. (1980). Animal studies also have

an inherent doubt about the applicability of the results to human populations. The lifespan, disease incidence, physiology and response of animals to certain situations are often different to our own and therefore the results cannot be guaranteed to apply to humans. In common with *in vitro* studies, the timescales tend to be short compared to the durations of exposure thought to create risk in humans and to compensate the light exposure tends to be artificially high, creating an environment which does not necessarily reflect the normal development of disease in humans.

Epidemiological studies have used questionnaires to attempt to assess light exposure over long periods of time but these are rather prone to inaccuracy, relying as they do on a person's recall of their history of light exposure and they are prone to other confounding factors such as lifestyle choices, use of sunhats and sunglasses which can significantly affect exposure levels (Cruickshanks et al., 1992; Ferrini et al., 1998; Threlfall and English, 1999) and other factors such as habitual light avoidance in those who may be most at risk of AMD (Delcourt et al., 2001), diet and smoking. There are studies which have attempted to correct for these variables as detailed in table 2.1, but there remains a degree of uncertainty. Nevertheless, there are studies which have found a correlation between light, MPOD and AMD, although others have not (Klein et al., 2004). A recent review and meta-analysis which considered 13 epidemiological studies concluded that light exposure is a significant risk factor for AMD (Sui et al., 2013)

Only one study identified has attempted to alter a human individual's light exposure and measure the effects on MPOD. The duration of this study and the light exposures involved was short at only five minutes and as there is known to be a significant time lag to changes

in MPOD, from weeks to months (Connolly et al., 2011), it is perhaps not surprising that this study did not find an effect (Wenzel et al., 2003).

Two studies have found an inverse relationship between lens density and MPOD although this was also strongly correlated with age. It is therefore unclear whether the relationship is causative or not. It may represent an effect the cataract has on MPOD or an effect that MP have on the lens (Berendschot et al., 2002; Hammond et al., 1997), the latter being more in line with current theories of the role of MP in the eye.

Cataract extraction has been used as a proxy for light exposure in two recent MPOD studies, one cross sectional study, (Demirel et al., 2013) and one longitudinal study (Obana et al., 2011), both of which found pseudophakic eyes had reduced MPOD compared to eyes with natural lenses. Both studies have potential confounding factors. In the study by Demirel (2003) the confounders are potential differences between conditions experienced by the two groups, for example differences in diet, light exposure, serum carotenoids and a small sample size. In the case of the study by Obana (2011) the confounders are differences in behaviour of the subjects after cataract removal with regard to diet or light avoidance and as the study was not randomised, there is the possibility of selection bias in the choice of IOL used.

Another longitudinal study by Nolan et al (2009) found that MPOD was augmented in eyes implanted with blue blocking IOLs when compared to both clear IOLs and the pre cataract removal results. This effect is counterintuitive given that the retinal blue light exposure through a blue blocking IOL would still be expected to be greater postoperatively than it was through the cataract preoperatively (Romano et al., 2011). The augmentation of MPOD observed may be due to systematic measurement differences in MPOD due to the

pseudophakia (although this was controlled for with measurements taken shortly before and after cataract removal), differences in behaviour between the groups or could be due to the mechanism for MP replacement being more complex than assumed.

These studies give useful information on how long it takes MPOD values to change following cataract removal, which occurs over months/years rather than days to weeks.

Another relevant recent study has found that MP is higher in those wearing UV blocking contact lenses, even when factors such as UV exposure are controlled for (Wolffsohn et al., 2012) despite minimal amounts of UV generally assumed in the adult eye to be reaching the retina. This again could point to a feedback mechanism governing MP uptake or deposition which is not purely governed by retinal light exposure.

| Author and year | Factor assessed | Type of study | relationship found with r value if stated | Variable parameters/exposure | Number of subjects |
|---|---|---|---|---|---|
| (Raman et al., 2012) | UV exposure and MPOD | case control *in vivo* | Inverse $r^2$= -0.12 | UV based on questionnaire | 33 with AMD, 29 controls |
| (Nolan et al., 2009) | Light exposure and MPOD | Case control *in vivo* | Inverse (augmentation of MPOD in BB group) | Blue blocking up to 500nm Vs UV blocking IOL | 21 BB, 21 UV |
| (Wenzel et al., 2003) | | Interventional *in vivo* | None | 5 minute exposure 7 $\mu W/cm^2$ 400-500nm | 2 |
| (Obana et al., 2011) | | Longitudinal *in vivo* | Inverse $R^2$=0.303 exposure:MPOD | Blue blocking and clear IOL implantation | 259, BB121, clear 138 |
| (Demirel et al., 2013) | | Cross sectional *in vivo* | Inverse $R^2$ =0.44 exposure:MPOD | HFP, cataract removal proxy for exposure | 37 subjects, 68 eyes |
| (Mares et al., 2006) | | Cohort *in vivo* | None | Light exposure based on questionnaire | 1698 women |
| (Mellerio et al., 2009) | | Cross sectional *in vivo* | Inverse exposure:MPOD | Light exposure based on questionnaire | 124 |
| (Ciulla et al., 2001) | | Cross sectional *in vivo* | positive $r^2$=.04 hat and sunglass use:MPOD | Sunglass and hat use as proxies for exposure | 280 |
| (Bone et al., 2001) | AMD and MPOD | Case control | Inverse MPOD: Donor eyes with AMD | MPOD in eyes with and without AMD | 224 eyes |
| (Obana et al., 2008) | | Case control | Inverse F=36.44 Presence of amd:MPOD | MPOD in eyes with and without AMD | 187 eyes, 97 subjects |
| (LaRowe et al., 2008) | | Cross sectional | None | MPOD in eyes with and without AMD | 1698 women |
| (Bernstein et al., 2002) | | Case control | Inverse AMD:MPOD | MPOD in eyes with and without AMD | 93 AMD eyes, 220 normal |
| (Jahn et al., 2005) | | Case control | none | MPOD in eyes at different AMD stages | 146, all with ARM |
| (Beatty et al., 2001) | Multiple AMD risk factors and MPOD | Case control | Inverse risk:MPOD. r=0.29 for mpod:age | MPOD in eyes at high AMD risk | 9 eyes at risk, 46 controls |
| (Nolan et al., 2007) | | Cross sectional | Inverse Risk:MPOD r2=0.25 | MPOD in eyes of variable risk | 800 subjects |
| (Nolan et al., 2010) | | Cross sectional | Inverse r=0.309 | MPOD in eyes of variable risk | 853 subjects |
| (Berrow et al., 2011) | | Case control | None | MPOD and age, smoking and diet (questionnaire) | 81 eyes, 16 with ARM |
| (Hammond and Caruso–Avery, 2000) | | Cross sectional | inverse r=-0.14 for age:MPOD | MPOD and age, gender, iris colour, smoking | 217 subjects |
| (Raman et al., 2012) | | Case control | inverse risk:mpod,diet; positive risk:smoking,BMI, UV | MPOD and smoking, BMI, diet. | 33 AMD, 29 controls |
| (Taylor et al., 1992) | Light exposure and AMD | Case control | Positive blue light exposure:AMD risk odds ratio, 1.36 | AMD and 20 year exposure based on history. | 838 subjects |
| (Delcourt et al., 2001) | | Cross sectional | None | AMD and light exposure (questionnaire) | 2584 subjects |
| (Cruickshanks et al., 1993) | | Cross sectional | Positive | AMD and light exposure, based on leisure time and hat/sunglasses use | 4926 subjects |
| (Arnault et al., 2013) | | *in vitro* | Positive, greatest damage @420, 430, 440 and 450 nm | A2E loaded cells exposed simulated sunlight at multiple wavelengths 18hrs. | |
| (Tomany et al., 2004) | | Cohort longitudinal | Positive light exposure:AMD RR for high exposure=2.20 | sunlight exposure (questionnaire) and AMD | 2764 subjects |
| (Fletcher, 2008) | | Cross sectional | none excepting those with low antioxidant intake | Blue light exposure (questionnaire) and AMD | 4753 subjects |
| (Cruickshanks et al., 2001) | | longitudinal | positive early exposure:amd risk | AMD and early light exposure before age of 30 (questionnaire) | 3684 subjects |

| | | | | | |
|---|---|---|---|---|---|
| (Berendschot et al., 2002) | cataract and MPOD | Cross sectional | Inverse, β = –.096 | Lens density @420NM and MPOD | 376 subjects |
| (Hammond et al., 1997) | | Case control | Inverse r = -0.47 | Lens density @420nm and MPOD | 39 case, 14 control |
| (Ciulla and Hammond Jr., 2004) | | Cross sectional | none | MPOD and cataract requiring extraction | 390, 22 with cataract |
| (areds report 22, 2007) | Dietary carotenoids and AMD | Case control | Inverse OR 0.45-0.73 depending on type of AMD | dietary carotenoids and AMD severity | 4519 |
| (Seddon et al., 1994) | | Case control | Inverse OR =0.57 between highest and lowest intake | dietary carotenoids and AMD | 356 case, 520 control |
| (Moeller et al., 2006) | | Cross sectional | Inverse | intermediate AMD and vegetable intake | 600, 71 with AMD |
| (Snellen et al., 2002) | | Case control | Inverse OR=1.7 antioxidant and 2.4 lutein intake | neovascular AMD and antioxidant/lutein intake | 72 case, 66 control |
| (Tan et al., 2008) | | Cohort | Inverse RR 0.35 neovascular AMD for top tertile carotenoids | AMD and dietary antioxidants | 2454 |
| (Flood et al., 2002) | | Cohort | None | early ARM and dietary antioxidants over 5 years | 1989 |
| (Cho et al., 2008) | | Cross sectional | None | carotenoids via food questionnaire and self-reported early AMD | 71494 women 41564 men |
| ( Eye Disease Case-Control Study Group," 1993) | serum carotenoids and AMD | Case control | Inverse | serum carotenoids and AMD | 421 case, 651 control |
| (Delcourt et al., 2006) | | Case control | Inverse OR=0.31 for ARM and plasma lutein | serum carotenoids and AMD/ARM | 640 |
| (Gale et al., 2003) | | Cross sectional | Inverse OR=2.0 for Low Zeaxanthin and AMD | serum carotenoids and AMD | 380 |
| (Mares-Perlman et al., 1995) | | Case control | none | serum carotenoids and AMD | 167 case, 167 control |
| (Malinow et al., 1980) | Animal studies of MP and AMD | Case control | Inverse xanthophyll/fluorescence | Foveal hyperfluoresence in xanthophyll deprived monkeys | 48 |
| (Leung et al., 2004) | | Case control | Inverse RPE cell count: dietary carotenoids | RPE cell counts in xanthophyll deprived monkeys | 18 case, 15 controls |
| (Thomson et al., 2002) | | Case control | Inverse zeaxanthin: photoreceptor apoptosis | 3200-lux white light 10x 1 hour | 16 quail |
| (Sasaki et al., 2012) | | Case control | Inverse lutein:macular thinning | 5000 lux for 3 hours | 14 case, 5 cont |
| (Ham et al., 1978) (Gouras et al., 2008) | | case control case control | Positive ( RPE lesion produced) Positive (drusen spatially associated with lipofuscin) | 1000 seconds 0f 62microW @ 441nm. Histological analysis of autofluorescence and drusen | 20 eyes rhesus. 12 eyes Rhesus |
| (Sparrow et al., 2004) | *In vitro* studies of MP/ light exposure and RPE damage | *In vitro* case control | Blue blocking IOL reduces cell death | Blue light Vs clear IOL on A2E loaded RPE cell apoptosis 390 to 750 nm; 246 mW/cm$^2$ | 3x7 different conditions |
| (Sparrow et al., 2000) | | *In vitro* case control | A2E increases light induced apoptosis | A2e infused Vs non infused human cells. 480nm and 540nm@ 75 and 200 mW/mm$^2$ 15-60 seconds | 2x3 different conditions |
| (Schütt et al., 2000) | | *In vitro* case control | A2E increases light induced damage | 400–500 nm A2e infused Vs non infused cells | 1x2 conditions |
| (Zhou and Sparrow, 2011) | | *In vitro* case control | Blue light filters attenuate apoptosis in A2E infused cells | 1 mW/cm$^2$, 30 min | 7 conditions |
| (Chucair et al., 2007) (Siems et al., 1999) | | *In vitro* case control *In vitro* case control | L and Z reduce oxidative RPE damage Photodegradation of macular carotenoids | Oxidative stress by h2o2 Natural sunlight | 7 filters |

Table 2.1: Studies investigating light exposure, macular pigment optical density and age-related macular degeneration.

### 2.1.1: Rationale behind study design

It has been shown that with aging the light transmittance of the lens gradually decreases (Sample et al., 1988) and it is thought that this may have an effect on MPOD. There are conflicting experimental results showing what the effect of cataract removal on MPOD is and if an effect exists, what the long term trend for MPOD following cataract removal would be. Differences between individuals makes unrandomised cohort study results unreliable as a number of factors such as UV exposure and diet can effect MPOD, but MPOD is known to be similar between the eyes of healthy individuals (Kanis et al., 2007).

One way to control for confounding factors which affect MPOD and light exposure is to use the same patients and compare the effects of light exposure at the same time. A way to do this is to study differences between the two eyes of individuals where the two maculae are receiving different levels of light exposure, in particular blue light. The need to control for other factors such as behaviour, sunglasses use, diet, obesity, and smoking is eliminated as they will affect both eyes, so each individual acts as their own control. Monocular effects such as ocular disease, direct effects of surgery or differences in physiology between the two eyes are left as possible confounders, as is the potential for measurement differences resulting from the IOL.

There are several possible ways to study individuals with different light exposure to the two eyes. Modification of the light exposure to one eye though for example tinted contact lenses is one method but is difficult to perform in the long term. Alternatively people who have an existing difference in light exposure between the eyes, for example those with significant anisocoria and those with different light transmission characteristics of the ocular media could be studied. The most obvious subjects would be those who have had just one cataract

removed. The advantage of studying subjects who are pseudophakic in one eye is that there are several studies which show that removing cataract has no immediate effect on MPOD or its measurement with HFP (Ciulla et al., 2001; Makridaki et al., 2009; Nolan et al., 2009) although pupil size has also been shown not to affect HFP readings (Wooten et al., 1999). It is well known the effect that cataract has on light transmission to the retina and the effect is stronger than the effect of pupil size (Charman, 2003). The weaknesses of this study design are the possibility that some factor effects both cataract development and MPOD in one eye only and the possibility that the cataract itself had a lasting effect on MPOD before it was removed. This second effect should be minimised with by the timescales involved in this study.

2.2: Research concerning macular thickness and MPOD

There are several mechanisms whereby macular thickness and MPOD may be correlated and several studies have investigated this relationship.

All studies identified have isolated the fovea or central macula as this is where MP is concentrated and none have investigated whether there is any thinning of the macula outside this area.

Some have found a positive relationship (Hammond Jr. et al., 1997; Liew et al., 2006; van der Veen et al., 2009) and (Zheng et al., 2013) positive only in myopes and others showing none or mixed  (Kanis et al., 2007; Kirby et al., 2009; Nolan et al., 2008; Palomo-Alvarez et al., 2010).  Only one study shows a negative correlation, between retinal thickness and MPOD at one and two degrees eccentricity (Westrup et al., 2014) with the same study showing no association with central MPOD.

Nolan et al (2008) found a relationship between foveal width, and not thickness, between individuals. These studies are summarised in table 2.2

A weak correlation between axial length and MPOD has been found in myopes (Tong et al., 2013) which represents a potential confounding factor although not all studies have found a relationship (Neelam et al., 2006). Macular thickness in those without pathological myopia have also been shown by some studies not to correlate with axial length (Göbel et al., 2001; Ooto et al., 2011; Wakitani et al., 2003) and to show a negative correlation in others (Song et al., 2010) and a positive correlation in others (Ueda et al., 2006; Wong et al., 2004). Correcting for axial length or other biometry (Neelam et al., 2006) when considering macular thickness and MPOD is therefore unnecessary.

| Author and year | Area studied | Correlation found | Number of subjects | OCT |
|---|---|---|---|---|
| Liew (2006) | central 1mm, fovea | r=033 (1mm) and r=0.4 (fovea) MPOD 0.5° | 612 eyes (all female twins under 50) | SD |
| Nolan (2008) | 1mm, fovea, fovea width | r=0.67 at 0.5° in nonwhites for fovea r=0.41 foveal width entire study group | n=18 nonwhites n=59 entire study group | SD |
| Van der Veen (2009) | fovea | r=0.359 fovea at 0.5° | n=40 | SD |
| Zheng (2013) | central 1mm, fovea | r=-0.66 1mm, r=0.67 fovea in myopes only no correlation for entire study group | n=27 myopic n=94 entire group | SD |
| Kanis (2007) | central 1mm, fovea | no correlation | 69 eyes | SD |
| Kirby (2009) | fovea | no correlation | n=16 | SD |
| Westrup (2014) | "retinal thickness" | no central correlation, negative correlation at 1° and 2° | 124 eyes | n/a |
| Palomo-Alvarez (2010) | foveal thickness | no correlation | 40 eyes with ARM | FD |

Table 2.2: studies which have investigated the relationship between macular thickness and macular pigment optical density. SD = spectral domain, FD = Fourier domain

There is some evidence from animal studies that there may be a relationship between light exposure and macular thickness that MP may modify. Light exposure sufficient to thin the retina in mice has been shown in a recent study to be attenuated by dietary intake of L (Sasaki et al., 2012) which may indicate that eyes with higher MPOD are less susceptible to age related thinning of the macula, hence eyes with higher MPOD as they age would be expected to have a thicker macula, however it is important to note that animal studies and those using acute light exposure do not necessarily translate to chronic light exposure effects in humans.

Figure 2.1: The effect of intense light exposure on retinal thickness in mice with and without lutein in the diet. From (Sasaki et al., 2012)

An investigation which measured the effect on macular thickness in individuals who had one blue blocking and one clear IOL implanted found no significant difference in macular thickness between the two groups after 5 years (Kara-Junior et al., 2011). Unfortunately this study did not measure MPOD, so any effect on MPOD is unknown. The study did however find there was no difference in low light VA, contrast sensitivity or colour vision between the blue blocking and clear IOL. Another recent study found that retinal thinning in the absence of visible pathology was associated with loss of contrast sensitivity (Richer et al., 2012) which may indicate that retinal thinning is an earlier indicator of age related disease than visible ARM.

In summary, there is conflicting evidence of the relationship between MPOD and foveal thickness and no investigations of a relationship between MPOD and the thickness of the entire macula. The investigations which have been identified have mostly used lower

resolution spectral domain OCT and radial scans rather than cube scans of the macula which may lead to lower precision.

## 2.2.1: Rationale behind study design:

There are conflicting results from previous studies which have investigated the relationship between macular thickness and MPOD. Studies investigating MPOD have mostly used spectral domain OCT and concentrated purely on the fovea and surrounding 1mm diameter of the macula where MPOD is highest. The advent of high definition Fourier domain OCT enables higher resolution imaging of the macula and may provide results which have greater precision than those used in many studies so far.

As no previous studies have investigated the relationship between MPOD and the thickness of the entire macula area, it is not clear whether the results found which indicate that thinner foveae are correlated with lower MPOD are part of a generalised thinning of the macula or whether the thinning is specific to the central macula.

Therefore this study aims to determine if there is a relationship between central macular thickness, foveal thickness or average macular thickness and MPOD. This will add to the available experimental information as to the relationship between retinal anatomy and MPOD. Although there are other studies into this there are conflicting results. This will in turn add to our understanding of the role of MPOD in the development of AMD and the role that foveal architecture has in influencing risk of AMD, potentially leading to improved identification of those most at risk of AMD and the advice which may be given to those at high risk, such as subject which display age related thinning of the macula.

## 2.3: Research relevant to the objective measurement of cataract using OCT

OCT imaging has not been previously used to provide objective measurements which could be used to grade cortical cataract, although it has been successfully used to image the lens cortex (DiCarlo et al., 1999) and also to grade nuclear cataract (Wong et al., 2009).

OCT has been used successfully to measure the thickness of the crystalline lens with better reproducibility than A-scan ultrasound although showing a systematically larger measurement than ultrasound measurements (Zeng et al., 2009).

In principle OCT can provide accurate cross sectional images of the lens cortex which can be referenced to slit lamp images or histology (DiCarlo et al., 1999). Whether this data can be used to provide objective measurements which could be useful to use as clinical information when making a decision as to whether to operate on cataract or how much energy might be required to emulsify the cataract remains unproven, although other techniques such as Scheimpflug imaging have been used for this purpose (Fujikado et al., 2004; Lee and Taylor, 1989; Ortiz et al., 2008) generally the ability to usefully grade cortical cataract is poor.

There are several ways to grade cataract objectively, the most common used for research is the LOCS III grading system (Chylack et al., 1993) which uses photographic grading scales.

Previous to the popular use of OCT, computer analysis of slit lamp images (Maclean and Taylor, 1981) and Scheimpflug imaging has been used in a similar way to that proposed here to image cataract and provide objective measurements of cataract (Masters, 1996; Sparrow et al., 1990). It has not been widely adopted for this purpose however, perhaps due to the Scheimpflug instruments not being particularly common. A device described as a dynamic light scattering device has been used similarly (Datiles III et al., 2002). Other similar

investigations have shown a strong correlation between optical aberrations with cataract and contrast sensitivity using wavefront analysis (Kuroda et al., 2002) although the association with cortical cataract was not strong.

Wong et al (2009) used images taken with a Zeiss Visante AS-OCT instrument to grade nuclear cataract and found a good correlation with LOCS III measurements of nuclear colour and nuclear opalescence. This investigation isolated the lens nucleus using software and measured the average pixel brightness across the image, which was itself a composite of two images taken (figure 2.1)



Aston University

Illustration removed for copyright restrictions

Figure 2.2: The lens nucleus measurements used by Wong et al (2009) to grade nuclear cataract using OCT.

None of these methods have gained purchase in routine optometric or ophthalmological practice, with subjective, non-standardised slit lamp assessment and visual acuity

measurements still being the most common assessment of cataract used in primary practice. No instrument thus far has demonstrated an ability to grade cortical cataract in a useful way.

## 2.3.1: Rationale behind study design:

This study follows the model of the technique used by Wong et al (2009) whereby the area of lens to be graded is scanned using OCT to create cross sectional lens images. The lens area of interest is then isolated and analysed using software (figure 2.2). This method has been used successfully to grade nuclear cataract using a similar instrument and it was noted in that paper that images of the lens cortex could also be acquired in the same way.

Previous studies have failed to grade cortical cataract in a way which correlates well with other measureable aspects of vision. It would be useful to be able to do so in primary practice and research.

Therefore this study aimed to determine the repeatability of cross sectional images through the lens cortex and the relationship between vision and lens density. Due to the irregular nature of cortical cataract both the lens density and the variability in lens density as represented by the standard deviation of the pixel brightness across the image will be measured.

## 2.4: Conclusion:

This chapter has set out the research which is relevant to the experimental chapters which follow and the rationale behind the research design. The following chapter is the first experimental chapter and will investigate the difference in MPOD between the eyes of individuals who have one natural aged lens and one clear artificial IOL.

Chapter Three

Experiment one: pseudophakia and macular pigment optical density.

In the previous chapter the evidence associating light exposure, MPOD, dietary carotenoid intake and AMD has been presented. The rationale for investigating the effect that pseudophakia has on MPOD using subjects who are pseudophakic in one eye has been explained in terms of good control of confounding factors. This experimental chapter will cover the investigation of that relationship and will begin with a summary of the evidence base for a relationship between MPOD, light exposure and pseudophakia.

3.1: Introduction:

Age-related macular degeneration is one of the leading causes of vision loss in the developed world  and is worldwide only second to cataract as a cause of vision loss (Congdon, 2003). Many elderly people undergo cataract removal and implantation with clear artificial IOLs (McCarty et al., 1999), as do refractive surgery candidates wishing to correct ametropa or presbyopia (Horgan et al., 2005). These usually block UV light but greatly increase the exposure of the retina to blue light which the aging lens absorbs (Mainster and Sparrow, 2003).

Blue light is thought to be a major hazard to the aging retina due to its high energy and photoreactivity with lipofuscin, an age related accumulation of lipid rich cellular waste and debris (Sparrow et al., 2000). When blue light reacts with lipofuscin it produces ROS which in turn damage the RPE which supports the health of the photoreceptors (Schütt et al., 2000). Blue light has been show to induce apoptosis of RPE *in vitro* both with and without the presence of lipofuscin fluorophore A2E (Rezai et al., 2008).

Oxidation caused by normal cellular metabolic processes and photooxidation is thought to contribute to the aging processes in the RPE which lead to macular degeneration through

the depositing of lipid rich material in Bruch's membrane (Suzuki et al., 2007). This can lead to either atrophy of the RPE and photoreceptors known as geographic atrophy or vascular proliferation in the choriocapillaris leading to haemorrhage and scarring, known as wet macular degeneration (Ambati et al., 2003). Light exposure may be positively correlated with AMD risk (Sui et al., 2013).

It is thought that cataract removal may increase the risk of developing AMD due to a combination of postoperative inflammation or an increase in light exposure (Klein et al., 2012). There is conflicting evidence from epidemiological studies as to whether the risk of AMD in pseudophakics does indeed increase although a recent meta-analysis concluded that it is a risk comparable to smoking (Chakravarthy et al., 2010). IOLs which block blue light theoretically offer some protection against blue light damage to the retina relative to a clear IOL but this remains to be proven (Cuthbertson et al., 2009).

MP is thought to offer barrier protection and antioxidant protection against the blue light hazard and ROS generated by metabolic processes (Kijlstra et al., 2012). It is selectively taken up into the central macula region from the serum and deposited in the retina anterior to the RPE where it can act as a barrier against blue light (Li et al., 2010).

MP cannot be measured directly in the living eye. Instead the optical density due to the MP is measured, the MPOD. There are several ways of measuring MPOD with both objective and subjective tests (Howells et al., 2011). The test chosen for this research is the psychophysical method of HFP, which is commonly used in research and has been proven to provide consistent results in the presence of cataract and in pseudophakics (Ciulla et al., 2001; Makridaki et al., 2009; Nolan et al., 2009). It is thought that low levels of MPOD may be a risk factor for AMD but research results are conflicting (table 2.1). Supplementation with MP has

been associated with increased MPOD and a reduced risk of AMD progression in those with low dietary intake (The Age-Related Eye Disease Study 2 (AREDS2) Research Group, 2013).

It is thought that MP is photobleached and destroyed by ROS and requires constant replacement (Mortensen and Skibsted, 1997). It is likely that increased light exposure will therefore reduce the MPOD unless there is a homeostatic mechanism in the retina to keep the MP levels stable. No such mechanism has been identified but has been proposed (Nolan et al., 2009).

This experiment is to determine if pseudophakia with a clear IOL reduces MPOD. The proposed mechanism for this change is that the greater light exposure to the retina following cataract removal may photobleach and oxidise MP in the retina, leading to lower MPOD. If there is no homeostatic mechanism for maintenance of MP levels in the retina it would be expected that pseudophakia would reduce MPOD.

Several studies have measured MPOD in pseudophakics and compared the levels to pre-operative results or a phakic control group. These studies vary in design with three longitudinal case control studies, one of short duration (Ciulla et al., 2001) which found no change in MPOD and two of longer duration (Nolan et al., 2009; Obana et al., 2011) both of which found a relative difference in MPOD between blue blocking IOLs and clear IOLs, but with different absolute results. Nolan et al using HFP found an increase in MPOD in individuals implanted with blue blocking IOLs and stable levels in those with clear IOLs. Obana et al using Raman spectroscopy found a decrease in MPOD with both blue blocking and clear IOLs which was less in the blue blocking group. A cross sectional study by Demirel (2013) found lower MPOD in those who where pseudophakic than those who were not although the size of the pseudophakic group was small, only 11 subjects.

No studies identified have compared the interocular difference in MPOD of those who are pseudophakic in one eye and phakic in the other. This chapter concerns the investigation of the effect that cataract removal has on MPOD. Subjects having a clear artificial intraocular lens and the other eye a natural aged crystalline lens in the other were studied. It is well established that this will result in increased exposure of the retina to light, particularly blue light, in the pseudophakic eye. MPOD was measured by flicker photometry.

3.2: Study aims:

The principal aim of this study was to determine if there is an *in vivo* effect of cataract removal and by inference light exposure on MPOD. The results of this study have potential implications for the risk of AMD after cataract removal and artificial intraocular lens implantation. Both the choice of lens implant and the advice given to patients considering this procedure may be influenced by the results of this study, particularly those who have only mild cataract but also have signs of ARM or those who have IOL implantation performed at a young age

3.3: Methods:

The tests used were carried out over two sessions, with the first being the patient's usual routine eye examination. During this examination, the patient's general suitability for the study was assessed. In patients who had one pseudophakic eye the probable suitability for the study was assessed before they were invited to take part in the second session using the tests carried out as part of a normal sight test.

3.3.1: Subjects:

The criteria used during this first session for invitation to the second stage were as follows:

Pseudophakia:

One eye only, of greater than 3 months duration. It is possible that shorter timescales would not allow any effect to be manifest as MPOD is often found to be relatively stable over the course of months or even years (Bernstein et al., 2012).

Age:

Over 50, to ensure a significant difference in blue light transmission through the two lenses due to there being sufficient lens yellowing for this to be the case in those over this age (Kessel et al., 2010).

Visual acuity:

Better than 0.4 LogMAR in the phakic eye and better than 0.1 logMAR in the pseudophakic eye. Subject with a VA less than 0.4 LogMAR were excluded. This figure is based on a study

showing no difference in MPOD measured with HFP shortly before and after cataract removal (Ciulla et al., 2001) which used Snellen 6/15 as their cut off point. The effect that greater cataract than this was likely to have on the ability to reliably perform the MPOD test is unknown. Similarly Nolan et al (2009) used a cut-off point of 0.5 logMAR.

Different exclusion criteria were used in the two eyes as it was likely in the subjects used that in the absence of any disease other than cataract, the VA would be better in the pseudophakic eye.

Amblyopia:

Patients with amblyopia were not considered due to possible effects on flicker sensitivity and fixation instability or inaccuracy which is often shown on visual field testing. Amblyopia was determined using binocular vision tests and the patient's historical record.

Ocular Health:

Patients with AMD were excluded from the study due to uncertainty as to whether this would affect the results. It is known that ARM affects the ability of the retina to detect flicker and any small scotomas may affect the ability of the patient to see the target accurately (Feigl, 2009).

Patients with visible drusen, pigmentary changes or RPE atrophy at the macula (2 disc diameters from the fovea) were not invited to take part in the study. Patients with drusen outside this area were not excluded as these are not thought to be associated with AMD (Munch et al., 2010; Postel et al., 2005).

During the second session using the OCT any subjects with RPE thickening, atrophy or drusen within the 6mm$^2$ scan area were excluded from the study, as were any patients with epiretinal membranes or other macular pathology within the scan area.

Patients with corneal opacities or significant vitreous floaters were excluded.

General health:

The MPS test is a psychophysical test which relies on good mental abilities to complete accurately. Patients with diagnosed or suspected dementia, those with arthritis affecting the hands, back or neck in a way which could cause difficulty in comfortably completing the test, those unable to complete an accurate visual field examination and vulnerable individuals who were unlikely to be able to consent were not considered suitable for inclusion. A good, accurate performance using the Zeiss FDT field screener was considered essential for inclusion. A patient unable to perform well on the FDT was unlikely to be able to perform the MPS test accurately as the FDT requires good reaction times and concentration, as does the MPS.

Anisocoria:

To be included the pupil in the pseudophakic eye should be no more than 1mm smaller than the fellow eye under scotopic conditions simulating daylight using the slit lamp.

Subjects taking supplements containing L, Z or MZ were also excluded.

If these criteria were satisfied, the patient was invited to participate in the study during a second session, typically of 40 minutes to one hour duration. Informed written consent as approved by the Aston University Ethics Committee was gained and the study was

performed in accordance with the Declaration of Helsinki. The consent form used is included in appendix four. No expenses or reward were offered for participation.

<u>3.3.2: Materials and procedures</u>

MPOD measurement:

After an explanation and consent being obtained, the subject was given a demonstration of the MPOD test before the measurements were taken to accustom them to the task.

MPOD was measured using a commercial MPOD screening device, the MPS, also variously known as the MPS 9000, Quantifeye, and most recently the name has been standardised across markets to MPS II. It has been confirmed with the distributor (Topcon UK) that all these devices are exactly the same except for colour and logos.



Figure 3.1: The MPS instrument used for this research project in position. The patient response button is to the left.

The device itself is based on the body of a Henson 7000 visual field screener. It measures one eye at a time, with the other occluded using an eye patch and is controlled via software on a connected PC and a patient response button. The test has two aspects, first a central test to measure MPOD and then a peripheral test, the purpose of which is largely to exclude the effects of lens yellowing. For this test the instrument was mounted on the translating

table of a combi unit with adjustable height chair as per figure 3.1. The unit itself also has a small range of movement to allow optimal positioning for the comfort of the subject.

The instrument is linked to a computer via USB and the results can be seen plotted in real time as the patient is performing the test, allowing the responses to be monitored. The 30 degree screen is LED illuminated with a uniform $250Cd/m^2$ and has 3 LED fixation targets, a central point (1 degree subtense) which provides the flickering stimulus and two red peripheral targets of size 1.75 degrees at 8 degrees from the centre. The left red target is used when testing the right eye and the right target when testing the left eye (figure 3.2).
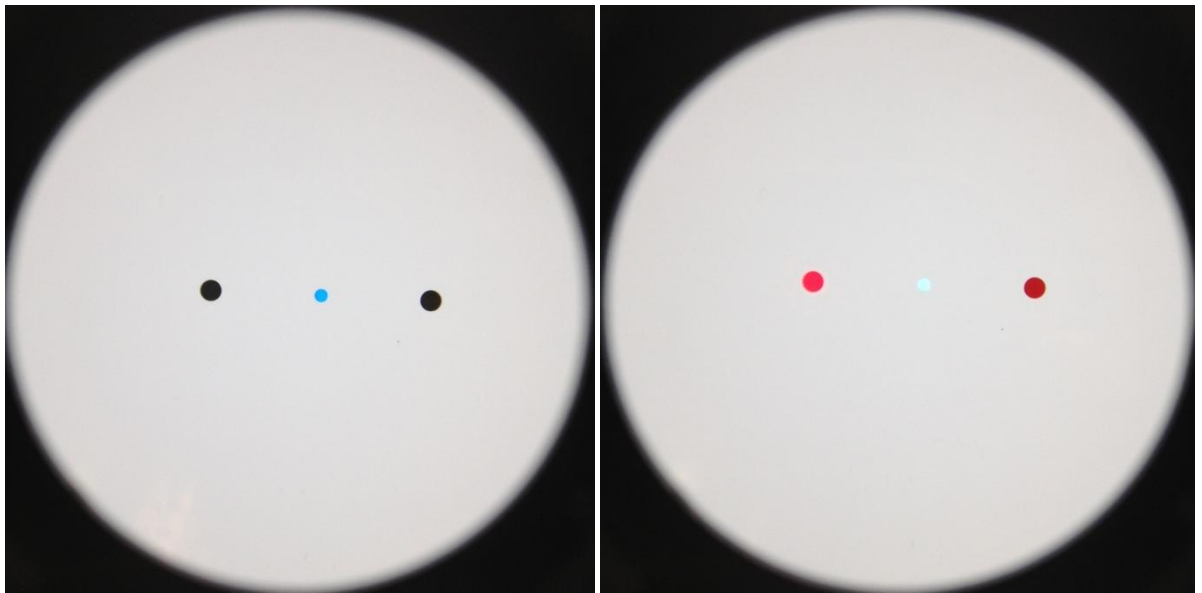


Figure 3.2: The subject's view of the M|POD test during central testing (L) and peripheral testing (R). The difference in colour between the red dots is an artefact.

The central target is composed of 2 LEDs, one blue (465 nm) and one green (530 nm). These two stimuli flicker initially at 60Hz and the instrument gradually ramps down the frequency to a minimum of 6Hz. At the point the subject was able to detect the flickering, they pressed the button, the instrument beeped to confirm the press and a corresponding point appeared

on the screen of the connected computer. The first five cycles serve to determine the flicker sensitivity of the subject in order to set the starting point for the test.

After a short pause the test begins, with each cycle repeated with a differing ratio of brightness of the blue and green LEDs. As the ratio changes, the flicker becomes initially harder, then after a minimum point, easier to detect at a given frequency. The blue:green brightness ratio at which the flicker is hardest to detect represents the point at which the brightness of the blue and green are perceptually equivalent, from which the amount of blue absorption of the retinal tissues can be deducted. The same test is then repeated with the subject looking at one of the peripheral targets (right target for left eye and vice versa) which places the blue light at eight degrees from the fovea where it is assumed there is little or no macular pigment.

This point gives the ratio of blue to green which is equally bright to the subject in the presence of minimal macular pigment. The difference between these two values is then used to calculate the effect of the blue absorption of the MP on the flicker perception, and hence, presuming the only variable between the two tests is the MPOD (figure 3.3). If, for example there was a complete absence of macular pigment, the curves should be the same although if this were to actually occur the more likely explanation would be a fixation error by the subject (figure 3.4).

The formula for deriving MPOD given by Snodderly et al. (1999)

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

Where $T_{MP}$ is the transmission of light through the MP, B fov is the blue light intensity required to minimise flicker at the fovea and B ref is the blue light intensity required to minimise flicker at the peripheral location.
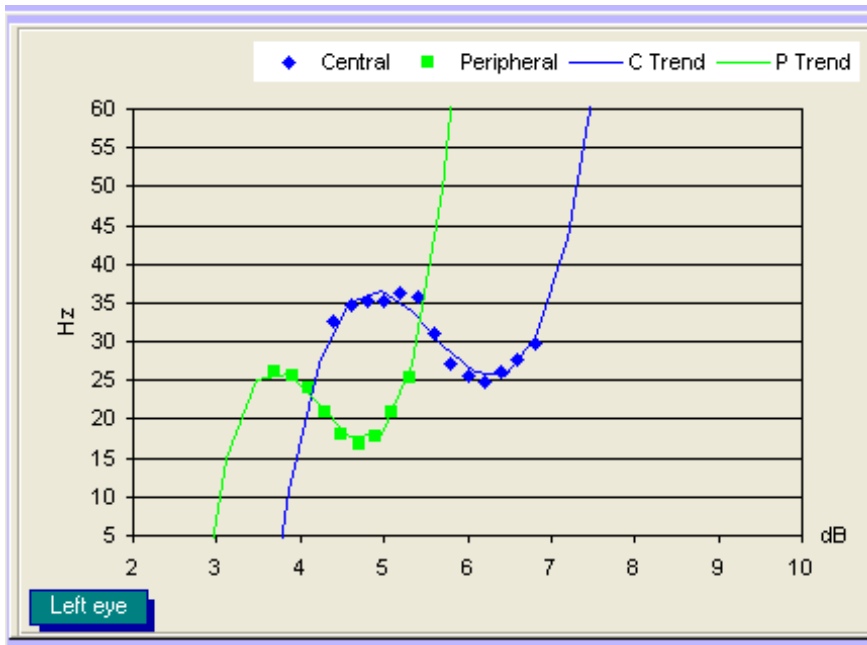


Figure 3.3: a plot of the central (blue) and peripheral (green) curves using the MPS
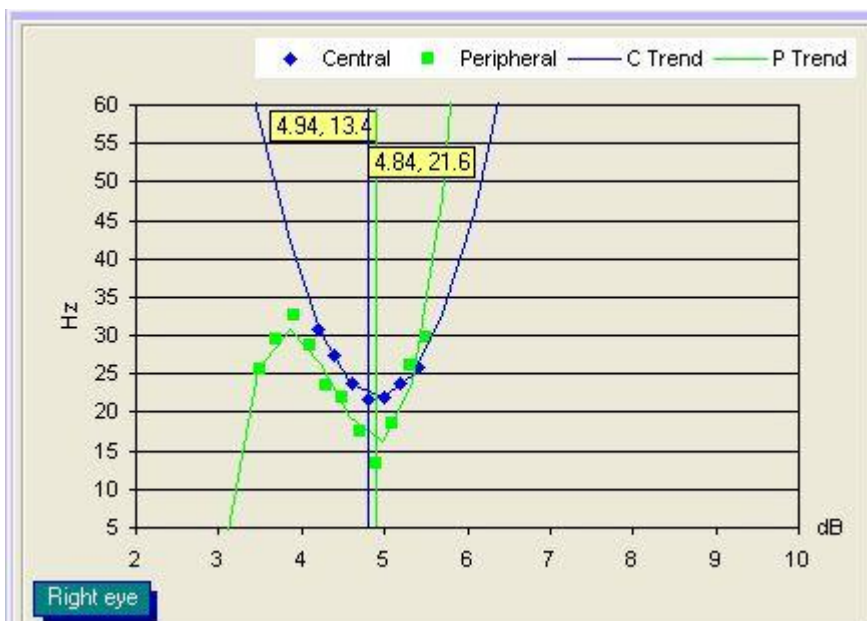


Figure 3.4: a probable fixation error plot or zero MPOD.

The test was conducted in the same room as the measurements taken during the subjects' routine sight test and slit lamp measurements and was quiet for the duration of the test aside from occasional advice/encouragement as deemed appropriate by the operator (MS).

The subjects were required to perform the test twice on each eye to allow assessment of the repeatability of individual measurements and allow averaged measurements to be used in the final calculations. The sequence of tests was as follows: Right central, right peripheral, Left central, Left peripheral, Break (at which point macular thickness measurements were taken using OCT), Left central, left peripheral, right central, right peripheral.

This sequence was chosen to minimise both learning and fatigue effects. Randomisation was provided by the fact that the eye which had an artificial IOL was independent of the test sequence and it was assumed that whether the right or left eye had developed the cataract which had been removed was random.

The fellow eye was covered with an eye patch to aid concentration and the subjects wore their habitual distance correction if required.

The measurements taken follow a similar protocol to the one suggested by (Howells et al., 2013b) and the data analysis also follows some of the suggestions from the same study to improve test reliability, namely rejection and repeating of curves not considered optimal and adjustment of curves with ambiguous minima. Subjects were advised to blink frequently and shift their fixation slightly within the red target to avoid the troxler effect where an image static on the retina can seem to disappear.

As the results are displayed in real time whilst the operator is watching, there is an opportunity to assess the accuracy of the result during the test. If the patient was producing

a curve which was not smooth or reliable, the test was stopped and the subject was given advice on performing the test accurately. If after 3 attempts a reliable result could not be obtained, the test was stopped and the results were not included in the final analysis

Visual acuity:

Visual acuity was measured using a directly projecting Nidek CP670 chart projector with the patient wearing their best correction immediately following subjective refraction. MPOD measurements and VA were taken with the same correction in place. Subjective refraction was performed with a Nidek RT-5200 computerised phoropter with normal room illumination and VA measurements taken through the same device.

Pupil Diameter:

Pupil diameter was measured using a slit lamp under diffuse illumination to simulate photopic conditions similar to those experienced in normal daylight and measured using a graticule on the slit lamp.

Age related maculopathy:

Two methods were used to detect age related macuopathy, fundus photography and OCT scans of the macula. Fundus photographs were taken with flash in darkened conditions using a Topcon NW6s fundus camera approximately 30-60 seconds apart as required to allow pupil redilation. The images were recorded using a Nikon D90 digital camera and Topcon imagebase software at a resolution of 3 megapixels and a 45 degree field of view, custom set white balance and using JPEG compression. White balance was set by taking a reading through the fundus camera with flash using a white card. These pictures were taken during the patients routine eye examination, not during the same session as the MPOD

measurements and research shows this is a reliable way to assess the fundus for AMD (Bartlett and Eperjesi, 2007). During the second session between the first and second set of MPOD measurements the central 6mm$^2$ of the macula was also analysed using OCT scans taken with a Zeiss Cirrus 4000 Optical Coherence Tomography instrument (Zeiss Meditec, Jena, Germany). 512x128 raster scans were taken from each eye. These were the same scans used to provide the macular thickness measurements used in chapter four.

### 3.3.3: Statistical analysis and Sample size calculations:

Using mean and standard deviation from Bartlett et al (2010) for MPOD it was determined that 18 subjects would be required to detect a difference of 0.1 between the two eyes using a paired t test. 30 subjects were recruited to allow for dropouts, those with disease detected on the second session and those unable to complete the MPS test.

Statistical analysis was undertaken using IBM SPSS version 20 for Windows and Microsoft Excel 2010 for Windows. Bland-Altman analysis was used to determine repeatability, correlations were determined using Pearson's or Spearman's correlation coefficients as appropriate depending on whether the data was normally distributed and means were compared using paired t tests. Two tailed test were used in all calculations.

3.4: Results:

Thirty subjects were recruited as planned for this study. Some were excluded from the final analysis for the following reasons:

ARM detected on OCT scan which was not visible on fundus photographs: 2 subjects. Inability to perform the MPS test accurately after three attempts on both eyes: three subjects.

Data from the remaining 25 subjects was included in the final analysis. Two of those subjects only managed to produce one accurate MPOD reading on one of their eyes, but in these cases the reading was considered sufficiently reliable to use based on the shape of the curves produced. The remaining 23 subjects managed to produce two smooth central and peripheral curves on both eyes with well-defined minima within three attempts at each curve.

The majority of the patients recruited were -0.1LogMAR or better in the pseudophakic eye as a consequence of the other exclusion criteria effectively eliminating anyone with any ocular disease which would reduce VA below these levels

Repeatability of MPOD measurements:

To determine the repeatability of the MPOD measurements, Bland-Altman analysis was applied to all the individual measurements where two good sets of curves were produced.

The coefficient of repeatability, representing the 95% confidence intervals of the individual measurements was 0.17. The mean of the difference between individual measurements was

0.02 +/-0.09.  There was no significant difference between the mean difference of the two

measurements and zero, one sample t test, t=1.75, p=0.86.
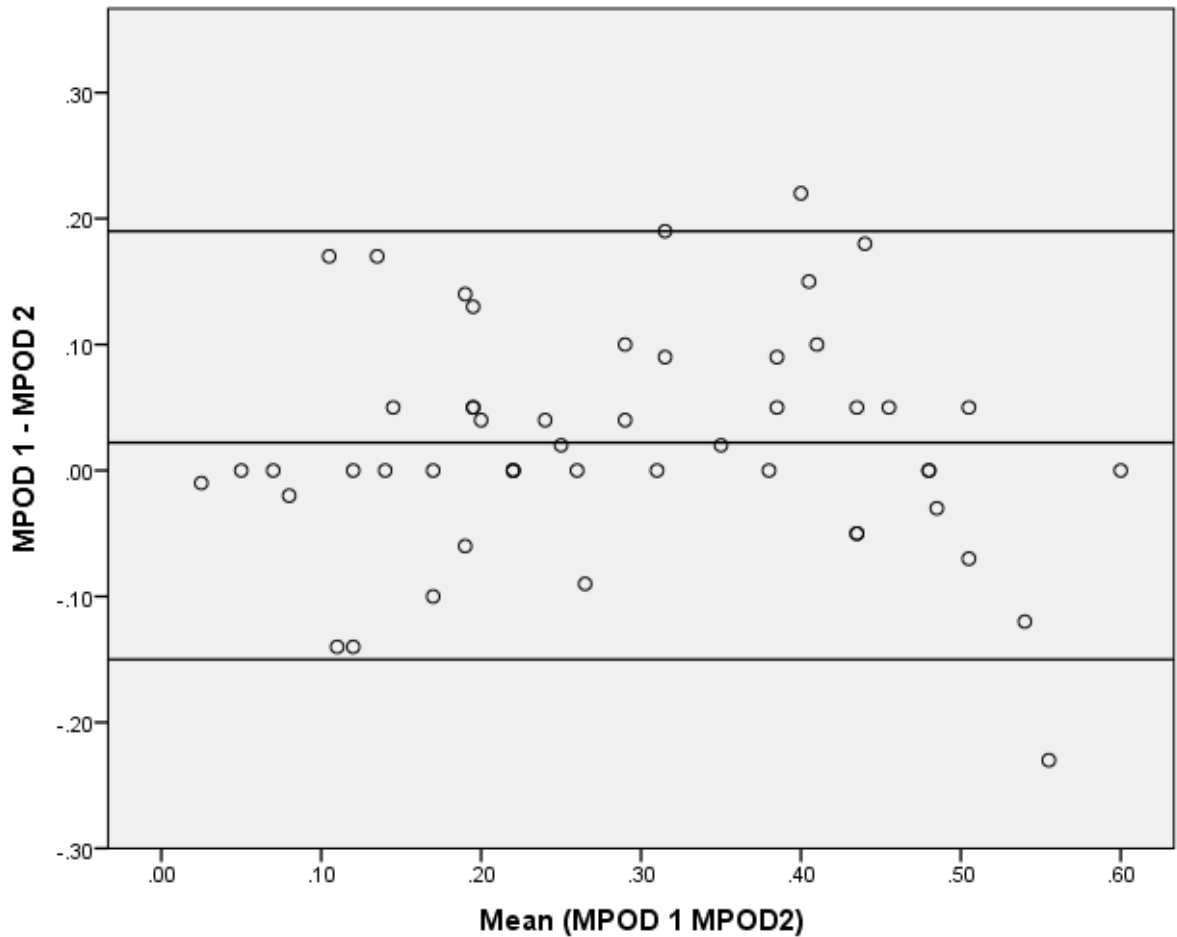


Figure 3.5: A Bland-Altman plot of the difference between the measurements against the mean of the two measurements. N=52

Age:

The median age of the subjects recruited was 73 years old, with a range from 52-89 years

old. The mean age of the subjects used in the data analysis was 69 years old.  A Shapiro-Wilk

test on the data showed the age data was not normally distributed (SW statistic = 0.851,

p=0.002).There was not a statistically significant correlation between age and the mean

MPOD of the two eyes. (Spearman's rank correlation coefficient r=0.059, p=0.779). For correlation graph see appendix 1.1.

Time since operation:

The median length of time since the cataract operation was 42.5 months, with a range from three months to 432 months, (36 years) although 20 of the 25 subjects had been pseudophakic less than 100 months. The time between the measurement and the operation was not normally distributed (Shapiro Wilk test: 0.69, p<0.001).

There was no correlation between the difference in MPOD between the pseudophakic eye and the fellow eye and the time since the operation (r=-0.16, p=0.940). For correlation graph see appendix 1.2.

If the results up to 100 months were calculated alone to bring the timescales involved in line with previous similar studies (Demirel et al., 2013; Nolan et al., 2009; Obana et al., 2011), there was a stronger correlation, (r=0.424) but this was still not statistically significant, (p=0.056, 2 tailed test, n=20), although this would have been significant if only an increase difference in MPOD with time had been hypothesised (one tailed test; p=0.028). For correlation graph see appendix 1.3

MPOD:

Mean MPOD for all eyes was 0.308 +/-0.16. Mean MPOD for the pseudophakic eyes was 0.254 +/-0.16 and for the fellow eye was 0.363 +/-0.15. There were 9 pseudophakic right eyes and 16left.

MPOD values were normally distributed for the MPOD values taken for each eye tested, (Shapiro-Wilk statistic 0.971, p=0.255) and also for the mean of the two MPOD values for each individual (Shapiro-Wilk statistic 0.965, p=0.517). .

There was a statistically significant difference between MPOD values in the pseudophakic eye compared to the fellow eye with the natural crystalline lens remaining. The mean difference between the pseudophakic and phakic eyes was 0.110 with a 95% Confidence Interval of 0.05 to 1.70 (Paired t test, t=3.777 p=0.001).
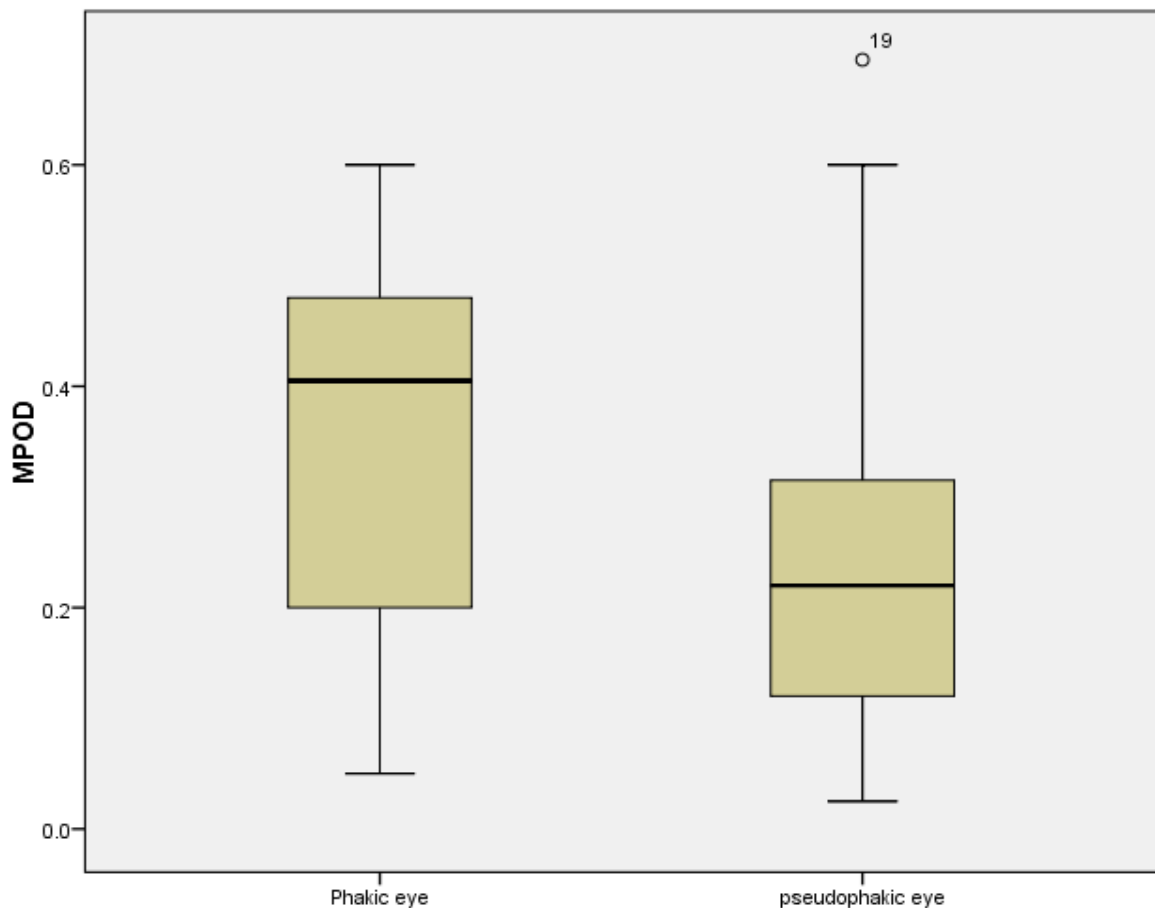


Figure 3.6: A box plot showing the difference in values between the phakic and pseudophakic eyes. The central line represents the median, the box represents the 25th to 75th percentile, the whiskers represent the minimum and maximum values and point 19 represented by the circle is an outlying value.  N=25

3.5: Discussion:

The coefficient of repeatability found here between the individual measurements is similar to other studies using the same instrument (Howells et al., 2013b; Loughman et al., 2012; van der Veen et al., 2009). Howells et al. (2013) found that using the average of two measurements reduced the coefficient of repeatability (CR) from 0.17 to 0.09 which is similar to other methods of MPOD measurement using HFP (Loughman et al., 2012). It was the average of two measurements used in the calculations of MPOD. There was no statistical difference between the first and second MPOD values.

The values for MPOD found in this study in phakic eyes are in line with a previous study which tested the MPS in a clinical setting (Bartlett et al., 2010b) whereas the values found for pseudophakic eyes are significantly lower. This result shows that there is a statistically significant relationship that MPOD is on average 0.11 lower in pseudophakic eyes than in the fellow phakic eye. The magnitude of the effect demonstrated here is similar to that found by the two studies discussed earlier that have found loss of MPOD post operatively (Demirel et al., 2013; Obana et al., 2011) of around 0.1.

The most likely cause of this result is the *in vivo* degradation or photobleaching of the macular pigments or due to the increased oxidative load which results from light exposure or inflammatory processes caused by the operation itself.

Given this result it is unlikely that there is any significant homeostatic mechanism in the retina to replace lost MP whereby the retina responds to lower MPOD by increasing uptake from the serum. It would appear that MPOD is therefore limited by the rate of renewal of the pigment as determined by blood serum concentrations, the efficiency of the transport

mechanisms that move MP from the serum to the retina and effectiveness of the binding mechanisms at the macula.

There are several implications of this result. Firstly it strengthens the theory that sunlight exposure is a risk factor for AMD. Secondly it has implications for the clinical decisions made when IOL are implanted and the advice given postoperatively. It also has implications for the behaviour of those at risk of AMD and strengthens the evidence base for the advice frequently given by optometrists and ophthalmologists that light exposure avoidance is desirable, particularly in old age and especially in those who have had IOLs implanted. It may also be advisable in light of this result for pseudophakics to supplement their diets with L, Z and MZ to counteract the effect of light exposure on their protective MPOD.

The lack of association between the time since the operation and the difference in MPOD readings would indicate that the MPOD tends to relatively quickly stabilise at a new, lower equilibrium between uptake and photobleaching rather than continuing to decline over many years. however when very long outliers are removed (over 100 months) to bring the timescale of this study in line with previous investigations there is a suggestion of a negative correlation between the variables (Demirel et al., 2013; Obana et al., 2011). This is not however statistically significant for the number of subjects left for inclusion. This correlation might be significant with a greater number of subjects and would be worth investigating further, particularly bearing in mind previous studies which have shown a time:effect relationship between MPOD and the time since the operation (Demirel et al., 2013; Nolan et al., 2009; Obana et al., 2011).

The limitations of this study are that it has a relatively small sample size and it is not known what the preoperative MPOD levels were, so it is impossible to determine if the values

measured have changed since the cataract removal as it is presumed they have. This also leaves the possibility of there being a measurement bias in the instrument towards giving lower readings in a pseudophakic eye.

3.6: Conclusions:

This research adds to current knowledge of the behaviour of MPOD in the eye, and the risk factors for AMD. It strengthens the case for the advice given to patients regarding sun protection with regard to AMD risk and indicates that L, Z and MZ supplementation or blue blocking IOLs may be a worthwhile intervention to prevent or delay AMD in pseudophakics.

Further research into this are may include long term trials of the effect of blue blocking IOLs or L, Z and MZ supplements on pseudophakics to assess whether there is any normalisation of the MPOD levels or reduction in AMD risk. It may also be useful to conduct a similar investigation to this one but with an attempt to measure MPOD both objectively and subjectively and to measure light transmission through the non pseudophakic eye to attempt to quantify the strength of the effect observed here. A study investigating the effect of anisocoria on MPOD may also have the potential to investigate the effects shown here, although the number of subjects would need to be large to take account of the smaller difference in exposure and the lack of blue light specificity as anisocoria reduces all wavelengths equally as opposed to cataract which selectively reduces blue light.

The next chapter concerns the experimental determination of the relationship between three macular thickness parameters and MPOD using Fourier domain OCT to measure thickness and the MPS to measure MPOD.

Chapter Four

Experiment two: To determine if there is a relationship between MPOD and macular

thickness.

In the previous chapter it has been determined that MPOD measures lower in pseudophakic eyes using HFP than in phakic eyes in the over 50s. This chapter concerns the experimental determination of the nature of the relationship between macular thickness and MPOD. Macular thickness will be determined by Fourier domain OCT and MPOD will be measured by HFP.

4.1: Introduction:

Macular pigment optical density (MPOD) represents the *in vivo* absorption of light by macular pigments, namely L, Z and MZ. These pigments are thought to perform an important role in the eye as antioxidants and blue light filters (Whitehead A, 2006). MPOD can be measured in several ways, using objective and subjective techniques (Howells et al., 2011). Of principal interest in this study is the thickness of the fovea where MPOD is highest. The thickness of this area is variable between individuals through a number of normal anatomical variations and possibly also disease or aging processes (Liu et al., 2011).

Macular pigment is taken from the blood serum via selective active transport (Li et al., 2010) and concentrated in the outer plexiform and Henle fibre layers (Trieschmann et al., 2007) where it acts to block blue light (Kernt et al., 2012) and neutralise ROS generated by photo oxidation and metabolic processes (Khachik et al., 1997).

Macular thinning may be associated with early age related disease of the macula (Richer et al., 2012). MPOD has also been shown in some studies to be reduced in those with AMD (Bernstein et al., 2012; Bone et al., 2001; Obana et al., 2008)

There are a number of mechanisms whereby MT and MPOD may be correlated. Both MPOD and macular thickness varies between individuals. The two may be correlated through

114

normal anatomical variations altering the capacity of the eye to bind macular pigment, for example through a thinner outer plexiform or Henle fibre layer altering the ability of the retina to bind MPOD. Alternatively early disease processes may reduce both macular thickness and MPOD concurrently as has been shown in AMD. There is also the possibility that a lack of MPOD increases the risk of disease processes which cause thinning of the retina.

The outer plexiform layer is known to undergo aging changes and thinning (Gartner and Henkind, 1981) and this may alter its capacity to carry MP with thinning leading to lower MPOD values. There is mixed evidence as to whether MPOD declines with age, with studies using Ramen spectroscopy more likely to show a difference (Howells et al., 2011) and it has been suggested that the decline is only found in people with AMD (Kaya et al., 2010)

Current studies examining the relationship between MPOD and macular thickness have produced conflicting results. Most have used time domain OCT which has lower resolution than the spectral domain OCT used here and may therefore have a reduced ability to determine subtle differences in macular thickness. Previous studies have also only measured the central fovea or 1mm diameter of the macula where MPOD is highest and have not determined if there is a relationship between MPOD and the thickness of the wider macula area. These studies are detailed in table 2.2.

Demonstrating the relationship between the thickness of the central retina and MPOD would help to increase our understanding of the effect that aging has on the retina and the influence of retinal anatomy on MPOD.

4.2: Methods:

4.2.1: Subjects:

Subjects were chosen from the two other experiments of this research project, into the effect of pseudophakia on MPOD and the measurement of cortical cataract.

This included 40 eyes from 20 subjects with some degree of cortical cataract from experiment three and 30 eyes with only moderate cataract from experiment one, so 70 eyes from 50 subjects in total were available for this study. Only one eye from each individual will be used as macular thickness will not be independent between the two eyes of an individual.

Not all of the subjects recruited for the cortical cataract study were able to perform a reliable MPOD test as the criteria for selection on this study is directed towards patients with cataract, so the total number of eyes available was be slightly lower than this number. Due to the need to gain a wide variation in cataract, some of the subject had cataracts too dense to perform the MPOD test reliably.

The methods for MPOD collection are as the other two studies, and differ slightly between the two groups. For the cortical cataract study the MPOD measurements are only taken as many times as is necessary to gain one set of reliable curves with the initial eye randomised. For the pseudophakia study the MPOD measurements are taken twice, with the right eye always taken first.

For the group with monocular pseudophakia, the methods for selecting patients and collecting data are as described in the preceding chapter. In addition to those criteria subjects with evidence of epiretinal membranes or damage to the foveal surface from a previous vitreous detachment, or any evidence of vitreoretinal traction were excluded, along

with epiretinal membranes or previous vitromacular surgery. Even if these were subtle enough to be deemed unlikely to affect the MPOD test, they may affect the results of the macular thickness measurements.

Figure 4.1: Examples of minor retinal defects which would invalidate macular thickness measurements: A: Mild lamellar damage from previous vitreomacular traction
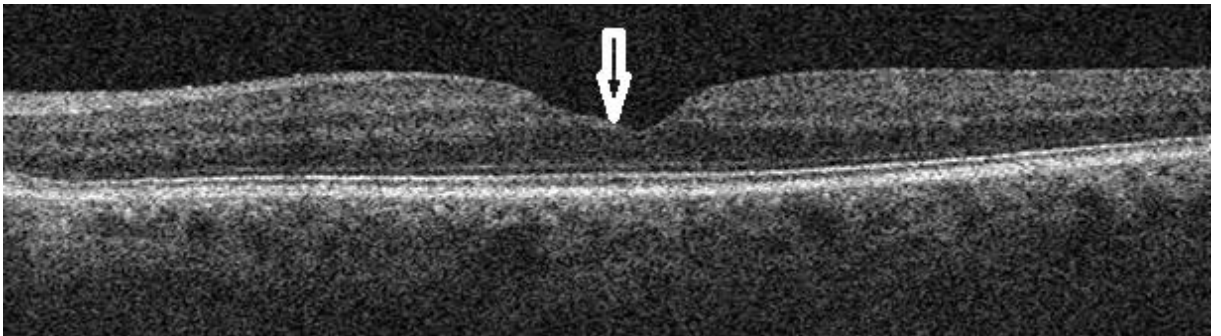


B: vitromacular traction
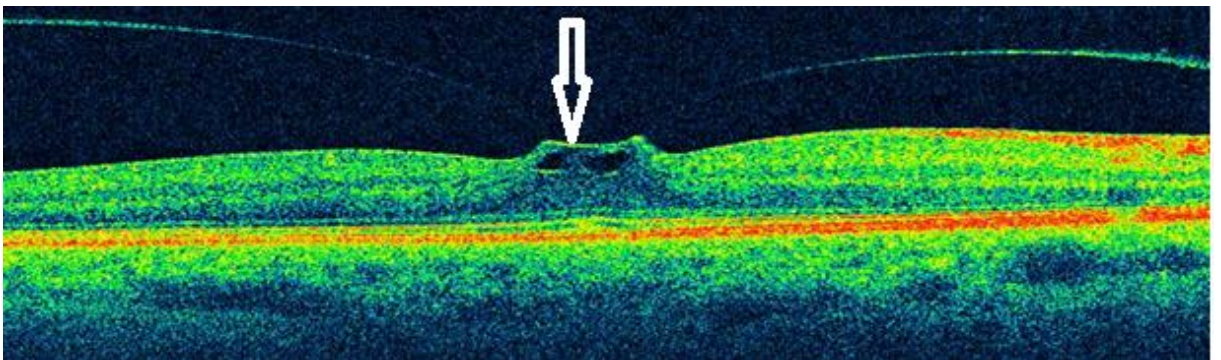


C: epiretinal membrane
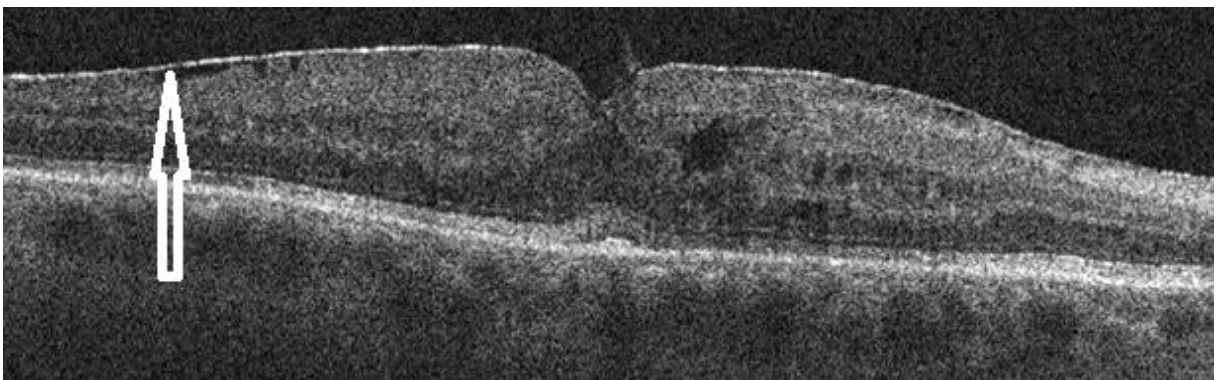
For the group recruited for cortical cataract assessment, MPOD data was collected from those subjects able to perform the test as determined by the same criteria in the experiment one, with the same exclusion criteria. Patients meeting the criteria for the cortical cataract assessment who did not meet the criteria for MPOD measurement did not perform the MPOD test. Those who were suitable performed the test 5 minutes after instillation of 0.5% tropicamide drops as required for the LOCS III grading for experiment three and before slit lamp and OCT tests were performed. The eye tested first was randomised using a random number generator. Due to the fact that the tropicamide drops were acting whilst the subject is performing the test there will be differences in pupil size as the tropicamide acts but this has been shown not to affect the results of the HFP test used (Wooten et al., 1999).

The MPS test was only performed once on these subjects due to time constraints and due to the risk of fatigue and photobleaching from the other tests affecting the results after the data collection for the cortical cataract measurements

The OCT measurements were taken using a Zeiss Cirrus OCT and analysed with version 6.1 software (Zeiss Meditec, Jena, Germany). Three measurements were recorded – the central foveal thickness as measured by the measurement tool integrated within the 6.1 software, the average central thickness over both the central area 1mm in diameter and the average thickness over the whole $6mm^2$ scan area as measured automatically by the instrument. These measurements have been shown previously in many studies using this instrument to have excellent repeatability and reproducibility and were therefore only taken once (Garcia-Martin et al., 2011; Giani et al., 2010; Ho et al., 2009; Huang et al., 2011) although it should be noted that these studies also tend to find poor agreement between instruments from

different manufacturers due to the different algorithms used to delineate and measure the

retinal layers.
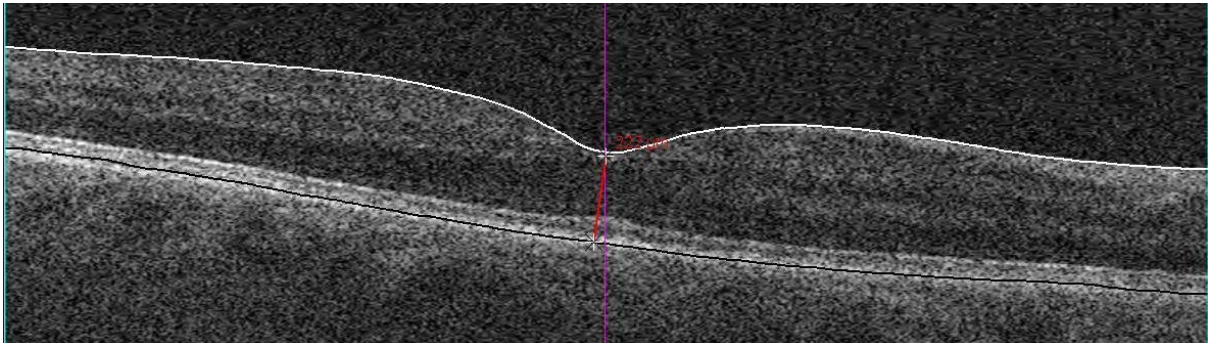


Figure 4.2: demonstration of measurements of foveal and macular thickness. The red line is

the foveal measurement and the macular thickness measurements are taken between the

white lines delineating the RPE and ILM.

As displayed in figure 4.2, the foveal thickness measurement was taken perpendicular to the

RPE from the centre of the RPE as automatically detected by the Cirrus instrument to the

bottom of the centre of the foveal pit. The instrument sometimes seems to underestimate

the depth of the foveal pit as shown in figure 4.3
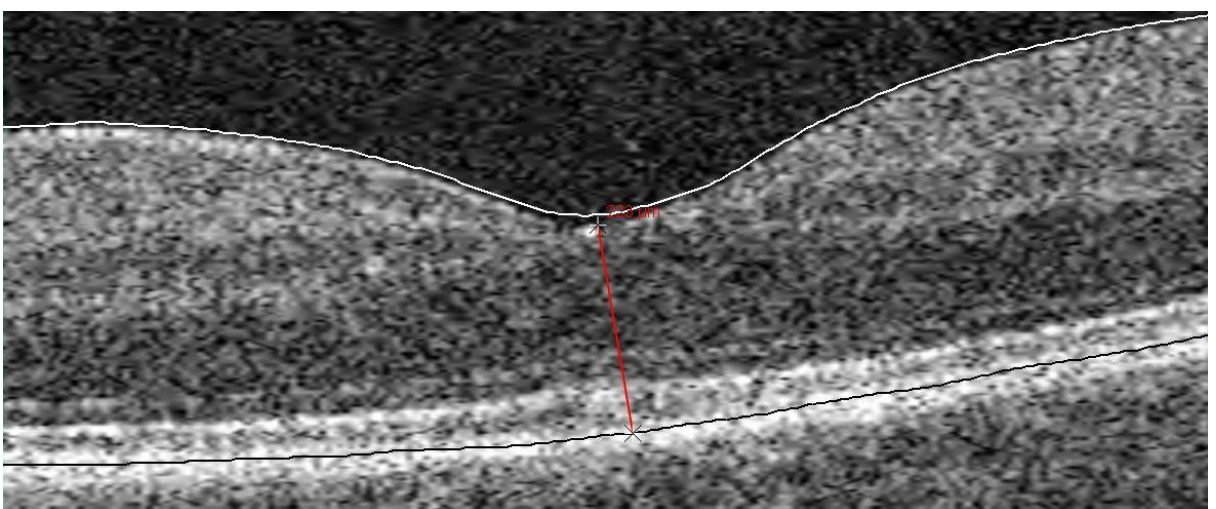


Figure 4.3: automatic segmentation line underestimating foveal pit depth where the retinal

surface does not quite correspond with the segmentation line.

In these cases the measurement was taken not to the segmentation line provided by the instrument but to the foveal pit surface as demonstrated by the red line in figure 4.3. In cases where the automatic segmentation failed due to poor signal strength or vitreous floaters only reliable measurements were recorded.

Age was recorded from the patient record and visual acuity data was taken during the patient's routine sight test as described in chapter three.

Before any measurements were taken the patient's informed consent to the procedures and use of their data was recorded using the consent form. The tests used which were not standard routine clinical tests were approved by Aston University ethics committee and all tenets of the Declaration of Helsinki were adhered to.

4.2.3: Statistical Analysis

The data were recorded on record cards, transferred to Microsoft Excel analysed using IBM SPSS version 20 for Windows. Pearson's correlation coefficient was used for parametric data and Spearman's rank correlation coefficient was used for non-parametric data. All correlations use 2 tailed data. Significance was taken as p≤0.05.

Sample size calculations were undertaken to assess the ability of this study to detect differences. Using values of macular thickness from Song et al. (2010.) which gives values of 275.66 ± 14.12 for overall average thickness and 253.92 ± 24.18 for central subfield thickness using the Cirrus instrument a sample size of 43 would be able to detect a 6μm difference in average thickness and a difference of 10μm in central retinal thickness for a power of 0.8. The number of subjects in this study will be sufficient to detect an r value of 0.30 at the 0.05 confidence level, or an effect which accounts for 9% of the difference between the variables.

Data from 50 subjects were available to the study in total, with 99 eyes being included for some measurements, one being excluded from all measurements due to corneal scarring. Age and visual acuity were taken for 99 eyes. 20 eyes were excluded from any macular thickness measurements due to macular abnormalities which would invalidate the thickness measurements. Data was then selected with one eye from each patient analysed. Where patients with pseudophakia were used, the phakic eye was used only, on the other subjects, the eye used was chosen at random where good data was taken from both eyes and where good data from only one eye was available, that eye was selected. This resulted in data from 43 eyes being used, with all macular thickness measurements, age and VA data available from all of these subjects and MPOD data from 41 eyes.

Macular thickness measurements

Three macular thickness measurements were taken, central foveal thickness, the central 1mm diameter macular thickness measured by the Cirrus software and the average macular thickness over the 6mm$^2$ scan area. The foveal thickness (FT) was not normally distributed (Shapiro Wilk statistic 0.945, p=0.048), the central macular thickness was normally distributed (Shapiro Wilk statistic 0.962, p=0.184) and average thickness (AVT) measurements were normally distributed, (Shapiro Wilk statistic 0.974, p=0.477). The median FT measurement was 210μm with a range from 169 to 276, MT measurements were 265μm +/-21 and AVT measurements were 277μm +/-13

All the macular thickness measurements were inversely correlated with age but only the central 1mm diameter measurement was statistically significant and the measurements were not significantly correlated with VA although the central macular thickness measurement was close to being significant (p=0.085). There was a positive correlation between all the macular thickness measurements and MPOD but only the average thickness over the entire scan area was statistically significant (p=0.039).

|  |  | MPOD | Age | VA |
|---|---|---|---|---|
| Foveal thickness (FT) | Correlation Coefficient | .148 | -.295 | -.138 |
|  | Sig. (2-tailed) | .356 | .055 | .376 |
|  | N | 41 | 43 | 43 |
| Central macular thickness (MT) | Correlation Coefficient | .185 | -.471 | -.266 |
|  | Sig. (2-tailed) | .246 | .001 | .085 |
|  | N | 41 | 43 | 43 |
| Average macular thickness (AVT) | Correlation Coefficient | .324 | -.208 | -.143 |
|  | Sig. (2-tailed) | .039 | .180 | .359 |
|  | N | 41 | 43 | 43 |

Table 4.1. Correlations involving macular thickness measurements. MT = central 1mm diameter thickness; FT = foveal thickness; AVT = 6mm diameter thickness. See appendix 2.1 for graph of macular thickness plotted against age.

MPOD

MPOD measurements were normally distributed (Shapiro-Wilk statistic 0.975, p=0.482) with a mean value of 0.36 +/- 0.15

There was a statistically significant correlation between the average thickness of the macula region and MPOD, but not between the foveal or central macular thickness measurements.

|  |  | MT | FT | AVT | Age | VA |
|---|---|---|---|---|---|---|
| MPOD | Correlation Coefficient | .185 | .148 | .324 | .064 | -.207 |
|  | Sig. (2-tailed) | .246 | .356 | .039 | .691 | .194 |
|  | N | 41 | 41 | 41 | 41 | 41 |

Table 4.2: Correlations with MPOD. MT = central 1mm diameter thickness; FT = foveal thickness; AVT = 6mm diameter thickness. See appendix 2.2 for graph of MPOD plotted against average thickness.

Age

Age was normally distributed (Shapiro-wilk statistic 0.976, p=0.524). Mean age was 72.7 +/- 8.7 years and had a range from 52 to 89 years old.

Age was significantly correlated with visual acuity and the central macular thickness measurements, but not MPOD.

|  |  | MPOD | FT | MT | AVT | VA |
|---|---|---|---|---|---|---|
| Age | Correlation Coefficient | .064 | -.295 | -.471 | -.208 | .472 |
|  | Sig. (2-tailed) | .691 | .055 | .001 | .180 | .001 |
|  | N | 41 | 43 | 43 | 43 | 43 |

Table 4.3: correlations with age

Visual acuity

VA was not normally distributed (Shapiro-wilk statistic 0.596, p<0.001). Median VA (LogMAR) was 0.00 with a range from -0.1 to 0.32.

VA was significantly correlated with age (r=0.472 p=0.001)

| | | MPOD | FT | MT | AVT | Age |
|---|---|---|---|---|---|---|
| VA | Correlation Coefficient Spearman's | -.207 | -.138 | -.266 | -.143 | .472 |
| | Sig. (2-tailed) | .194 | .376 | .085 | .359 | .001 |
| | N | 41 | 43 | 43 | 43 | 43 |

Table 4.4: correlations with VA

4.4: Discussion:

This data indicates that there is an association between macular thickness parameters and MPOD. The association was found between MPOD and the thickness of the entire macula region and not between MPOD and the areas where the MP is concentrated, weakening the hypothesis that there is a causal link between the two variables. It may indicate rather that there is a general decline of MPOD with the health of the macular region in general, rather than the availability of the binding sites being a limiting factor on MPOD.

It may be that maculae that are less healthy have less pigment and a general thinning of the retina, possibly caused by early dysfunction of the RPE affecting MP uptake rates. If the thinning was being caused by a lack of MP directly it would be likely that a preferential thinning of the central $1mm^2$ of the macula would be seen as this is the area protected by the MPOD. Another possibility is that this finding represents early RPE dysfunction which has not yet affected the central $1mm^2$ of the retina due to the protective effect of the MPOD. This may be related to the phenomenon of foveal sparing seen in many patients with geographic atrophy of the macula which tends to start in the perifovea where MP is lowest. These results must also be interpreted with caution as multiple comparisons were made and the correlation between the AVT measurements and MPOD was not very strongly significant. Had a criteria of $p \leq 0.01$ been used instead to take account of the multiple comparisons this would not have been considered a significant result. Further study with a larger number of subjects is needed to confirm this finding.

The methods for the MPOD test varies between the subjects recruited for the two main wings of the study in that in the subjects recruited with cortical cataract, the test was only performed once. It would have been more desirable to perform this test twice as with the

other study but this would probably only have been feasible with a third visit by the subjects, with the inconvenience involved which it was decided outweighed the benefits, particularly with a study which involved a number of elderly subjects. The difference in methods did not appear to affect the results of the MPOD test as both the mean and standard deviation were similar, and close to the values reported by similar studies (Bartlett et al., 2010b). The Bland-Altman analysis of the data in chapter 3 shows the individual MPOD readings had a CR of 0.17 and no systematic difference between the first and second readings.

Although the statistical power of this study is not as great as some previous investigations, this is the only study identified which compared the thickness of the entire macula region with MPOD, rather than just the small central area in which MPOD accumulates, and which some previous studies have found has a relationship with MPOD. The majority of the previous studies have used time rather than spectral domain OCT which has a lower resolution. Longitudinal studies measuring thickness and MPOD may be able to determine the relationship between these variables. No significant correlation between MPOD and any of the other variables was found, indicating that MPOD is not significantly affected with age within the limited range tested and is not significantly affected by foveal or central macular thickness within the power of this study.

Significant correlations were found between macular thickness and age, which is in line with a previous study using the same instrument and with a similar rate of change detected (Song et al., 2010.). Visual acuity was also shown to decline with age as might be expected, particularly in the group of patients selected many of whom had cataract, an age related disease (Klein et al., 1991).

These results indicate that there is no significant effect of foveal architecture on the accumulation of macular pigment. This indicates that the limiting factors on MPOD do not include the foveal architecture and by implication the ability of the macula to bind MPOD, indicating that factors such as dietary intake, uptake rates from the serum and clearance rates through oxidation and bleaching caused by light exposure are more important.

This study specifically excluded any subjects with traces of macular disease, and this may be why there is no association between the two variables found. It may be that studies showing an association between MPOD and central macular thickness (Hammond Jr. et al., 1997; Liew et al., 2006; van der Veen et al., 2009) are detecting differences which are due to the early stages of AMD, which is known to reduce MT and MPOD and this may also explain the relationship found between AVT and MPOD here which may both be a result of early RPE dysfunction due to oxidative stress, light exposure or hypoxic stress. The lack of a relationship between central macular thickness and MPOD may be due to the relative sparing of the central macula from stress by the presence of macular pigment. The results also indicate that the decline in MPOD seen in pseudophakic patients is not due to a change in macular thickness.

4.5: Conclusion:

This data does not support the hypothesis that MPOD is determined by central macular thickness, but does confirm that macular thickness declines with age and does find that there is a relationship between average macular thickness and MPOD which may not be causal.

This result agrees with some published studies (Kanis et al., 2007; Kirby et al., 2009; Nolan et al., 2008) which have found no association between MPOD and central and foveal macular thickness but contradicts others (Hammond Jr. et al., 1997; Liew et al., 2006; van der Veen et al., 2009). This appears to be the first study associating average macular thickness with MPOD. Further study into this area might investigate whether the effects of L, Z and MZ supplementation on MPOD are affected by macular thickness and whether any correlation independent of factors such as cataract between visual acuity and macular thickness is present. It might also be interesting to conduct a larger study into whether the effect found here is repeatable and whether any significant correlation between central macular thickness and MPOD in a study of subject without macular disease can be shown or whether the effects demonstrated in earlier studies are attributable to the presence of subjects with AMD.

Since OCT instruments have become more widespread, collecting data on macular thickness is a routine practice in some optometry practices now and this bank of data might be used in further studies. Turning this newly widespread technology on other areas of the eye such as the crystalline lens is an obvious area of potential study, which is where the next chapter progresses to.

Chapter Five: Objective measurements of cortical cataract using Optical Coherence

Tomography

In the previous chapter OCT was used to measure various parameters of macular thickness in order to determine their relationship with MPOD and a statistically significant correlation was found between MPOD and the thickness of the 6mm diameter region of the macula.

This Chapter concerns an attempt to perform objective quantitative grading of cortical cataract using a commercial Optical Coherence Tomography (OCT) instrument with a wavelength of 840nm. Cortical cataract assessed using the popular LOCS III grading scales was compared to lens density readings obtained using a Zeiss Cirrus OCT instrument scanning through the lens in cross section.

5.1: Introduction

OCT is a relatively new (Huang et al., 1991) but rapidly maturing technology with various applications in manufacturing (Dunkers et al., 1999), dentistry (Otis et al., 2000), vascular imaging (Brezinski et al., 1996) and in its most mature application, ophthalmology. It is analogous to ultrasound using light rather than sound waves and creates non-invasive cross sectional images through semi-transparent tissues, with the depth, field of view and resolution being determined by the tissue to be examined, the optics of the instrument and the wavelength and bandwidth of the light used (Wang et al., 2003).

Its resolution is superior to that of magnetic resonance imaging (MRI), computed tomography (CT) or ultrasound, and less invasive with no danger from metallic objects, ionising radiation or contact via coupling solutions, although these other techniques all have their advantages such as the ability to image posterior to the sclera and their differing abilities to measure certain types of tissue (Huang et al., 1991).

The eye is an ideal subject for the technology with various parts being transparent by necessity in order to let light penetrate to the photoreceptors at the posterior pole. The cornea, lens and retina are all multi-layered semi-transparent tissues which can be reliably imaged with OCT. Anterior segment OCT was first described by Izatt et al., (1994) and generally uses an illumination source of a superluminescent diode of wavelength 1310nm as opposed to posterior segment instruments, which generally now also have anterior segment capabilities, which use wavelengths around 840nm. The anterior segment OCT uses the longer wavelength due to the greater penetration of the sclera at this wavelength, allowing limited imaging of the ciliary body and the greater field of view it provides. Posterior/ multi-application OCT uses the shorter wavelength to improve the resolution for accurate imaging of the layers of the retina and to reduce light scattering in the anterior and posterior chambers.
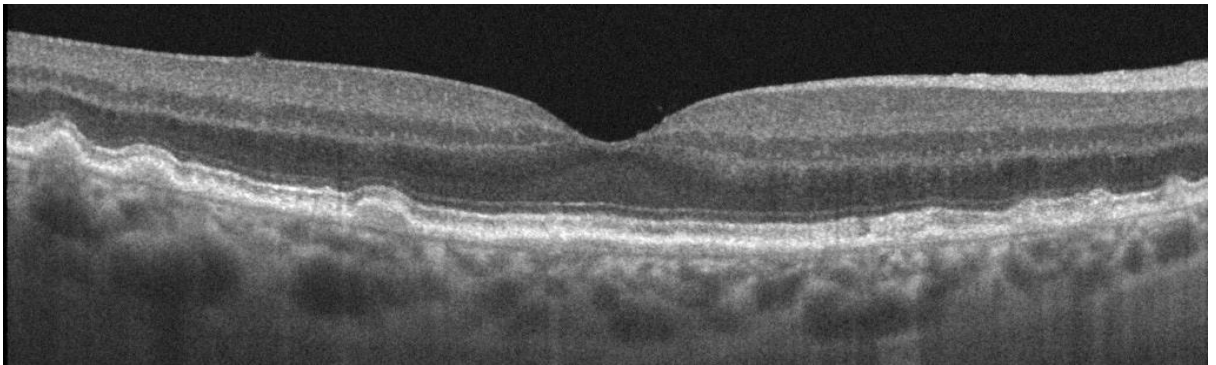


Figure 5.1: OCT image of the retina at 840nm

Figure 5.2: OCT of the anterior chamber at 1310nm. From www.meditec.zeiss.com
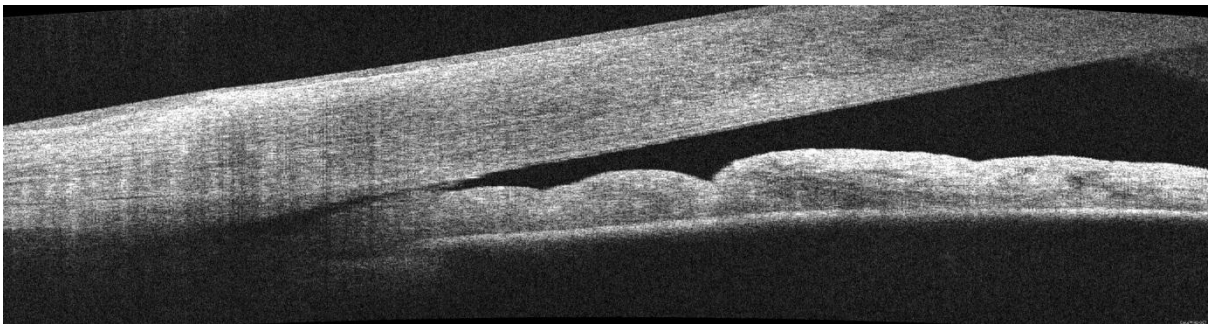


Figure 5.3: OCT of a narrow anterior chamber angle at 840nm. Note the small field of view,

higher resolution and shallower tissue penetration compared to figure 5.2.

Cortical cataract is cataract which affects the outer layers of the lens, the cortex. It is known

to be associated with UV light exposure (Cruickshanks et al., 1992; Taylor et al., 1988). It

often appears as radially arranged spokes of opacity with relatively clear lens in between

(figure 5.4)
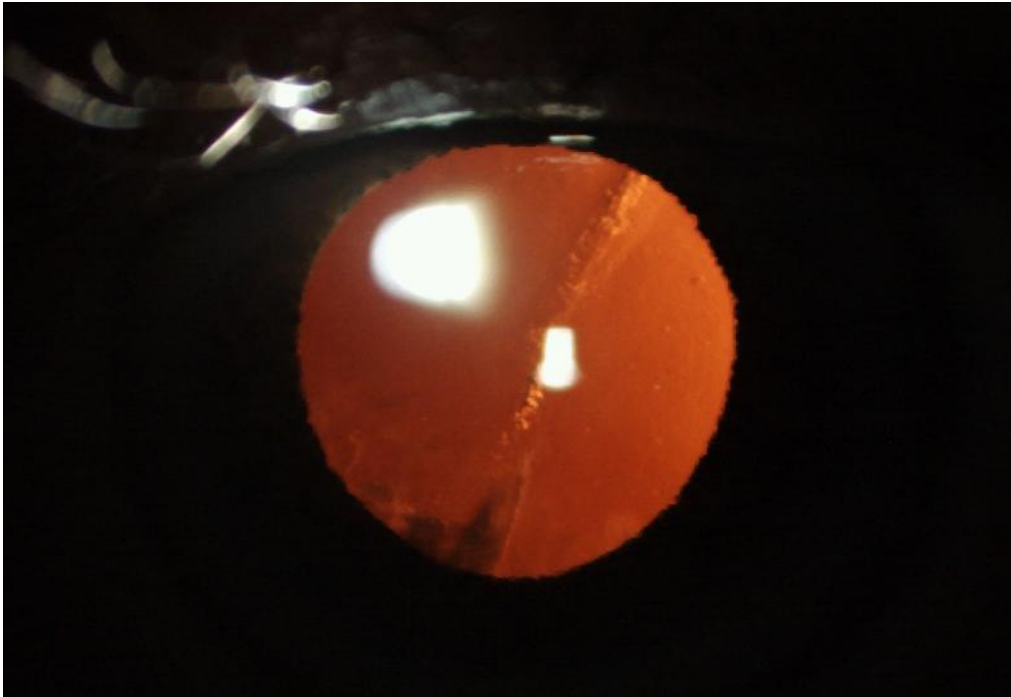
Figure.5.4: Cortical cataract viewed with retroilllumination.

This makes it challenging to grade in a way that reflects the visual effects which often vary depending on the location and type of opacity. Grading scales of cataract do not always relate well to visual acuity (Wong et al., 2009), but it is visual acuity which is used as one of the criteria to assess NHS patients for eligibility for cataract removal and is also used as one of the legal minimum standards for vision required for driving. This leads to the question of whether grading scales for cataract or visual acuity is the better assessment of whether an individual should have their cataract removed. Although symptoms are also heavily weighted in clinical decisions, these also often correlate poorly with objective measurements of vision and cataract.

The instrument used was an 840nm, posterior segment based instrument with anterior segment capabilities via a 60D additional lens, the Zeiss Cirrus (Zeiss meditec) The reason this instrument was chosen is that these instruments are increasingly widespread in primary

practice and ophthalmology units, making it likely that routine cataract grading in primary or secondary practice using OCT would use instruments of this type. The depth of the cortex increases with age and the scan depth of the 5 line anterior raster scan, which using the instruments internal measuring tool is 2mm is sufficient to image the entirety of the lens cortex reliably (Dubbelman et al., 2003).



Figure 5.5: Imaging of the anterior segment using an experimental OCT with a wavelength of 1050μm showing the lens as a whole: From (Li et al., 2013).

It was proposed that images of the central cortex and an analysis of the density of those images might provide information which is more pertinent to the visual status of the subject than the LOCS III grading scales. LOCS III enables subjective grading of cortical cataract on the presence of lens opacities visible when the pupil is dilated to over 6mm which in many cases might lie outside the area of the lens through which the subject is seeing, being hidden behind the iris when the pupil is undilated.  Grading cataract with a slit lamp through an

undilated pupil however is often impossible due to senile miosis and the light mediated constriction of the pupil when the bright slit lamp beam is passed through the lens. OCT overcomes these difficulties as the pupil can be physiologically dilated by scanning in darkness and the scanning light itself is in the infrared spectrum and therefore does not cause significant pupil constriction. Although senile miosis which constricts the pupil below 3mm would affect the area of lens available to scan through, it is also true that the lens area available to scan would be the same as the lens area the patient is looking through. Proving in principle that cortical cataract can be graded by simple imaging through undilated pupils may lead to more accurate assessments of cataract in primary practice and more accurate referrals of those most in need.

5.2: Methods:

5.2.1: Subjects:

Patients were selected during their routine eye examination due to the presence of cortical lens opacities and sufficient overall lens opacities to require pupil dilation to obtain a good fundus view and assessment of cataract. Patients were invited to take part in the study and if appropriate also perform a macular pigment optical density (MPOD) screening test, as described in chapter three. Not all of the subjects met the criteria for the MPOD screening test due to poor VA or macular disease, but these subjects were still assessed for cataract due to the need to obtain a wide range of cataract states, some of which would preclude VA sufficient to perform the MPOD testing and the fact that the presence of macular disease would not affect the cataract grading, indeed the reason some of the denser cataracts had not been removed was due to the presence of macular disease in that eye.

Inclusion criteria was the presence of cortical cataract visible on slit lamp examination in one or both eyes in an undilated state during the patient's routine eye examination which clinically warranted pupil dilation to adequately assess the fundus and the cataract itself.

Exclusion criteria for cataract measurement:

- The presence of corneal disease (for example Fuch's endothelial dystrophy) which may affect OCT scan intensity, or any corneal opacity within the central 4mm diameter of the cornea.

- Ataxia or an inability to undergo a slit lamp or OCT examination due to, for example, neck or back arthritis.

- Vulnerable people, those with dementia or mental health problems were excluded.

- Medications which can affect the opacity of the cornea

- A history of iritis or uveitis, angle closure glaucoma or narrow anterior chamber angles.

- synechae

- IOP over 21mmHg

- Van Hericks grade 1 (narrow) or 0.

- Pupils noted in records to be resistant to dilation

Exclusion criteria for MPOD measurement were the same as described in chapter three.

Measurements used in this research were taken during the patient's follow up dilation appointment.

Before any measurements were taken the patient's informed consent to the procedures and use of their data was recorded using the consent form. The tests used which were not standard routine clinical tests were approved by Aston University Ethics Committee and all tenets of the Declaration of Helsinki were adhered to.

5.2.2: Materials and procedures:

Before dilation, IOP measurements and a Van Herick's angle assessment was undertaken to exclude those with a risk of angle closure or abnormal IOP. The patients were dilated using 1 drop of 0.5% tropicamide in each eye and the MPOD measurements for chapter three taken whilst the pupils dilated. MPOD measurements were only taken once and only taken on suitable candidates, as outlined in chapter three. Patients unsuitable for MPOD testing were asked to wait for approximately 20 minutes after instillation of the drops until the pupils were dilated over 6mm.

The OCT measurements were taken using a Zeiss Cirrus OCT and analysed with version 6.1 software (Zeiss meditec). Measurements from only the front surface of the lens were used as images from the back surface tend to be rather faint due to absorption of the scan from the anterior cortex, nucleus and anterior lens surface, and also because visually significant cortical opacities tend to occur in the anterior cortex of the lens, probably due to the greater effect of UV exposure. It is also difficult to accurately define the anterior surface of the posterior lens cortex on many of the images, as with figure 5.5.

The settings for the OCT were left as per the factory defaults for brightness, contrast and the 'enhance' function which adjusts the polarisation of the scanning beam. Fourier-domain OCT has a peculiarity whereby the image intensity is dependent on where in the frame the scan is positioned (Spaide et al., 2008). To ensure this effect does not affect the apparent lens density, the scans were positioned with the anterior surface of the lens as high as possible in the scan window to achieve consistency between the scans (figure 5.6). This was in any case necessary to ensure that the entire cortex was imaged within the 2mm window of the OCT scan available. The size of this effect was also estimated using two patients from the study and analysing corneal images at various scan heights. It is also apparent that the centre of the lens curvature does not always coincide with the centre of the pupil as might be expected. In these cases the lens was scanned through the centre of the pupil, this being the centre of the area through which the subject is looking, rather than the centre of curvature of the lens, with the lens again being positioned as far forward as possible.
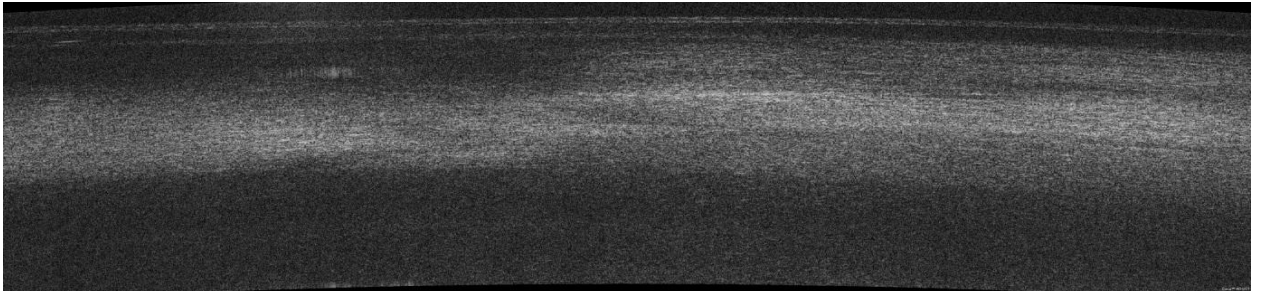
Figure 5.6: OCT section of lens cortex showing lens positioning in the scan window.

Ten 3mm wide by 2mm depth high definition raster scans were taken from each lens, five horizontal and five vertical with the centre measurement positioned on the centre of the pupil (figure 5.7). This generates images which can be exported for analysis as either .BMP or .jpg files of size 4096x1024. The phenomenon of lens dewarping artefacts noted by Wong et al. (2009) is not an issue with these scans as the cirrus software, unlike the visante, does not attempt to correct for the warping of the image caused by the refraction at the air-cornea and cornea-aqueous interfaces (Leung and Weinreb, 2011)
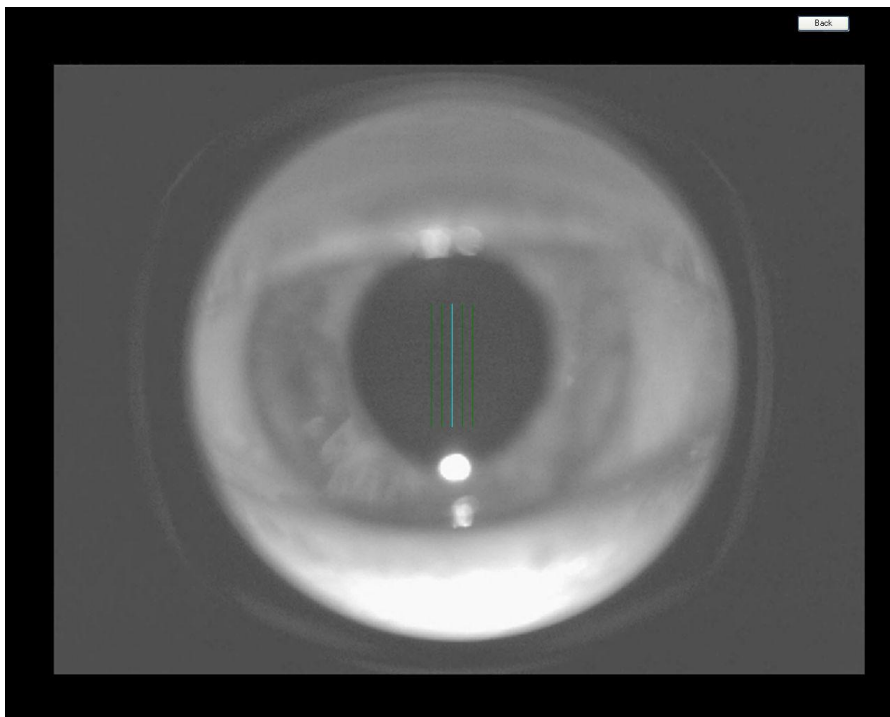


Figure 5.7 Placement of vertical scans (green lines)

Once pupils were dilated past 6mm, cataract was graded conventionally using the LOCS III photographic grading scales. Images were graded whilst the patient was at the slit lamp using the images provided in the LOCS III paper and photographs taken for records. Images were taken using a Topcon SL-D7 slit lamp equipped with a DV-1 camera which records images through the right hand eyepiece via a beam splitter. Camera settings were chosen for accurate colour rendition in the photographs and jpeg images of size 1280x960 recorded using Topcon imagenet ibase software version 3.7.4. The slit lamp was positioned on a translating table on a combi unit and connected to a PC VIA USB giving instant display of the images taken to ensure the images accurately reflected the view through the slit lamp. The measurements were taken in darkened room lighting.

Three good images that accurately represented the view through the slit lamp were taken on each eye, to record nuclear opacity (NO), nuclear colour (NC) and cortical cataract (C). For the NC and NO images the slit width was set to 0.2mm, the height to full and the angle to 45 degrees, as per the methods set out in the LOCS III paper (Chylack et al., 1993). The brightness was adjusted by trial and error to give accurate colour rendition and not subsequently adjusted. The slit beam was focussed on the centre of the lens to record NO (figure 5.8) and the rear of the lens to record NC (figure 5.9). For the C images the lens was retroilluminated using a spot and the cortical opacities analysed using the method described by Chylack et al. (1993). The LOCSIII grade between 0.1 and 5.9 was recorded. Posterior capsular cataract was not graded.
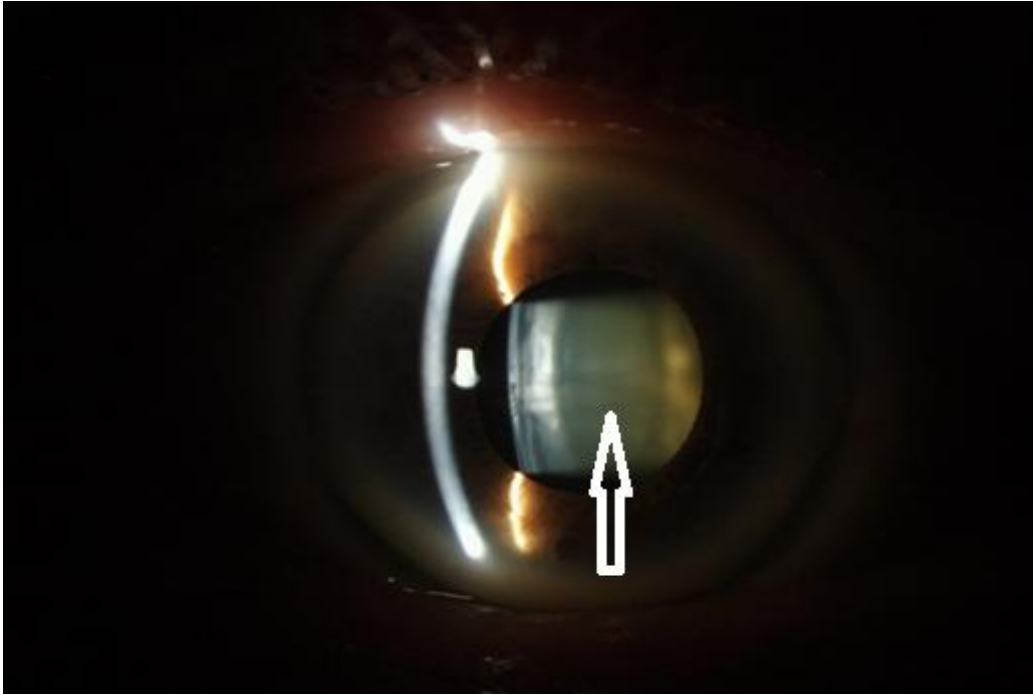
Figure 5.8: image showing slit beam positioned for nuclear opalescence measurement: the back scatter of the slit beam from the lens nucleus is used for grading
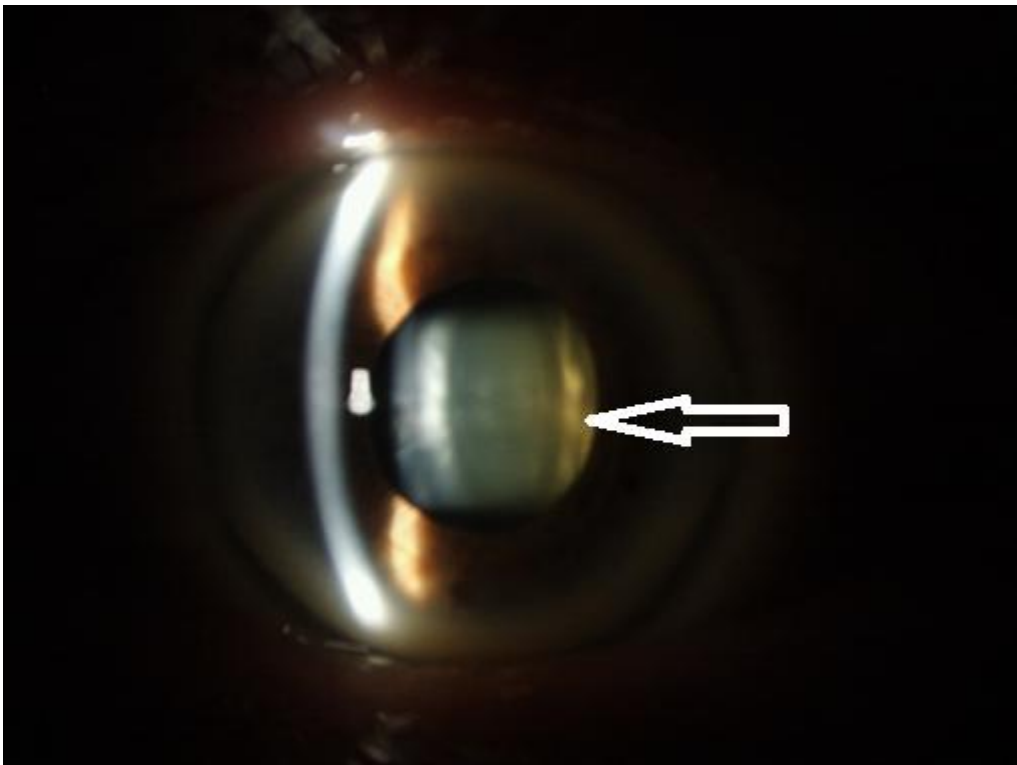


Figure 5.9: nuclear colour measurement: the colour of the posterior lens is used for grading.
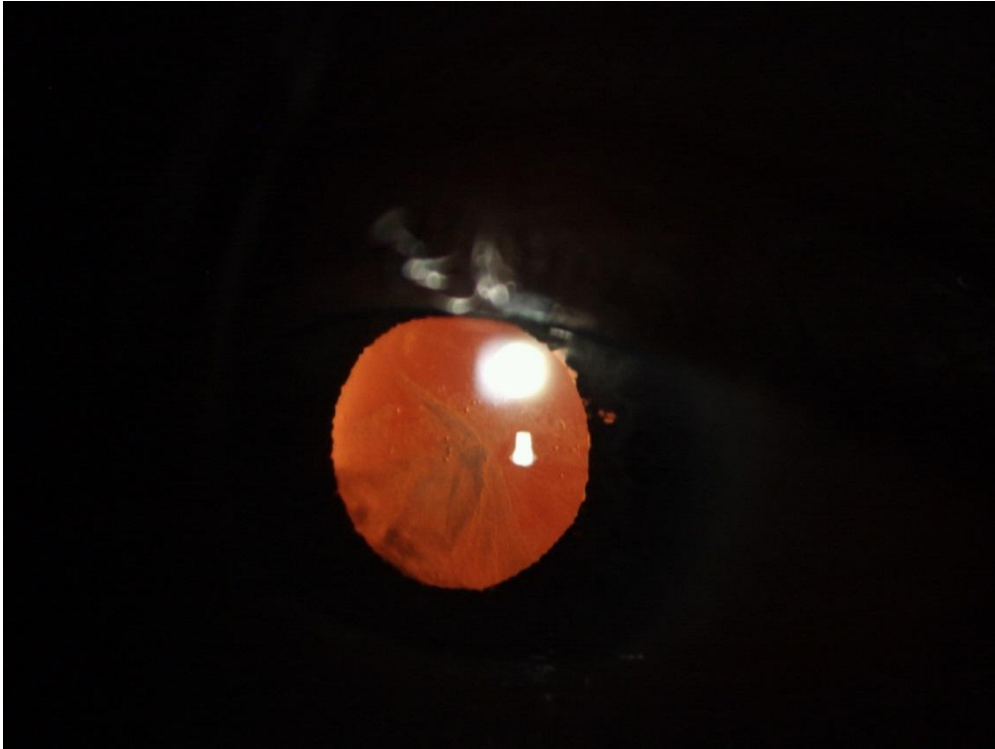
Figure 5.10: retroillumination showing cortical opacities: the area covered by the opacity is used for grading.

Analysis of the OCT images was blinded, with patient identifying details removed. The images were imported to ImageJ software where the lens cortex was isolated from the image, excluding the strong reflection from the anterior lens capsule (figure 5.11)
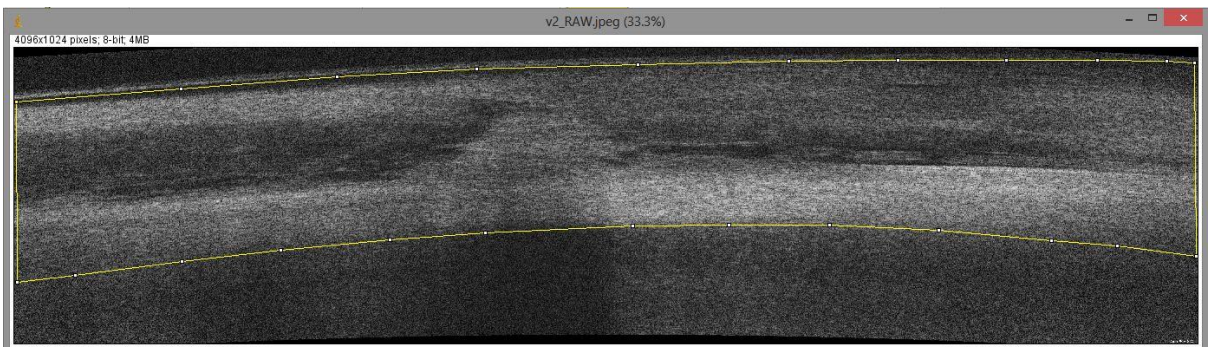


Figure 5.11: OCT scan of lens cortex isolated using the polygon selection tool in ImageJ software.

ImageJ retains the selection from one image to the next, and this was adjusted as required between images and invariably required completely redrawing when switching between eyes or between the horizontal and vertical sections. The analysis of the corneal images was performed the same way.

To investigate whether image format had any bearing on the results of the analysis, one set of 10 images (5 horizontal, 5 vertical) from one eye was analysed in the two formats the Cirrus OCT permits single images to be saved as, JPEG and Bitmap. The file size of the bitmap images is approximately 10 times that of the JPEG images due to the greater compression of the JPEGs and it is possible that this would lead to differences in the data. No significant difference was found in any of the measurements using a 2 tailed paired t test, for the mean pixel intensity values $p=0.9985$. As no significant difference was found, the higher compression JPEG images were used for convenience.

The five horizontal images and the five vertical images are also used to assess reproducibility. Although the images are of slightly different parts of the lens, they are analogous to minor differences in scan placement and will be used to assess the test-retest reliability of the measurements. If there is little correlation between the five scans taken 0.25mm apart, the test-retest reliability is likely to be poor. The selected area of each image was analysed to record mean pixel intensity, the standard deviation of the mean pixel intensity and the integrated density of the selection.

<u>5.2.3: Statistical analysis:</u>

It was determined that a sample size of 40 eyes would be more than sufficient to detect a correlation which would be clinically significant, based partly on the sample size chosen by Wong et al (2009) of 55 eyes.

Statistical analysis was performed using Microsoft Excel and IMB SPSS version 20 for Windows.

Statistical significance was chosen at the 0.05 level and one or two tailed tests were chosen as appropriate.

Bland-Altman analysis was used to determine repeatability, correlations were determined using Pearson's or Spearman's correlation coefficients as appropriate depending on whether the data was normally distributed and means were compared using t tests.

5.3: Results:

Effect of scan height:

A set of twelve images of the cornea of two patients at various heights were analysed to estimate the effect that scan height has on the pixel intensity. It was found that scan height is significantly correlated with mean pixel intensity (r=-0.981, p<0.001). These results were graphed (figure 4.12).
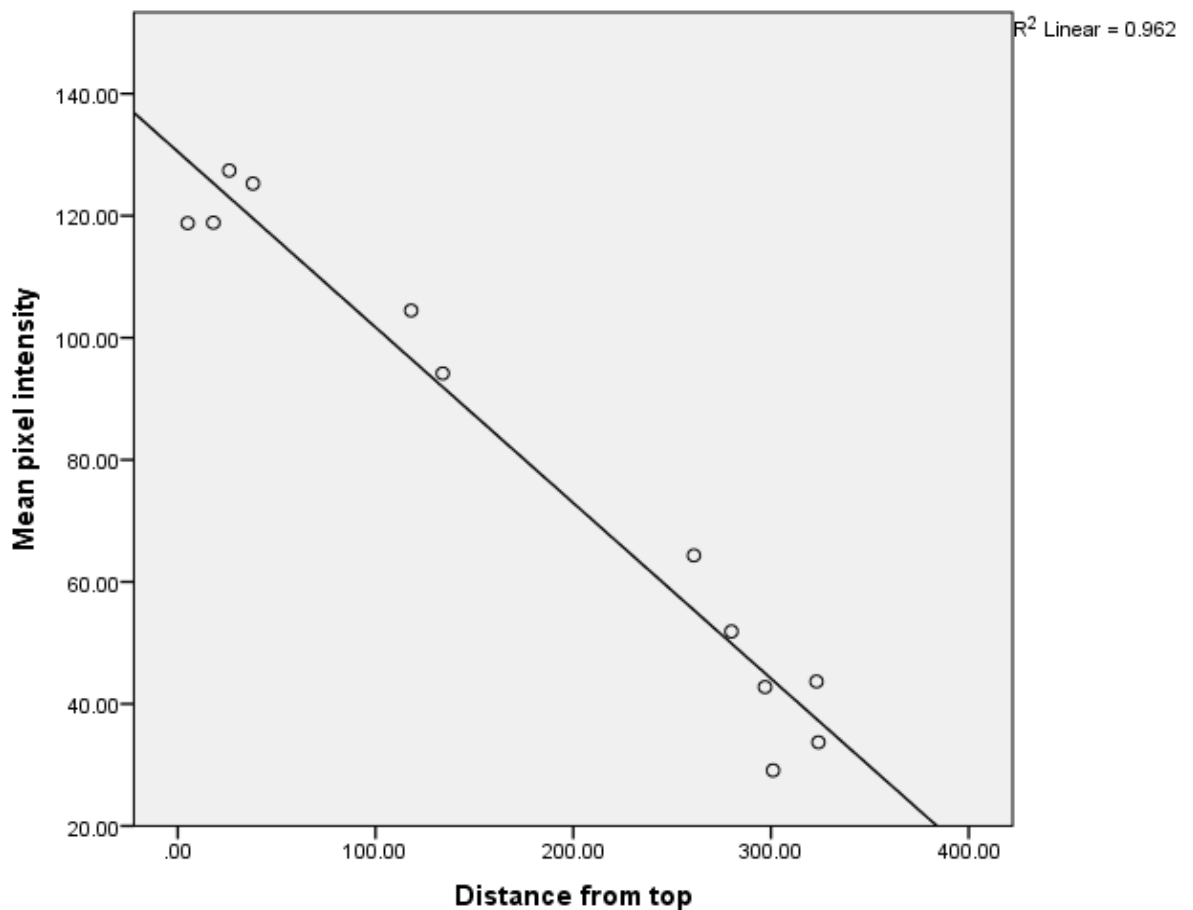


Figure 5.12: mean pixel intensity against image position in scan window (N=12)

The data was first tested using a Shapiro-Wilk test for normality. The mean average of the 10 measurements from each eye was used for this purpose. Results are displayed in table 5.1:

**Tests of Normality**

| | Shapiro-Wilk | | |
|---|---|---|---|
| | Statistic | N | Sig. |
| MT | .893 | 26 | .011 |
| MPOD | .952 | 27 | .237 |
| NC | .822 | 39 | .000 |
| NO | .888 | 39 | .001 |
| C | .888 | 39 | .001 |
| Age | .920 | 39 | .008 |
| VA | .659 | 39 | .000 |
| Mean | .964 | 39 | .244 |
| Standarddeviation | .944 | 39 | .053 |
| IntDen | .973 | 39 | .468 |

Table 5.1: normality tests. MT=central 1mm macular thickness, NC=nuclear colour, NO=nuclear opalescence, C=cortical opacity, mean=mean pixel intensity, Standarddeviation= standard deviation of pixel intensity, IntDen= Integrated density of pixel intensity.

Repeatability

Three sets of images were assessed to test for repeatability of the images – the second and third scans of both the horizontal and vertical sections were compared to test for repeatability when images were taken through slightly different parts of the lens as would be expected to occur in test-retest conditions and the central scan from the horizontal and vertical sections were compared to test for repeatability after repositioning, through a different aspect of the lens.

A Bland-Altman test of the mean pixel brightness data was used to assess repeatability, comparing the central scan with the previous scan placed 0.25mm away. This test was performed on both the horizontal and vertical sections. Additionally, the central scan from the horizontal and vertical scans were compared against each other in the same way.

Repeatability, horizontal scans 2 and 3: A one sample t test was performed which showed there was no statistically significant difference between the difference between the two scans and zero ( t=1.901p=0.065, CI=-0.0647-2.0604.) The mean difference between the two measurements was 0.99 +/-3.28 and the coefficient of repeatability (CR) was 6.42.
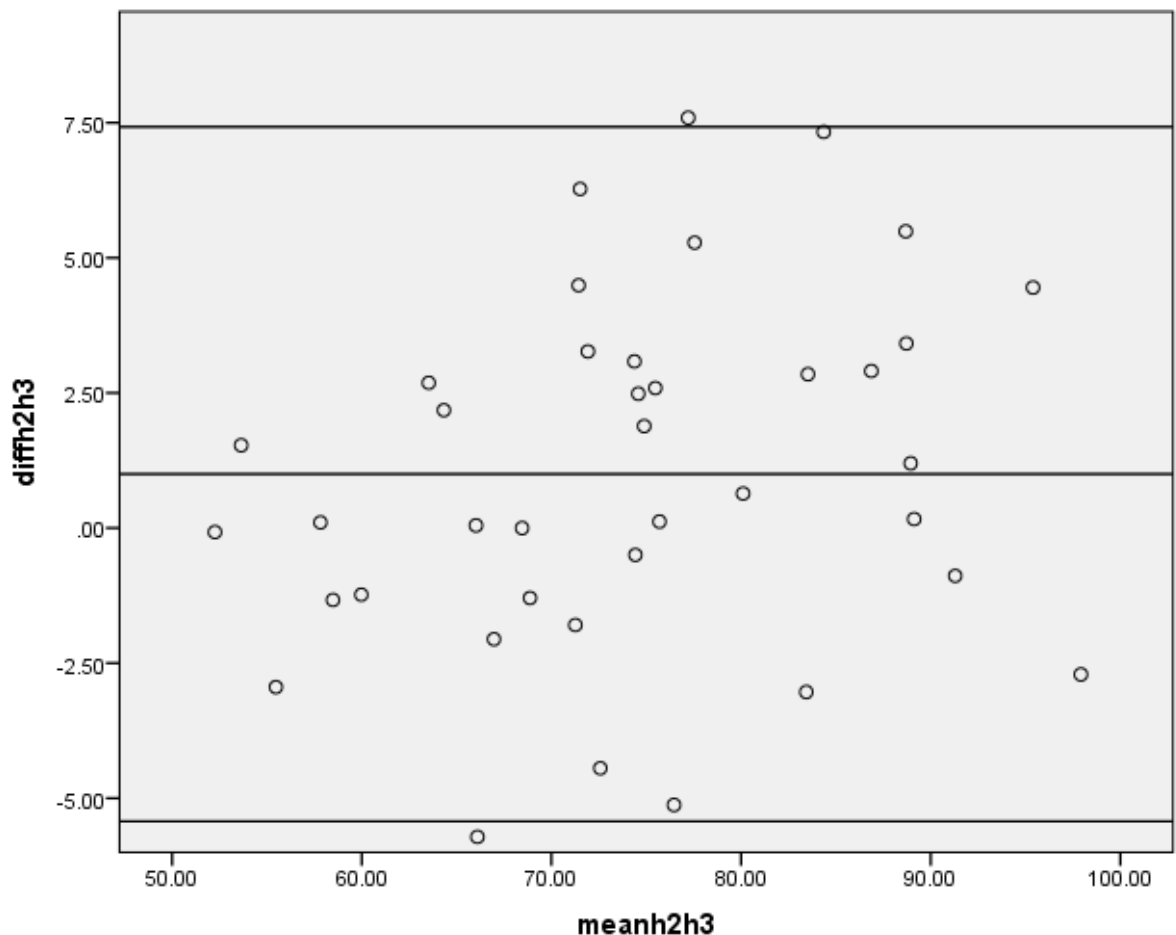


Figure 5.13: A Bland-Altman plot of the difference between the mean of the second and third horizontal scans plotted against the mean of the same 2 scans. N=39.

Repeatability, Vertical scans 2 and 3: A one sample t test was performed which showed there was no statistically significant difference between the difference between the two scans and zero ( t=-1.404 p=0.168, CI=--1.5435-.2794).

The mean difference between the two measurements was -0.63 +/- 2.81 and the CR was 5.51.
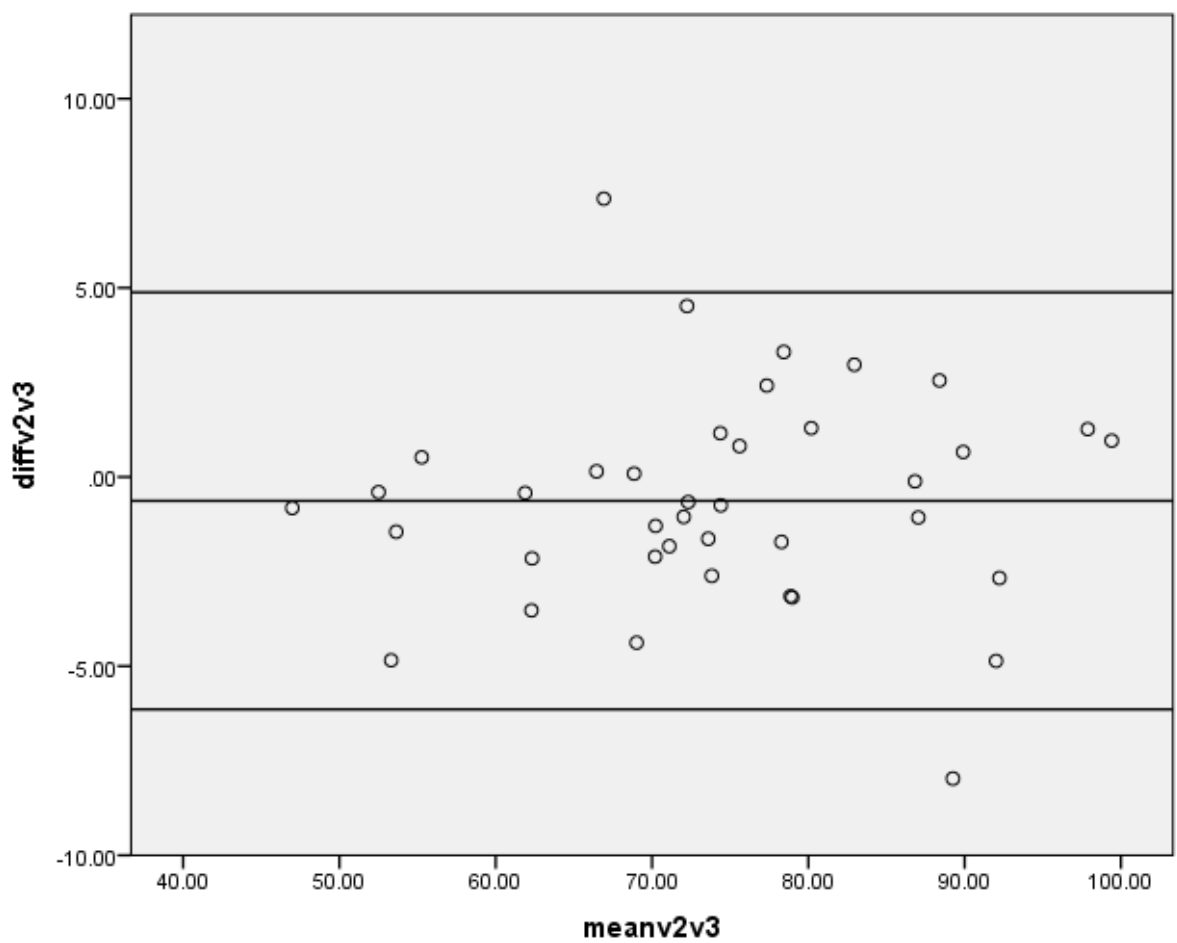


Figure 5.14: A Bland-Altman plot of the difference between the mean of the second and third vertical scans plotted against the mean of the same 2 scans. N=39.

Repeatability, horizontal centre scan Vs vertical centre scan: A one sample t test was performed which showed there was no statistically significant difference between the difference between the two scans and zero (t=-0.439 p=0.663, CI=-4.2317-.2.7242).

The mean difference between the two measurements was -0.75 +/-10.73 and the CR was 21.03.
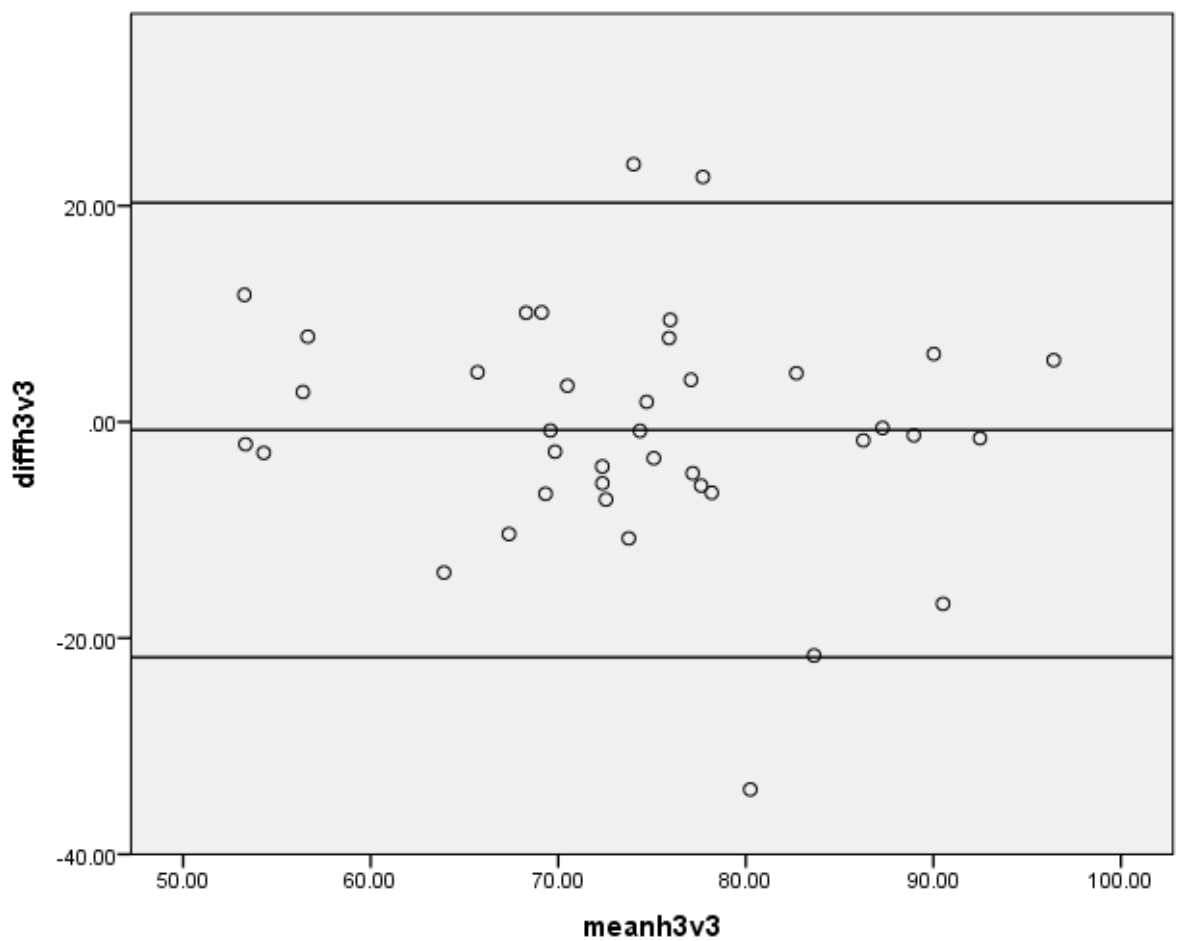


Figure 5.15: A Bland-Altman plot of the difference between the mean of the third horizontal and third vertical scans plotted against the mean of the same 2 scans N=39

Comparison of OCT scan data with LOCS III grading data:

Several aspects of the OCT scan data were collected: the mean pixel intensity, the standard deviation of the pixel intensity, and the integrated density of the images.

LOCS III data on nuclear opalescence (NO) nuclear colour (NC) and cortical opacities (C) was recorded, along with age, VA, MPOD where applicable and MT as automatically measured by OCT where applicable.

The data was analysed using Pearson's correlation coefficient for data which was normally distributed (Mean pixel intensity, standard deviation of pixel intensity, integrated density, C, MPOD) and Spearman's rank correlation coefficient for data which was not normally distributed (NC, NO, MT, age, VA).

Visual Acuity:

Median VA was 0.1 with a range from -0.1 to 1.0.

Visual acuity was correlated significantly with all 3 LOCS III measurements, moderately with NC and weakly with NO and C. VA was also correlated significantly with MT.

| | | C | MPOD | Mean | Standard Deviation | IntDen | NC | NO | MT | Age |
|---|---|---|---|---|---|---|---|---|---|---|
| VA | Correlation Coefficient | .328* | -0.044 | -0.102 | 0.168 | 0.12 | .534** | .418** | -.433* | 0.255 |
| | Sig. (1 or 2-tailed as appropriate) | 0.021 | .0.828 | 0.538 | 0.306 | 0.468 | 0 | 0.004 | 0.028 | 0.059 |
| | | 1-tailed | 2-tailed | 2-tailed | 2-tailed | 2-tailed | 1-tailed | 1-tailed | 2-tailed | 1-tailed |
| | N | 39 | 27 | 39 | 39 | 39 | 39 | 39 | 26 | 39 |

Table 5.2: correlations for VA

Age

The age range of the study was 63 to 89 years with a median age of 77 years. Age was correlated significantly with NC and NO but not with C. Age was inversely correlated with macular thickness. Integrated density of the images was also weakly correlated with age

| | | C | MPOD | Mean | Standard Deviation | Int Den | NC | NO | MT | VA |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | Correlation Coefficient | .207 | .027 | .252 | .291* | .347* | .426** | .354* | -.522** | .255 |
| | Sig. (1 or 2-tailed as appropriate) | .103 1-tailed | .894 2-tailed | .122 2-tailed | .072 2-tailed | .03 2-tailed | .003 1-tailed | .014 1-tailed | .006 2-tailed | .059 1-tailed |
| | N | 39 | 27 | 39 | 39 | 39 | 39 | 39 | 26 | 39 |

Table 5.3: correlations for age

MPOD

Mean MPOD was 0.37 +/- 0.16. MPOD was not significantly correlated with age, macular thickness or any of the cataract measurements taken (p>0.05).

| | | C | Mean | Standard Deviation | IntDen | NC | NO | MT | Age | VA |
|---|---|---|---|---|---|---|---|---|---|---|
| MPOD | Correlation Coefficient | .285 | 0.251 | .194 | .176 | .292 | .159 | .290 | .027 | -.044 |
| | Sig. (1or 2-tailed as appropriate) | .232 2-tailed | .394 2-tailed | .332 2-tailed | .380 2-tailed | .140 2-tailed | .428 2-tailed | .170 2-tailed | .894 2-tailed | .828 2-tailed |
| | N | 27 | 27 | 27 | 27 | 27 | 27 | 24 | 27 | 27 |

Table 5.4: correlations for MPOD

Mean pixel intensity

The mean pixel intensity of all the cortex images was 73.87+/-12.06.

The mean C value was 2.60 +/-1.02, the median NC value was 2.6 with a range from 1.9 to 5.9 and the median NO value was 2.4 with a range from 1.8 to 5.9.

Mean pixel intensity was only significantly correlated with the NC measurement (r=0.324, p=0.044) and was not significantly correlated with the C scores (r=0.251, p=0.122)

| | | C | MPOD | NC | NO | MT | Age | VA |
|---|---|---|---|---|---|---|---|---|
| mean | Correlation Coefficient | .251 | .260 | .324[*] | .143 | -.176 | .252 | -.102 |
| | Sig. (1 or 2-tailed) | .122 2-tailed | .190 2-tailed | .044 2-tailed | .368 2-tailed | .390 2-tailed | .122 2-tailed | .538 2-tailed |
| | N | 39 | 27 | 39 | 39 | 26 | 39 | 39 |

Table 5.5: correlations for mean pixel intensity

The standard deviation of the pixel intensity

The mean value for the standard deviation of the pixel intensity of each image was 34.72 +/- 5.80 The standard deviation of the pixel intensity was weakly correlated with the C scores (r=0.354, p=.028) . This measure was not significantly correlated with VA (r=0.168, p=0.153)

| | | C | NC | NO | MT | Age | VA |
|---|---|---|---|---|---|---|---|
| Standard deviation | Correlation Coefficient | .354[*] | .409[**] | .190 | -.378[*] | .291[*] | .168 |
| | Sig. (2-tailed) | .028 | .010 | .246 | .056 | .072 | .306 |
| | N | 39 | 39 | 39 | 26 | 39 | 39 |

Table 5.6: correlations for standard deviation of pixel intensity.

In addition, NC and NO measurements were significantly correlated with each other, (r=0.678, p=<0.001)

5.4: Discussion

The results of the corneal scans suggest that the image height has a major effect on the pixel intensity for the cornea which may have implications for how the lens cortex images measure and display; particularly that cortex depth may affect overall image intensity.

The LOCS III measurements are all significantly correlated with VA. The strength of the correlation is in line with previous studies of LOCS III measurements and VA (Donnelly et al., 2004; Elliott and Situ, 1998; Wong et al., 2009)

Macular thickness was correlated with VA as has been found in previous studies (Eriksson and Alm, 2009).

The results suggest that none of the quantitative measurements from 5 line raster scans taken with OCT used in his study are good metrics which can be used to grade visually significant cortical opacity.

The standard deviation of the pixel density was significantly correlated with the LOCS III cortical opacity score. This may reflect the greater variability of density found in the cortex of an eye with cortical cataract but this correlation was weak, only accounting for 13% of the variability in the scores and therefore is not a useful grading tool on its own. Furthermore, this metric was not as well correlated with VA as the LOCS III C score. The most significant correlation with VA is the NC score.

This highlights the other issue with measuring one aspect of the cataract in isolation which is that all the subjects also had other forms of cataract which it would appear were affecting their vision as well as, and possibly more than, the cortical cataract. To obtain a reliable metric an overall lens density score may be required, but as yet the only OCT instruments

capable of imaging the whole lens are experimental, such as the instrument used in figure 5.5. Other instruments such as those using the Scheimpflug principal can image the whole crystalline lens simultaneously, but as these lack the capability to be used in as versatile a manner as OCT, it is more likely that OCT technology will advance to the point where whole lens imaging is available to practitioners, rather than Scheimpflug instruments become commonplace.

A larger study might be able to isolate the effect of the C from the NC and NO by controlling for the effect t of the other types of cataract.

The Bland-Altman plots show that there is no systematic error between any of the variables, with the one sided t test showing no significant difference between the difference between the two data sets and zero. However, there is considerable greater variability between the horizontal and vertical centre scans than between the second and third scans of either the horizontal or vertical data sets, with the CR being considerably larger. This may be explained by the nature of cortical cataract which tends to occur in radial 'spokes' which if they intersect with one set of scans but not the other can give a considerable difference in pixel intensity between the horizontal and vertical scans. Part of the variability may also be due to the repositioning of the patient that occurs between the horizontal and vertical scan sets, but not between the individual scans in a single scan set which is likely to lead to greater differences between the two.

This data suggests that the repeatability of the individual scans is good, but the difference between the horizontal and vertical scans can be large. This may be due to the nature of cortical lens opacities, indicating that possibly a 200x200 cube scan of the lens cortex may give more complete results than single raster scans. Unfortunately these scans tend to be

prone to movement artefacts to an extent that the posterior cube scans are not. This is due to greater parallax between the anterior segment and the scanning beam compared to the posterior segment and the lack of a movement correction feature on the anterior segment scans as is applied to the posterior segment.

The images taken provide measurements which appear to be repeatable, within the bounds of the tests performed. It would be useful to establish repeatability with single identical scans with full repositioning to establish how much of the difference between the variability of the horizontal and vertical scans is due to repositioning, and how much is due to genuine differences in lens density that occur as a result of the spoke patterns of the cortical opacities.

There are a number of reasons for the images not providing useful metrics for cataract grading which involve the nature of cortical opacity and the nature of images generated by the OCT. Cortical cataract is often different in nature to nuclear cataract, and this may lead to difficulties with objective grading. As it is usually less uniform than nuclear cataract, the precise placement of the opacities determine the visual effect as well as the density.

The nature of the OCT scan is that a dense opacity will cast a shadow on the tissue below it. This often means that the image below a dense opacity will be darker than it would be without the opacity. This effect can easily be seen on a scan of the retina where blood vessels cast a shadow down to the RPE, and RPE atrophy will lead to brighter scan image of the vessels of the choroid than can be obtained with an intact RPE. This tends to mean that a lens with a dense opacity to the anterior surface will measure less bright on an OCT scan than a lens with identical density but a more posterior opacity.
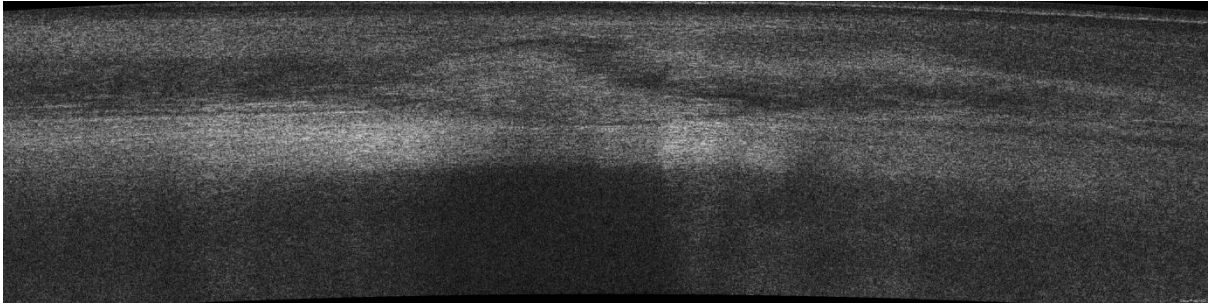
Figure 5.16: a dense central opacity with darker region below caused by shadowing of the scan beam.
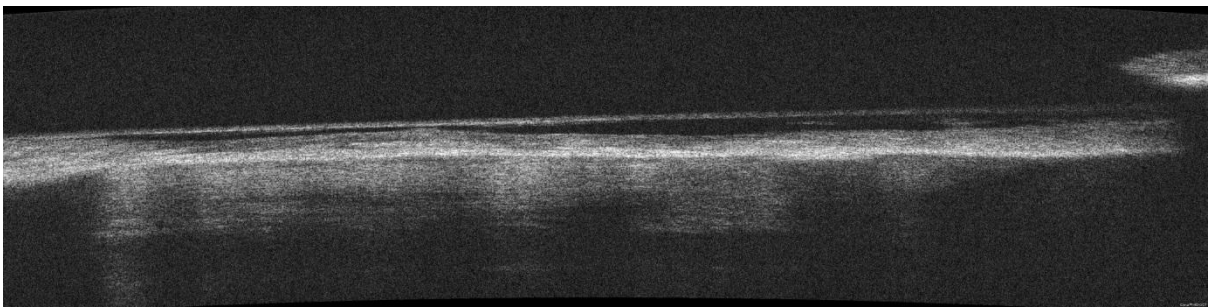


Figure 5.17: shadows cast by a very dense opacity from a white opaque cataract to the extent that the posterior cortex is not visible.
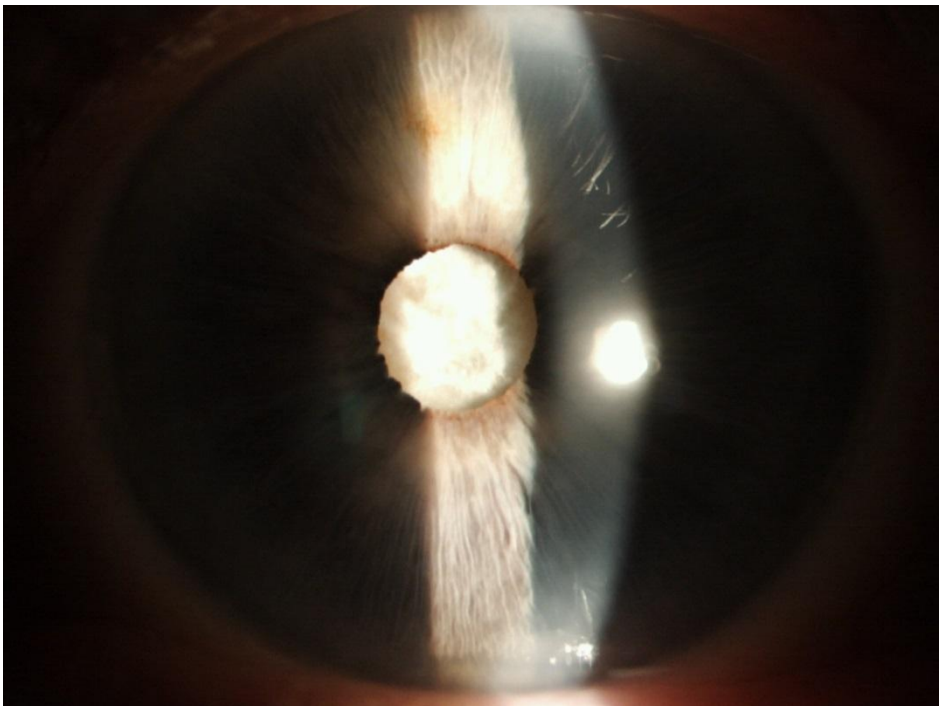


Figure 5.18: the lens in figure 4.17 viewed with a slit lamp.
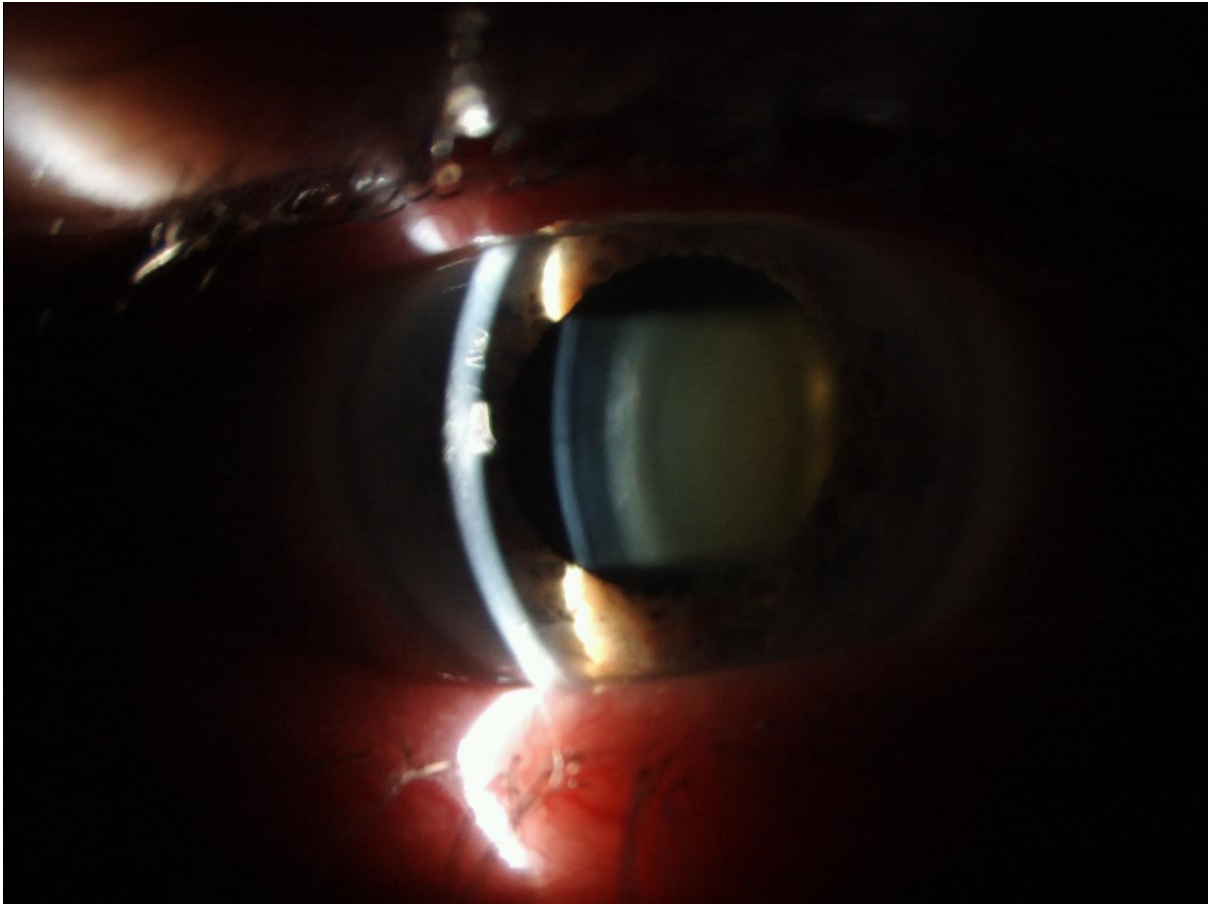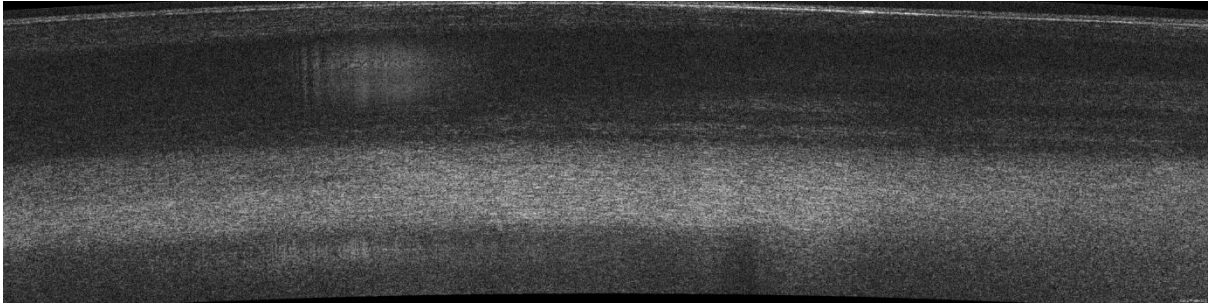
Figure 5.19: a very clear anterior lens which will cause an increase in posterior cortex scan intensity and a slit lamp image of the same lens.

Another issue is the highly variable nature of cortical lens opacities. Nuclear opacities tend to manifest as more or less even increases in lens density, ideal to measure with OCT as previously demonstrated (Wong et al., 2009). cortical opacities can also manifest as even dense opacities, but areas of a lens cortex with cataract can also be very clear, areas known

as water clefts. Both of these will cause visual impairment as light tends to be scattered and subject to aberrations which cannot be corrected optically but very clear areas of lens will not lead to an increase in overall scan density.
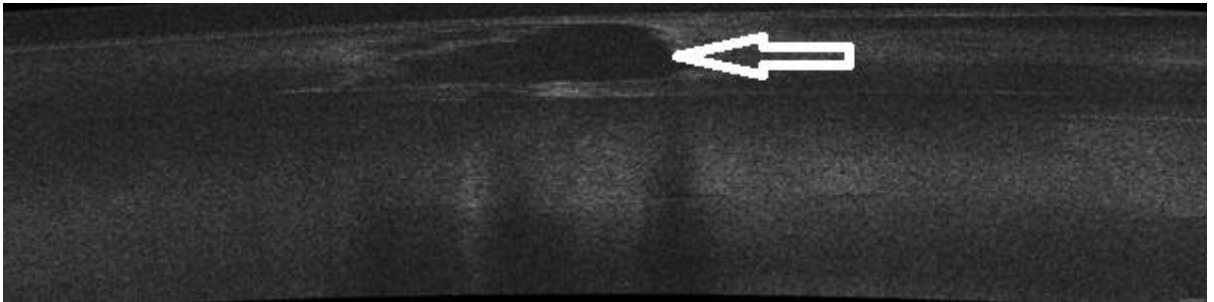


Figure 5.20: water clefts

In addition, any scans which do not pass through the opacity will not record increased density. This is also an issue with other measures of cortical opacities such as the LOCS III grades, which tend also to correlate poorly with the usual measures of vision used in practice.

Another possible confounding factor is the influence of the cornea on the brightness of the scan of the lens cortex. It is possible that anterior chamber depth, corneal reflectivity and corneal thickness influence the scan brightness and that inverted images of the cornea caused by the nature of the Fourier analysis the instrument uses to produce the images could produce artefacts in the cortex images at critical values. The depth of field the Cirrus instrument provides is not sufficient to measure anterior chamber depth so this effect could not be investigated in this study. The issue identified using the corneal sections that the image brightness is heavily dependent on the scan height could potentially overestimate the density of the front of the anterior cortex whilst underestimating the density of the back of

the anterior cortex. Further investigation, possibly using a time domain OCT image as comparison, may reveal the strength of this effect.

OCT measurements of lens density are probably accurate enough to measure differences in cortex density that occur generally with age, but not to distinguish between different degrees of cortical cataract as this causes specific difficulties that are not easily resolved into a single metric. The significant correlation between the standard deviation of the pixel intensity and the cortical cataract scores gives a hint as to how OCT could give some insight into cortical cataract as it provides some measure of the variation in density that is seen on the cross sectional images and causes the local variations in refractive index which can cause aberrations, However, the correlation is too weak to be useful as a grading on its own. OCT is a useful technology for imaging cataract in a non-invasive way to aid clinical decision making in a similar way to slit lamp imaging and produces images which an experienced operator can use to assess cortical cataract in ways which slit lamp imaging can sometimes be difficult, particularly without pupil dilation. This method may yet become useful when instruments become capable of imaging the whole lens as a cube and more complex data analysis can be performed.

Further investigations in this area may include using 200x200 scan cubes to analyse local differences in lens density as this may provide information that is difficult to extract from individual scans. It is more likely to be an analysis of variations in lens density through a single lens rather than absolute variations in density between different lenses which provides an index which can be used to grade visually significant cortical cataracts. Other analysis which might be useful is comparing OCT measurements with other measures of vision such as small letter contrast sensitivity which have been shown previously to correlate

better with cataract scores than high contrast letter acuity such as the snellen or LogMAR

standard.

5.5: Conclusion

This experiment concerned the use of a commercial OCT instrument to grade cortical cataract. It was found that the standard deviation of the pixel intensity was weakly correlated with the LOCS III cortical score. This correlation was statistically significant but not strong enough to be useful as a grading tool.

Ultimately, grading cataract has to consider the lens as a whole if good correlations with vision or symptoms are to be sought. Current OCT instruments on the market are not capable of performing this task. In the near future instruments which can image the entire crystalline lens may become available. This may provide the tools to usefully grade cataract using OCT although objective findings, however accurate, may not account for differences that are often observed in practice to exist in subjective symptoms between those with objectively similar cataracts.

The next chapter will summarise the findings of the experimental chapters and explore the implications for the results and possible future directions for research.

Chapter Six


General Conclusion

The previous three experimental chapters have explored the effect of pseudophakia on MPOD, the relationship between macular thickness and MPOD and the use of OCT to image and grade cortical cataract. This chapter will summarise the reasons the experiments were conducted, the findings of the experiments and possible directions for future research.

In an aging population, age related eye diseases are becoming more common and most people will be affected to some extent by cataract and many by AMD (Evans and Wormald, 1996). Recent advances in IOL technology and an increasing market for elective lens replacement for refractive reasons are also meaning more people are having cataracts removed earlier, and with increasing life expectancy can expect to live longer afterwards.

The implications of this are not fully understood, but research indicates that removing the natural crystalline lens and replacing it with a clear, UV blocking IOL increases the risk of AMD (Chakravarthy et al., 2010). This is likely to be due to the increase in light exposure to the retina and in particular blue light as well as possible activation of inflammatory processes in susceptible people (Cuthbertson et al., 2009). One of the possible mechanisms behind this is degradation of the MP by the increased light exposure, reducing both the barrier protection and antioxidant protection that MPOD is thought to provide.

New instruments to measure MPOD such as desktop HFP instruments and new instruments to measure physical parameters of the macula such as spectral domain OCT can provide insights into the *in vitro* behaviour of the aspects of the macula investigated in this study. Spectral domain OCT can also be used to examine the cataract itself, which provides a novel way to examine this common age related disease with the potential to extract data not

easily available from slit lamp examination. Initial trials to examine the lens cortex with OCT showed that images could be produced of the lens cortex which appeared to correlate well with cross sections of the lens obtained through slit lamp examination, so the question of whether these images could be used to objectively grade cataract in a way which correlate well with other methods of cataract assessment was raised.

At the time this study was devised, few studies had been performed which attempted to compare MPOD in eyes with different levels of light exposure, and those where the two variables had been compared were subject to possible confounding factors. An experimental design which compared MPOD in eyes which were subject to exactly the same conditions save light exposure was proposed, and the one common procedure which is often carried out monocularly and affects retinal light exposure levels is cataract removal. A study was therefore devised to compare levels of MPOD with a procedure which has been proven not to be affected by the removal of cataract in the short term, HFP. Subjects who had one of their cataracts removed were recruited and MPOD compared between the two eyes.

This study design has some potential confounding factors such as there being a pre-operative difference in MPOD or a physical difference between the eyes which leads to both low MPOD and cataract but in general there are different confounders to those in previous studies. This study design therefore offers an original contribution to knowledge as to the differences in MPOD between a phakic eye and a pseudophakic eye

The subjects of these two studies were all being examined with OCT to examine the macula as this is an excellent way to assess macular health and this provides thickness measurements of the macula which can be used as a proxy for macular health, as macular thickness has been previously shown to decline with age and macular disease (Eriksson and

Alm, 2009; Liu et al., 2011). A further investigation was devised to use this data to compare these parameters with MPOD as previous studies in this area have been inconclusive and many had used time domain OCT with a lower resolution than SD-OCT.

The three main questions posed by this study were therefore:

1) Does pseudophakia affect MPOD?

2) Is MPOD correlated with macular thickness?

3) Can SD-OCT be used to measure cortical cataract severity?

Chapter three dealt with the question of whether MPOD is affected by the presumed increased retinal light exposure that results from cataract removal and implantation of a visible light clear IOL. In the time whilst this study has been being conducted, two other studies have asked the same question, albeit using substantially different methods, and have come to the conclusion that MPOD is lower in pseudophakic eyes than the fellow phakic eye, and this study comes to the same conclusion. This represents a substantial body of evidence that MPOD is reduced by pseudophakia. The most likely reason for this effect is the increased light exposure. This outcome makes it likely that reduced MPOD may be in part responsible for the increase in macular degeneration rates previously reported following cataract removal.

There are several implications of this result. The first is that it strengthens the case for using blue blocking IOLs during routine cataract surgery, particularly in patients with signs of AMD and young patients who can expect a considerable lifespan post operatively. Secondly, it strengthens the advice often given postoperatively that eye protection from sunlight is desirable, namely sunhats, sunglasses and general sun avoidance. Thirdly it implies that light

exposure is likely to be a risk factor for macular degeneration regardless of whether the person is pseudophakic and fourthly it indicates that, particularly for those with low dietary carotenoid intake, dietary modification or supplementation may protect against AMD, particularly following a cataract removal.

The use of blue blocking lenses has been controversial with disputes over their use. It has been proposed that blue blocking lenses could affect colour vision, circadian rhythm and scotopic sensitivity (Mainster MA, 2005). These concerns are unlikely to be well founded. A blue blocking IOL still transmits similar levels of blue light as the natural lens of a 50 year old and therefore although relative to a clear IOL the level of transmitted blue light is low, it is in the vast majority of cases going to be higher than the levels of blue light the cataract removed was transmitting (Romano et al., 2011). In addition, one recent study found no difference in scotopic sensitivity or colour vision between the two eyes of subjects who had one blue blocking IOL and one clear IOL (Kara-Junior et al., 2011). The question of whether blue blocking lenses have any benefit remains unanswered and as they are more expensive than clear IOLs the cost-benefit ratio is uncertain. A large scale long term study of AMD progression on those implanted with blue blocking IOLs and clear IOLs would be useful to answer this question. Such a study could be used also to measure the effect of pseudophakia and blue blocking lenses on MPOD, preferably using both psychophysical and objective methods.

The recommendation to use sunhats, sunglasses and L, Z and MZ supplements after a cataract operation also has an uncertain cost benefit ratio, although in this case the costs are borne directly by the subject rather than by the health service provider. Many patients find the increase in light levels after cataract removal necessitate the use of sun protection in any

case. The effect of dietary supplements of L Z and MZ for pseudophakics has not been investigated and therefore although there is a theoretical basis for advising their use any recommendation must be with the caveat that there is a lack of evidence in this area.

There are several directions that this research could potentially lead. The response to supplementation in pseudophakics would be interesting to determine, in order to see if supplementation with L Z and MZ has a greater relative effect on MPOD than it does in phakics. It may be that there is a homeostatic mechanism for replacement of MP but in pseudophakics it is overwhelmed. It would also be interesting to see if the result here is consistent across different genetic groups. There appear to be racial differences in MPOD which could lead to differences in MPOD response to photooxidation or metabolic oxidative stress. It may be that Nolan et al (2009) studied a group of people with a more effective response to degradation of MP than the groups studies by Obana et al (2011) and Demirel et al. (2009) and the group of subjects studies here leading to the different conclusions.

The measurements taken with the OCT and MPS instruments during this study enabled investigation into other possible limiting factors on MPOD such as retinal architecture, which leads into the investigations carried out in chapter three.

In Chapter four the relationship between MT and MPOD was investigated. Current evidence is inconclusive, but this is the first study which has looked at the entire macular area as opposed to just the fovea or central macula where the MP accumulates (see table 2.2). This research indicates that the thickness of the entire macular area is correlated with MPOD, and that all measures of macular thickness decline with age. It did not find that central or foveal macular thickness measurements are significantly correlated with MPOD.

This result indicates that macular thickness and MPOD decline together, but it is unlikely that they are dependent on each other. It is likely that both decline as a function of macular health, possibly as MP uptake is reduced in a retina which is under stress. This could be due to the RPE becoming less efficient at the uptake and transport of MP as the cellular machinery ages. Alternatively greater oxidation in an aging RPE due to lipofuscin accumulation could denature the MP faster, leading to less being present in the retina.

This makes it less likely that the MPOD in the retina is governed by the availability of binding sites but that factors which affect macular thickness also affect the ability of the RPE to transport MP into the retina or people with low serum levels of MP also tend to have thinner maculae. Further investigation is required to determine which of these is the case. The result here must be viewed with the limitations of the study in mind, in particular the small sample size, the multiple comparisons made and the degree of correlation found which was not particularly strong. This investigation has revealed a possible area for further study but cannot be taken as conclusive in isolation. A larger scale study would be required to confirm if the effect found here can be replicated.

Further research in this area is required to understand the factors which govern MPOD levels in the retina and the cause effect relationship between them. Previous research has found that macular width is associated with MPOD differences and that there are differences in the relationship between macular measurements between races (Nolan et al., 2008). The relationship between the variables may be complex and it may be that the limiting factors for MPOD differ between individuals. It is possible that in some the ability to transport MP into the retina is a limiting factor and in others the thickness and distribution of binding sites

is the limiting factor. It may also be the case that the factors which govern MPOD are different in a healthy and a diseased retina.

A long term study of macular thickness in those who have had their cataracts removed and replaced with clear IOLs may give worthwhile insights into changes in macular thickness in those with lower MPOD but it is likely that such studies would need to be conducted in those who have been pseudophakic for more than five years as Kara-Junior et al (2011) have already found no effect over five years. This could be conducted as a longitudinal study, though dropout rates may be an issue, or it could be conducted as a cross sectional or case control study, though problems of confounding factors can be an issue.

The OCT instrument used to obtain the retinal measurements used in this part of the study does so by providing images which are graded on reflectiveness which is used in this case to delineate the retinal layers, as different tissues have different levels of reflectiveness. An important aspect of interest in the design of the study detailed in chapter two is that the optical density of the lens increases with age as cataract develops.

Wong et al (2009) have found that time domain AS-OCT images can be used to grade nuclear cataract density, but no studies have attempted to use SD-OCT designed for posterior segment imaging for this purpose and no studies have attempted to grade cortical cataract using OCT. It was proposed that these instruments could be used to obtain images of the lens cortex and the optical density of these images as displayed by the instrument might be used to grade cortical cataract, and this leads us to chapter four.

Chapter five investigated whether objective grading of cortical cataract could be carried out using a commercial OCT instrument primarily designed for posterior segment imaging, the

Zeiss Cirrus OCT (Zeiss Meditec, Jena, Germany). Cross sectional images of the lens cortex were successfully obtained from a number of subjects with a range of cortical cataract grades and these were compared using image analysis software to conventional grading techniques and to visual acuity measurements. Though the images obtained were repeatable, it was found that the image density correlated poorly with either the LOCS III cortical cataract grade or to the visual acuity of the subject. Several reasons were found for this result, some of which are specific to the type of cataract and some to the properties of SD-OCT. The variability of the density of the lens in cortical cataract presents a challenge when grading as the overall density of the lens may not reflect the effect it has on the vision of the patient. SD-OCT instruments are also prone, as Scheimpflug instruments are, to shadowing from dense opacities affecting the overall lens density.

These difficulties may be overcome as the technology progresses. There is potential for OCT to be a useful technology in the investigation of cataract in the future and it can be used to obtain sectional images through the lens which might be able to be used to chart the change in lens density in an individual over time.

These investigations further our knowledge of the behaviour of MP *in vivo*, the changes that occur in the macular and the lens of the aging eye and the potential and limitations of spectral domain optical coherence tomography as a tool in general optometric practice. They have in this respect accomplished the objectives of the thesis.

There are further research questions that lead from this research. As OCT instrumentation develops, continued investigations into assessment of cataract could prove useful as the limitations of the instruments revealed in this study are overcome. Instruments which obtain images of the whole lens and instruments which correct for the artefacts inherent in SD-OCT

instruments such as the reflected secondary image and the relationship between the scan height and the image brightness would be key to solving the issues identified here.

Further research is needed to establish whether any feedback mechanism for replacement of MP exists and how blue blocking lenses influence the MPOD postoperatively as current research is contradictory. Current research points towards MPOD reducing with both blue blocking and clear IOL implantation with blue blocking lenses reducing this effect but with a limited number of studies so far this is not certain. These investigations may be through the investigation of the cell chemistry of the RPE, larger studies investigating MPOD over longer timescales after cataract removal and investigations into the relative effect of supplementation on those with cataract and those who are pseudophakic. For example, if a feedback between light exposure and MP uptake exists, it might be expected that the effect of supplementation would be greater on a pseudophakic eye than a fellow eye in the same subject with cataract. Long term epidemiological studies which assess light exposure more accurately than retrospective questionnaires might also be useful. These could possibly involving new technology trends such as wearable video cameras to actually measure light exposure over time or comparing MPOD over time in people who move to significantly different latitudes to investigate the relationship between light exposure and MPOD.

Further research into the effect of foveal architecture on MPOD might include larger scale studies into the relationship found here between average macular thickness and MPOD and whether in larger scale studies a significant relationship between foveal and central macular thickness in the same eyes where a change in average thickness is found. It would also be useful to confirm using objective techniques the effects found using HFP here. OCT could be also used to measure specific retinal layers to identify if the thinning observed here occurs in

all retinal layers or if it more specific. Further investigations may also attempt to discover if chronic high light exposure causes retinal thinning or if the effects of light exposure on MPOD and the relationship between MPOD and retinal thickness are independent.

As OCT technology progresses the limitations of the instrument found here in the grading of lens opacities may be overcome to some extent. A scan of the density of the entire thickness of the lens, over the lens area exposed by the pupil may give useful measurements of lens density which may relate to vision. If the disadvantages of current instruments are overcome and future instruments have the ability to measure density irrespective of scan height or shadowing the methods used here might yield more useful results. Investigation of how these measurements correlate to other methods of assessing vision in cataract such as contrast sensitivity, symptoms and glare may also provide some insight into whether OCT can be used to assess patients for cataract removal in an objective way.

Access to new technology in the practice of primary care practitioners such as OCT and desktop MPOD measuring instruments is leading to new ways in which these practitioners can investigate the health of their patients' eyes and advise them on how they might best maintain their vision. It also provides a potential reservoir of information which can be used for research but is currently largely untapped. It is vital that primary care practitioners have up to date information on how procedures like cataract removal may affect the future visual status of their patients.

References:

Ahuja, A.K., Behrend, M.R., 2013. The Argus™ II retinal prosthesis: Factors affecting patient selection for implantation. Prog. Retin. Eye Res. 36, 1–23. doi:10.1016/j.preteyeres.2013.01.002

Al-Hussaini, H., Kam, J.H., Vugler, A., Semo, M., Jeffery, G., 2008. Mature retinal pigment epithelium cells are retained in the cell cycle and proliferate in vivo. Mol. Vis. 14, 1784–1791.

Aleman, T.S., Duncan, J.L., Bieber, M.L., De Castro, E., Marks, D.A., Gardner, L.M., Steinberg, J.D., Cideciyan, A.V., Maguire, M.G., Jacobson, S.G., 2001. Macular pigment and lutein supplementation in retinitis pigmentosa and usher syndrome. Invest. Ophthalmol. Vis. Sci. 42, 1873–1881.

Alfonso, J.F., Fernández-Vega, L., Ortí, S., Montés-Micó, R., 2010. Refractive lens exchange with distance-dominant diffractive bifocal intraocular lens implantation. Graefes Arch. Clin. Exp. Ophthalmol. 248, 1507–1514. doi:10.1007/s00417-010-1345-6

Algvere, P.V., Marshall, J., Seregard, S., 2006. Age-related maculopathy and the impact of blue light hazard. Acta Ophthalmol. Scand. 84, 4–15. doi:10.1111/j.1600-0420.2005.00627.x

Alves-Rodrigues, A., Shao, A., 2004. The science behind lutein. Toxicol. Lett. 150, 57–83. doi:10.1016/j.toxlet.2003.10.031

Ambati, J., Ambati, B.K., Yoo, S.H., Ianchulev, S., Adamis, A.P., 2003. Age-Related Macular Degeneration: Etiology, Pathogenesis, and Therapeutic Strategies. Surv. Ophthalmol. 48, 257–293. doi:10.1016/S0039-6257(03)00030-4

Azuara-Blanco, A., Katz, L.J., Spaeth, G.L., Vernon, S.A., Spencer, F., Lanzl, I.M., 2003. Clinical agreement among glaucoma experts in the detection of glaucomatous changes of the optic disk using simultaneous stereoscopic photographs. Am. J. Ophthalmol. 136, 949–950. doi:10.1016/S0002-9394(03)00480-X

Bakri, S.J., Kaiser, P.K., 2004. Verteporfin ocular photodynamic therapy. Expert Opin. Pharmacother. 5, 195–203. doi:10.1517/14656566.5.1.195

Barker, F.M., Snodderly, D.M., Johnson, E.J., Schalch, W., Koepcke, W., Gerss, J., Neuringer, M., 2011. Nutritional Manipulation of Primate Retinas, V: Effects of Lutein, Zeaxanthin, and n−3 Fatty Acids on Retinal Sensitivity to Blue-Light–Induced Damage. Invest. Ophthalmol. Vis. Sci. 52, 3934–3942. doi:10.1167/iovs.10-5898

Bartlett, H., Acton, J., Eperjesi, F., 2010a. Clinical evaluation of the MacuScope macular pigment densitometer. Br. J. Ophthalmol. 94, 328–331. doi:10.1136/bjo.2009.167213

Bartlett, H., Eperjesi, F., 2004a. An ideal ocular nutritional supplement? *. Ophthalmic Physiol. Opt. July 2004 24, 339–349.

Bartlett, H., Eperjesi, F., 2004b. Dietary analysis and patterns of nutritional supplement use in normal and age-related macular disease affected subjects: a prospective cross-sectional study. Nutr. J. 3, 16. doi:10.1186/1475-2891-3-16

Bartlett, H., Eperjesi, F., 2005. Possible contraindications and adverse reactions associated with the use of ocular nutritional supplements. Ophthalmic Physiol. Opt. May 2005 25, 179–194.

Bartlett, H., Eperjesi, F., 2007. Use of Fundus Imaging in Quantification of Age-related Macular Change. Surv. Ophthalmol. 52, 655–671. doi:10.1016/j.survophthal.2007.08.022

Bartlett, H., Stainer, L., Singh, S., Eperjesi, F., Howells, O., 2010b. Clinical evaluation of the MPS 9000 Macular Pigment Screener. Br. J. Ophthalmol. 94, 753–756. doi:10.1136/bjo.2009.175901

Bartlett, H.E., Eperjesi, F., 2007. Effect of lutein and antioxidant dietary supplementation on contrast sensitivity in age-related macular disease: a randomized controlled trial. Eur. J. Clin. Nutr. 61, 1121–1127. doi:10.1038/sj.ejcn.1602626

Beatty, S., Boulton, M., Henson, D., Koh, H.-H., Murray, I.J., 1999. Macular pigment and age related macular degeneration. Br. J. Ophthalmol. 83, 867–877. doi:10.1136/bjo.83.7.867

Beatty, S., Murray, I.J., Henson, D.B., Carden, D., Koh, H.-H., Boulton, M.E., 2001. Macular Pigment and Risk for Age-Related Macular Degeneration in Subjects from a Northern European Population. Invest. Ophthalmol. Vis. Sci. 42, 439–446.

Berendschot, T.T.J.., DeLint, P.J., Norren, D. van, 2003. Fundus reflectance—historical and present ideas. Prog. Retin. Eye Res. 22, 171–200. doi:10.1016/S1350-9462(02)00060-5

Berendschot, T.T.J.M., Goldbohm, R.A., Klöpping, W.A.A., Kraats, J. van de, Norel, J. van, Norren, D. van, 2000. Influence of Lutein Supplementation on Macular Pigment, Assessed with Two Objective Techniques. Invest. Ophthalmol. Vis. Sci. 41, 3322–3326.

Berendschot TM, Broekmans WR, Klöpping-Ketelaars IA, Kardinaal AM, van Poppel G, van Norren D, 2002. Lens aging in relation to nutritional determinants and possible risk factors for age-related cataract. Arch. Ophthalmol. 120, 1732–1737. doi:10.1001/archopht.120.12.1732

Bernstein, P.S., Ahmed, F., Liu, A., Allman, S., Sheng, X., Sharifzadeh, M., Ermakov, I., Gellermann, W., 2012. Macular Pigment Imaging in AREDS2 Participants: An Ancillary Study of AREDS2 Subjects Enrolled at the Moran Eye Center. Invest. Ophthalmol. Vis. Sci. 53, 6178–6186. doi:10.1167/iovs.12-10275

Berrow, E.J., Bartlett, H.E., Eperjesi, F., 2011. Do lutein, zeaxanthin and macular pigment optical density differ with age or age-related maculopathy? E-SPEN Eur. E-J. Clin. Nutr. Metab. 6, e197–e201. doi:10.1016/j.eclnm.2011.05.003

Bhosale, P., Bernstein, P.S., 2005. Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation. Biochim. Biophys. Acta BBA - Mol. Basis Dis. 1740, 116–121. doi:10.1016/j.bbadis.2005.02.002

Bhosale, P., Larson, A.J., Frederick, J.M., Southwick, K., Thulin, C.D., Bernstein, P.S., 2004. Identification and Characterization of a Pi Isoform of Glutathione S-Transferase (GSTP1) as a Zeaxanthin-binding Protein in the Macula of the Human Eye. J. Biol. Chem. 279, 49447–49454. doi:10.1074/jbc.M405334200

Bhutto, I.A., McLeod, D.S., Hasegawa, T., Kim, S.Y., Merges, C., Tong, P., Lutty, G.A., 2006. Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration. Exp. Eye Res. 82, 99–110. doi:10.1016/j.exer.2005.05.007

Bird, A.C., Bressler, N.M., Bressler, S.B., Chisholm, I.H., Coscas, G., Davis, M.D., de Jong, P.T.V.M., Klaver, C.C.W., Klein, B.E.K., Klein, R., Mitchell, P., Sarks, J.P., Sarks, S.H., Soubrane, G., Taylor, H.R., Vingerling, J.R., 1995a. An international classification and grading system for age-related maculopathy and age-related macular degeneration. Surv. Ophthalmol. 39, 367–374. doi:10.1016/S0039-6257(05)80092-X

Bird, A.C., Bressler, N.M., Bressler, S.B., Chisholm, I.H., Coscas, G., Davis, M.D., de Jong, P.T.V.M., Klaver, C.C.W., Klein, B.E.K., Klein, R., Mitchell, P., Sarks, J.P., Sarks, S.H., Soubrane, G., Taylor, H.R., Vingerling, J.R., 1995b. An international classification and grading system for age-related maculopathy and age-related macular degeneration. Surv. Ophthalmol. 39, 367–374. doi:10.1016/S0039-6257(05)80092-X

Blackmore-Wright, S., Eperjesi, F., 2012. Blue-light Filtering Intraocular Lenses. Eur. Ophthalmic Rev. 6, 104–107.

Bochow TW, W.S., 1989. Ultraviolet light exposure and risk of posterior subcapsular cataracts. Arch. Ophthalmol. 107, 369–372. doi:10.1001/archopht.1989.01070010379027

Bone, R.A., Landrum, J.T., Cao, Y., Howard, A.N., Alvarez-Calderon, F., 2007. Macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. Nutr. Metab. 4, 12. doi:10.1186/1743-7075-4-12

Bone, R.A., Landrum, J.T., Dixon, Z., Chen, Y., Llerena, C.M., 2000. Lutein and Zeaxanthin in the Eyes, Serum and Diet of Human Subjects. Exp. Eye Res. 71, 239–245. doi:10.1006/exer.2000.0870

Bone, R.A., Landrum, J.T., Friedes, L.M., Gomez, C.M., Kilburn, M.D., Menendez, E., Vidal, I., Wang, W., 1997. Distribution of lutein and zeaxanthin stereoisomers in the human retina. Exp. Eye Res. 64, 211–218. doi:10.1006/exer.1996.0210

Bone, R.A., Landrum, J.T., Hime, G.W., Cains, A., Zamor, J., 1993. Stereochemistry of the human macular carotenoids. Invest. Ophthalmol. Vis. Sci. 34, 2033–2040.

Bone, R.A., Landrum, J.T., Mayne, S.T., Gomez, C.M., Tibor, S.E., Twaroska, E.E., 2001. Macular pigment in donor eyes with and without AMD: A case-control study. Invest. Ophthalmol. Vis. Sci. 42, 235–240.

Bone, R.A., Landrum, J.T., Tarsis, S.L., 1985. Preliminary identification of the human macular pigment. Vision Res. 25, 1531–1535. doi:10.1016/0042-6989(85)90123-3

Bosma, T.L., Dole, J.M., Maness, N.O., 2003. Optimizing Marigold ( L.) Petal and Pigment Yield. Crop Sci. 43, 2118. doi:10.2135/cropsci2003.2118

Boulton, M., Rózanowska, M., Rózanowski, B., 2001. Retinal photodamage. J. Photochem. Photobiol. B 64, 144–161. doi:10.1016/S1011-1344(01)00227-5

Bressler, N., Bressler, S., West, S., Fine, S., Taylor, H., 1989. The grading and prevalence of macular degeneration in Chesapeake Bay watermen. Arch. Ophthalmol. 107, 847–852.

Bressler, S.B., 2009. Introduction: Understanding the role of angiogenesis and antiangiogenic agents in age-related macular degeneration. Ophthalmology 116, S1–7. doi:10.1016/j.ophtha.2009.06.045

Brezinski, M.E., Tearney, G.J., Bouma, B.E., Izatt, J.A., Hee, M.R., Swanson, E.A., Southern, J.F., Fujimoto, J.G., 1996. Optical Coherence Tomography for Optical Biopsy Properties and Demonstration of Vascular Pathology. Circulation 93, 1206–1213. doi:10.1161/01.CIR.93.6.1206

Brown, N.A., Hill, A.R., 1987. Cataract: the relation between myopia and cataract morphology. Br. J. Ophthalmol. 71, 405–414. doi:10.1136/bjo.71.6.405

Chakravarthy, U., Wong, T.Y., Fletcher, A., Piault, E., Evans, C., Zlateva, G., Buggage, R., Pleil, A., Mitchell, P., 2010. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. BMC Ophthalmol. 10, 31. doi:10.1186/1471-2415-10-31

Chan A, D.J., 2006. Normal macular thickness measurements in healthy eyes using stratus optical coherence tomography. Arch. Ophthalmol. 124, 193–198. doi:10.1001/archopht.124.2.193

Charman, W.N., 2003. Age, lens transmittance, and the possible effects of light on melatonin suppression. Ophthalmic Physiol. Opt. 23, 181–187. doi:10.1046/j.1475-1313.2003.00105.x

Chew, E.Y., Clemons, T.E., Agrón, E., Sperduto, R.D., Sangiovanni, J.P., Kurinij, N., Davis, M.D., Age-Related Eye Disease Study Research Group, 2013. Long-Term Effects of Vitamins C and E, β-Carotene, and Zinc on Age-Related Macular Degeneration: AREDS Report No. 35. Ophthalmology. doi:10.1016/j.ophtha.2013.01.021

Chew, E.Y., Sperduto, R.D., Milton, R.C., Clemons, T.E., Gensler, G.R., Bressler, S.B., Klein, R., Klein, B.E.K., Ferris III, F.L., 2009. Risk of Advanced Age-Related Macular Degeneration after Cataract Surgery in the Age-Related Eye Disease Study: AREDS Report 25. Ophthalmology 116, 297–303. doi:10.1016/j.ophtha.2008.09.019

Chua, B.E., Mitchell, P., Cumming, R.G., 2004. Effects of cataract type and location on visual function: The Blue Mountains Eye Study. Eye 18, 765–772. doi:10.1038/sj.eye.6701366

Chucair, A.J., Rotstein, N.P., SanGiovanni, J.P., During, A., Chew, E.Y., Politi, L.E., 2007. Lutein and Zeaxanthin Protect Photoreceptors from Apoptosis Induced by Oxidative Stress: Relation with Docosahexaenoic Acid. Invest. Ophthalmol. Vis. Sci. 48, 5168–5177. doi:10.1167/iovs.07-0037

Chung, H.-Y., Rasmussen, H.M., Johnson, E.J., 2004. Lutein Bioavailability Is Higher from Lutein-Enriched Eggs than from Supplements and Spinach in Men. J. Nutr. 134, 1887–1893.

Chylack, L.T., Wolfe, J.K., Singer, D.M., Leske, M.C., Bullimore, M.A., Bailey, I.L., Friend, J., Mcarthy, D., Wu, S.-Y., 1993. The Lens Opacities Classification System III. Arch. Ophthalmol. 111, 831–836.

Ciulla, T.A., Hammond, B.R., Yung, C.W., Pratt, L.M., 2001. Macular Pigment Optical Density before and after Cataract Extraction. Invest. Ophthalmol. Vis. Sci. 42, 1338–1341.

Congdon NG, F.D., 2003. Important causes of visual impairment in the world today. JAMA 290, 2057–2060. doi:10.1001/jama.290.15.2057

Connolly, E., Beatty, S., Thurnham, D., Loughman, J., Howard, A., Stack, J., Nolan, J., 2010. Augmentation of Macular Pigment Following Supplementation with all Three Macular Carotenoids: an Exploratory Study. Articles.

Connolly, E.E., Beatty, S., Loughman, J., Howard, A.N., Louw, M.S., Nolan, J.M., 2011. Supplementation with All Three Macular Carotenoids: Response, Stability, and Safety. Invest. Ophthalmol. Vis. Sci. 52, 9207–9217. doi:10.1167/iovs.11-8025

Cook, C.D., Stulting, A.A., 1995. Prevalence and incidence of blindness due to age-related cataract in the rural areas of South Africa. South Afr. Med. J. Suid-Afr. Tydskr. Vir Geneeskd. 85, 26–27.

Cruickshanks, K.J., Klein, B.E., Klein, R., 1992. Ultraviolet light exposure and lens opacities: the Beaver Dam Eye Study. Am. J. Public Health 82, 1658–1662. doi:10.2105/AJPH.82.12.1658

Curcio, C.A., Allen, K.A., 1990. Topography of ganglion cells in human retina. J. Comp. Neurol. 300, 5–25. doi:10.1002/cne.903000103

Cuthbertson, F.M., Peirson, S.N., Wulff, K., Foster, R.G., Downes, S.M., 2009. Blue light–filtering intraocular lenses: Review of potential benefits and side effects. J. Cataract Refract. Surg. 35, 1281–1297. doi:10.1016/j.jcrs.2009.04.017

Datiles III, M.B., Ansari, R.R., Reed, G.F., 2002. A Clinical Study of the Human Lens with a Dynamic Light Scattering Device. Exp. Eye Res. 74, 93–102. doi:10.1006/exer.2001.1106

Davison, J.A., Patel, A.S., Cunha, J.P., Schwiegerling, J., Muftuoglu, O., 2011. Recent studies provide an updated clinical perspective on blue light-filtering IOLs. Graefes Arch. Clin. Exp. Ophthalmol. 249, 957–968. doi:10.1007/s00417-011-1697-6

De Kinkelder, R., van der Veen, R.L.P., Verbaak, F.D., Faber, D.J., van Leeuwen, T.G., Berendschot, T.T.J.M., 2011. Macular pigment optical density measurements: evaluation of a device using heterochromatic flicker photometry. Eye 25, 105–112. doi:10.1038/eye.2010.164

Delahunt, P.B., Webster, M.A., Ma, L., Werner, J.S., 2004. Long-term renormalization of chromatic mechanisms following cataract surgery. Vis. Neurosci. 21, 301–307. doi:10.1017/S0952523804213025

Delcourt C, C.I., 2000. Light exposure and the risk of cortical, nuclear, and posterior subcapsular cataracts: The pathologies oculaires liées à l'age (pola) study. Arch. Ophthalmol. 118, 385–392. doi:10.1001/archopht.118.3.385

Delcourt C, Carrière I, Ponton-Sanchez A, et al, 2001. Light exposure and the risk of age-related macular degeneration: The pathologies oculaires liées à l'age (pola) study. Arch. Ophthalmol. 119, 1463–1468. doi:10.1001/archopht.119.10.1463

Delcourt, C., Carrière, I., Delage, M., Barberger-Gateau, P., Schalch, W., 2006. Plasma Lutein and Zeaxanthin and Other Carotenoids as Modifiable Risk Factors for Age-Related Maculopathy and Cataract: The POLA Study. Invest. Ophthalmol. Vis. Sci. 47, 2329–2335. doi:10.1167/iovs.05-1235

Delori, F.C., Fleckner, M.R., Goger, D.G., Weiter, J.J., Dorey, C.K., 2000. Autofluorescence Distribution Associated with Drusen in Age-Related Macular Degeneration. Invest. Ophthalmol. Vis. Sci. 41, 496–504.

Delori, F.C., Goger, D.G., Hammond, B.R., Snodderly, D.M., Burns, S.A., 2001. Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. J. Opt. Soc. Am. A 18, 1212–1230. doi:10.1364/JOSAA.18.001212

Demirel, S., Bilici, S., Batıoglu, F., Ozmert, E., 2013. The effect of age and cataract surgery on macular pigment optic density: a cross-sectional, comparative study. Graefes Arch. Clin. Exp. Ophthalmol. 1–6. doi:10.1007/s00417-013-2424-2

Dennison, J.L., Stack, J., Beatty, S., Nolan, J.M., 2013. Concordance of macular pigment measurements obtained using customized heterochromatic flicker photometry, dual-

wavelength autofluorescence, and single-wavelength reflectance. Exp. Eye Res. 116, 190–198. doi:10.1016/j.exer.2013.08.014

DiCarlo, C.D., Roach, W.P., Gagliano, D.A., Boppart, S.A., Hammer, D.X., Cox, A.B., Fujimoto, J.G., 1999. Comparison of Optical Coherence Tomography Imaging of Cataracts With Histopathology. J. Biomed. Opt. 4, 450–458. doi:10.1117/1.429951

Dietzel, M., Pauleikhoff, D., Holz, F.G., Bird, A.C., 2013. Early AMD, in: Holz, F.G., Pauleikhoff, D., Spaide, R.F., Bird, A.C. (Eds.), Age-Related Macular Degeneration. Springer Berlin Heidelberg, pp. 101–109.

Dillon, J., Wang, R.-H., Atherton, S.J., 1990. Photochemical and Photophysical Studies on Human Lens Constituents. Photochem. Photobiol. 52, 849–854. doi:10.1111/j.1751-1097.1990.tb08692.x

Donnelly, W.J., Pesudovs, K., Marsack, J.D., Sarver, E.J., Applegate, R.A., 2004. Quantifying scatter in Shack-Hartmann images to evaluate nuclear cataract. J. Refract. Surg. 20, S515–S522.

Dorey, C.K., Wu, G., Ebenstein, D., Garsd, A., Weiter, J.J., 1989. Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. Invest. Ophthalmol. Vis. Sci. 30, 1691–1699.

Drasdo, N., Millican, C.L., Katholi, C.R., Curcio, C.A., 2007. The length of Henle fibers in the human retina and a model of ganglion receptive field density in the visual field. Vision Res. 47, 2901–2911. doi:10.1016/j.visres.2007.01.007

Drexler W, Sattmann H, Hermann B, et al, 2003. ENhanced visualization of macular pathology with the use of ultrahigh-resolution optical coherence tomography. Arch. Ophthalmol. 121, 695–706. doi:10.1001/archopht.121.5.695

Dubbelman, M., Van der Heijde, G.L., 2001. The shape of the aging human lens: curvature, equivalent refractive index and the lens paradox. Vision Res. 41, 1867–1877. doi:10.1016/S0042-6989(01)00057-8

Dubbelman, M., Van der Heijde, G.L., Weeber, H.A., Vrensen, G.F.J.M., 2003. Changes in the internal structure of the human crystalline lens with age and accommodation. Vision Res. 43, 2363–2375. doi:10.1016/S0042-6989(03)00428-0

Dubis, A.M., Hansen, B.R., Cooper, R.F., Beringer, J., Dubra, A., Carroll, J., 2012. Relationship between the Foveal Avascular Zone and Foveal Pit Morphology. Invest. Ophthalmol. Vis. Sci. 53, 1628–1636. doi:10.1167/iovs.11-8488

Duncan, J.L., Aleman, T.S., Gardner, L.M., De Castro, E., Marks, D.A., Emmons, J.M., Bieber, M.L., Steinberg, J.D., Bennett, J., Stone, E.M., MacDonald, I.M., Cideciyan, A.V., Maguire, M.G., Jacobson, S.G., 2002. Macular pigment and lutein supplementation in choroideremia. Exp. Eye Res. 74, 371–381. doi:10.1006/exer.2001.1126

Dunkers, J.P., Parnas, R.S., G. Zimba, C., Peterson, R.C., Flynn, K.M., Fujimoto, J.G., Bouma, B.E., 1999. Optical coherence tomography of glass reinforced polymer composites. Compos. Part Appl. Sci. Manuf. 30, 139–145. doi:10.1016/S1359-835X(98)00084-0

Elliott, D.B., Situ, P., 1998. Visual acuity versus letter contrast sensitivity in early cataract. Vision Res. 38, 2047–2052. doi:10.1016/S0042-6989(97)00382-9

Eriksson, U., Alm, A., 2009. Macular thickness decreases with age in normal eyes: a study on the macular thickness map protocol in the Stratus OCT. Br. J. Ophthalmol. 93, 1448–1452. doi:10.1136/bjo.2007.131094

Ermakov, I.V., Ermakova, M.R., McClane, R.W., Gellermann, W., 2001. Resonance Raman detection of carotenoid antioxidants in living human tissues. Opt. Lett. 26, 1179–1181. doi:10.1364/OL.26.001179

Evans, J., Wormald, R., 1996. Is the incidence of registrable age-related macular degeneration increasing? Br. J. Ophthalmol. 80, 9–14. doi:10.1136/bjo.80.1.9

Family, F., Mazzitello, K.I., Arizmendi, C.M., Grossniklaus, H.E., 2011. Dynamic Scaling of Lipofuscin Deposition in Aging Cells. J. Stat. Phys. 144, 332–343. doi:10.1007/s10955-011-0178-y

Feigl, B., 2009. Age-related maculopathy – Linking aetiology and pathophysiological changes to the ischaemia hypothesis. Prog. Retin. Eye Res. 28, 63–86. doi:10.1016/j.preteyeres.2008.11.004

Ferrini, R.L., Perlman, M., Hill, L., 1998. American college of preventive medicine practice policy statement: Skin protection from ultraviolet light exposure. Am. J. Prev. Med. 14, 83–86. doi:10.1016/S0749-3797(97)00006-8

Fleming, P.A., Braekevelt, C.R., Harman, A.M., Beazley, L.D., 1996. Retinal pigment epithelium and photoreceptor maturation in a wallaby, the Quokka. J. Comp. Neurol. 370, 47–60. doi:10.1002/(SICI)1096-9861(19960617)370:1<47::AID-CNE5>3.0.CO;2-L

Freund, K.B., -Van Ho, I., Barbazetto, I.A., Koizumi, H., Laud, K., Ferrara, D., Matsumoto, Y., Sorenson, J.A., Yannuzzi, L., 2008. TYPE 3 NEOVASCULARIZATION. Retina 28, 201–211. doi:10.1097/IAE.0b013e3181669504

Friedman, D.S., Katz, J., Bressler, N.M., Rahmani, B., Tielsch, J.M., 1999. Racial differences in the prevalence of age-related macular degeneration: The Baltimore eye survey. Ophthalmology 106, 1049–1055. doi:10.1016/S0161-6420(99)90267-1

Fujikado, T., Kuroda, T., Maeda, N., Ninomiya, S., Goto, H., Tano, Y., Oshika, T., Hirohara, Y., Mihashi, T., 2004. Light scattering and optical aberrations as objective parameters to predict visual deterioration in eyes with cataracts. J. Cataract Refract. Surg. 30, 1198–1208. doi:10.1016/j.jcrs.2003.12.023

Garcia-Martin, E., Pinilla, I., Idoipe, M., Fuertes, I., Pueyo, V., 2011. Intra and interoperator reproducibility of retinal nerve fibre and macular thickness measurements using Cirrus Fourier-domain OCT. Acta Ophthalmol. (Copenh.) 89, e23–e29. doi:10.1111/j.1755-3768.2010.02045.x

Gartner, S., Henkind, P., 1981. Aging and degeneration of the human macula. 1. Outer nuclear layer and photoreceptors. Br. J. Ophthalmol. 65, 23–28. doi:10.1136/bjo.65.1.23

Giani, A., Cigada, M., Choudhry, N., Deiro, A.P., Oldani, M., Pellegrini, M., Invernizzi, A., Duca, P., Miller, J.W., Staurenghi, G., 2010. Reproducibility of Retinal Thickness Measurements on Normal and Pathologic Eyes by Different Optical Coherence Tomography Instruments. Am. J. Ophthalmol. 150, 815–824.e1. doi:10.1016/j.ajo.2010.06.025

Glasser, A., Campbell, M.C.W., 1998. Presbyopia and the optical changes in the human crystalline lens with age. Vision Res. 38, 209–229. doi:10.1016/S0042-6989(97)00102-8

Göbel, W., Hartmann, F., Haigis, W., 2001. [Determination of retinal thickness in relation to the age and axial length using optical coherence tomography]. Ophthalmol. Z. Dtsch. Ophthalmol. Ges. 98, 157–162.

Gorrand, J.M., 1979. Diffusion of the human retina and quality of the optics of the eye on the fovea and the peripheral retina. Vision Res. 19, 907–912. doi:10.1016/0042-6989(79)90025-7

Gouras, P., Ivert, L., Mattison, J.A., Ingram, D.K., Neuringer, M., 2008. Drusenoid maculopathy in rhesus monkeys: autofluorescence, lipofuscin and drusen pathogenesis. Graefes Arch. Clin. Exp. Ophthalmol. 246, 1403–1411. doi:10.1007/s00417-008-0911-7

Haila, K.M., Lievonen, S.M., Heinonen, M.I., 1996. Effects of Lutein, Lycopene, Annatto, and γ-Tocopherol on Autoxidation of Triglycerides. J. Agric. Food Chem. 44, 2096–2100. doi:10.1021/jf9504935

Hammond, B.R., Caruso–Avery, M., 2000. Macular Pigment Optical Density in a Southwestern Sample. Invest. Ophthalmol. Vis. Sci. 41, 1492–1497.

Hammond, B.R., Jr, Wooten, B.R., Snodderly, D.M., 1997. Density of the human crystalline lens is related to the macular pigment carotenoids, lutein and zeaxanthin. Optom. Vis. Sci. Off. Publ. Am. Acad. Optom. 74, 499–504.

Hammond, B.R., Wooten, B.R., Snodderly, D.M., 1998. Preservation of visual sensitivity of older subjects: association with macular pigment density. Invest. Ophthalmol. Vis. Sci. 39, 397–406.

Hammond, J., Fuld, K., Snodderly, M.D., 1996. Erratum: Iris Color and Macula Pigment Optical Density. Exp. Eye Res. 62, 713–720. doi:10.1006/exer.1996.0082

Hammond Jr., B.R., Wooten, B.R., Snodderly, D.M., 1997. Individual variations in the spatial profile of human macular pigment. J. Opt. Soc. Am. A Opt. Image Sci. Vis. 14, 1187–1196.

Ho, A.C., Maguire, M.G., Yoken, J., Lee, M.S., Shin, D.S., Javornik, N.B., Fine, S.L., 1999. Laser-induced drusen reduction improves visual function at 1 year. Ophthalmology 106, 1367–1374. doi:10.1016/S0161-6420(99)00735-6

Ho, J., Sull, A.C., Vuong, L.N., Chen, Y., Liu, J., Fujimoto, J.G., Schuman, J.S., Duker, J.S., 2009. Assessment of Artifacts and Reproducibility across Spectral- and Time-Domain Optical Coherence Tomography Devices. Ophthalmology 116, 1960–1970. doi:10.1016/j.ophtha.2009.03.034

Hoerauf, H., Gordes, R., Scholz, C., Wirbelauer, C., Koch, P., R, E., J, W., H, L., R, B., 1999. First experimental and clinical results with transscleral optical coherence tomography. Ophthalmic Surg. Lasers 31, 218–222.

Holz FG, S.G., 1994. ANalysis of lipid deposits extracted from human macular and peripheral bruch's membrane. Arch. Ophthalmol. 112, 402–406. doi:10.1001/archopht.1994.01090150132035

Horgan, N., Condon, P.I., Beatty, S., 2005. Refractive lens exchange in high myopia: long term follow up. Br. J. Ophthalmol. 89, 670–672. doi:10.1136/bjo.2004.052720

Howells, O., Eperjesi, F., Bartlett, H., 2011. Measuring macular pigment optical density in vivo: a review of techniques. Graefes Arch. Clin. Exp. Ophthalmol. 249, 315–347. doi:10.1007/s00417-010-1577-5

Howells, O., Eperjesi, F., Bartlett, H., 2013a. Macular Pigment Optical Density in Young Adults of South Asian Origin. Invest. Ophthalmol. Vis. Sci. 54, 2711–2719. doi:10.1167/iovs.12-10957

Howells, O., Eperjesi, F., Bartlett, H., 2013b. Improving the repeatability of heterochromatic flicker photometry for measurement of macular pigment optical density. Graefes Arch. Clin. Exp. Ophthalmol. 251, 871–880. doi:10.1007/s00417-012-2127-0

Huang, D., Swanson, E.A., Lin, C.P., Schuman, J.S., Stinson, W.G., Chang, W., Hee, M.R., Flotte, T., Gregory, K., Puliafito, C.A., Et, A., 1991. Optical coherence tomography. Science 254, 1178–1181. doi:10.1126/science.1957169

Huang, J., Liu, X., Wu, Z., Guo, X., Xu, H., Dustin, L., Sadda, S., 2011. Macular and Retinal Nerve Fiber Layer Thickness Measurements in Normal Eyes With the Stratus OCT, the Cirrus HD-OCT, and the Topcon 3D OCT-1000. J. Glaucoma 20, 118–125. doi:10.1097/IJG.0b013e3181d786f8

Iannaccone, A., Mura, M., Gallaher, K.T., Johnson, E.J., Todd, W.A., Kenyon, E., Harris, T.L., Harris, T., Satterfield, S., Johnson, K.C., Kritchevsky, S.B., 2007. Macular Pigment Optical Density in the Elderly: Findings in a Large Biracial Midsouth Population Sample. Invest. Ophthalmol. Vis. Sci. 48, 1458–1465. doi:10.1167/iovs.06-0438

Igras, E., Loughman, J., Ratzlaff, M., O'Caoimh, R., O'Brien, C., 2013. Evidence of lower macular pigment optical density in chronic open angle glaucoma. Br. J. Ophthalmol. bjophthalmol–2013–303153. doi:10.1136/bjophthalmol-2013-303153

Izatt, J., Hee, M., Swanson, E., Lin, C., Huang, D., Schuman, J., Puliafito, C., Fujimoto, J., 1994. Micrometer-scale resolution imaging of the anterior eye in vivo with optical coherence tomography. Arch. Ophthalmol. 112, 1584–1589.

Jager, R.D., Mieler, W.F., Miller, J.W., 2008. Age-Related Macular Degeneration. N. Engl. J. Med. 358, 2606–2617. doi:10.1056/NEJMra0801537

Jonas, J.B., Schneider, U., Naumann, G.O.H., 1992. Count and density of human retinal photoreceptors. Graefes Arch. Clin. Exp. Ophthalmol. 230, 505–510. doi:10.1007/BF00181769

Kanis, M.J., Berendschot, T.T.J.M., van Norren, D., 2007. Interocular agreement in melanin and macular pigment optical density. Exp. Eye Res. 84, 934–938. doi:10.1016/j.exer.2007.01.020

Kara-Junior, N., Espindola, R.F., Gomes, B.A.F., Ventura, B., Smadja, D., Santhiago, M.R., 2011. Effects of blue light–filtering intraocular lenses on the macula, contrast sensitivity, and color vision after a long-term follow-up. J. Cataract Refract. Surg. 37, 2115–2119. doi:10.1016/j.jcrs.2011.06.024

Kassoff, A., Kassoff, J., Buehler, J., Eglow, M., Kaufman, F., Mehu, M., Kieval, S., Mairs, M., Graig, B., Quattrocchi, A., Jones, D., Locatelli, J., Ruby, A., Capon A., J., Garretson, B., Hassan, T., Trese,

M.T., Williams, G.A., Regan, V., Manatrey, P., Streasick, P., Szydlowski, L., McIver, F., Bridges, C., Stanley, C., Cumming, K., Lewis, B., Zajechowski, M., Margherio, R.R., Cox, M.S., Werner, J.C., Falk, R., Siedlak, P., Neubert, C., Klein, M.L., Stout, J.T., O'Malley, A., Lauer, A.K., Robertson, J.E., Wilson, D.J., Beardsley, C., Anderson, H., Wallace, P., Smith, G., Howard, S., Dreyer, R.F., Ma, C., Chenoweth, R.G., Zilis, J.D., Johnson, M., Rice, P., Daniel, H., Crider, H., Parker, S., Sherman, K., Martin, D.F., Aaberg T.M., S., Sternberg P., J., Curtis, L.T., Ju, B., Gilman, J., Myles, B., Strittman, S., Gentry, C., Yi, H., Capone A., J., Lambert, M., Meredith, T., Aaberg T.M., J., Saperstein, D., Lim, J.I., Stribling, B., Armiger, D., Swords, R., Orth, D.H., Flood, T.P., Civantos, J., Debustros, S., Packo, K.H., Merrill, P.T., Cohen, J.A., Figliulo, C., Morrison, C., Bryant, D.A., Doherty, D., McVicker, M., Drefcinski, T., Seddon, J.M., Pinnolis, M.K., Davis, N., Burton, I., Taitsel, T., Walsh, D., Dubois-Moran, J., Callahan, C., Evans, C., Snow, K.K., Jones-Devonish, D.A., Crouse, V.D., 2001. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch. Ophthalmol. 119, 1417–1436.

Kaya, S., Weigert, G., Dragostinoff, N., Werkmeister, R., Pemp, B., Sacu, S., Garhöfer, G., Schmidt-Erfurth, U., Schmetterer, L., 2010. The influence of lutein supplementation on macular pigment optical density in patients with dry AMD compared with healthy control subjects. BMC Pharmacol. 10, A40. doi:10.1186/1471-2210-10-S1-A40

Kernt, M., Walch, A., Neubauer, A.S., Hirneiss, C., Haritoglou, C., Ulbig, M.W., Kampik, A., 2012. Filtering blue light reduces light-induced oxidative stress, senescence and accumulation of extracellular matrix proteins in human retinal pigment epithelium cells. Clin. Experiment. Ophthalmol. 40, e87–97. doi:10.1111/j.1442-9071.2011.02620.x

Kessel, L., Lundeman, J.H., Herbst, K., Andersen, T.V., Larsen, M., 2010. Age-related changes in the transmission properties of the human lens and their relevance to circadian entrainment. J. Cataract Refract. Surg. 36, 308–312. doi:10.1016/j.jcrs.2009.08.035

Khachik, F., Bernstein, P.S., Garland, D.L., 1997. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. Invest. Ophthalmol. Vis. Sci. 38, 1802–1811.

Khachik, F., Moura, F.F. de, Zhao, D.-Y., Aebischer, C.-P., Bernstein, P.S., 2002. Transformations of Selected Carotenoids in Plasma, Liver, and Ocular Tissues of Humans and in Nonprimate Animal Models. Invest. Ophthalmol. Vis. Sci. 43, 3383–3392.

Khan, J.C., Shahid, H., Thurlby, D.A., Bradley, M., Clayton, D.G., Moore, A.T., Bird, A.C., Yates, J.R.W., 2006. Age related macular degeneration and sun exposure, iris colour, and skin sensitivity to sunlight. Br. J. Ophthalmol. 90, 29–32. doi:10.1136/bjo.2005.073825

Kijlstra, A., Tian, Y., Kelly, E.R., Berendschot, T.T.J.M., 2012. Lutein: More than just a filter for blue light. Prog. Retin. Eye Res. 31, 303–315. doi:10.1016/j.preteyeres.2012.03.002

Kilbride, P.E., Alexander, K.R., Fishman, M., Fishman, G.A., 1989. Human macular pigment assessed by imaging fundus reflectometry. Vision Res. 29, 663–674. doi:10.1016/0042-6989(89)90028-X

Kirby, M.L., Galea, M., Loane, E., Stack, J., Beatty, S., Nolan, J.M., 2009. Foveal Anatomic Associations with the Secondary Peak and the Slope of the Macular Pigment Spatial Profile. Invest. Ophthalmol. Vis. Sci. 50, 1383–1391. doi:10.1167/iovs.08-2494

Klein, B.E.K., Howard, K.P., Lee, K.E., Iyengar, S.K., Sivakumaran, T.A., Klein, R., 2012. The Relationship of Cataract and Cataract Extraction to Age-related Macular Degeneration: The Beaver Dam Eye Study. Ophthalmology 119, 1628–1633. doi:10.1016/j.ophtha.2012.01.050

Klein, B.E.K., Klein, R., Linton, K.L.P., 1992. Prevalence of Age-related Lens Opacities in a Population: The Beaver Dam Eye Study. Ophthalmology 99, 546–552. doi:10.1016/S0161-6420(92)31934-7

Klein, R., Klein, B., Linton, K., De Mets, D., 1991. The Beaver Dam Eye Study: visual acuity. Ophthalmology 98, 1310–1315.

Klein, R., Klein, B.E.K., Knudtson, M.D., Meuer, S.M., Swift, M., Gangnon, R.E., 2007. Fifteen-Year Cumulative Incidence of Age-Related Macular Degeneration: The Beaver Dam Eye Study. Ophthalmology 114, 253–262. doi:10.1016/j.ophtha.2006.10.040

Klein, R., Peto, T., Bird, A., Vannewkirk, M.R., 2004. The epidemiology of age-related macular degeneration. Am. J. Ophthalmol. 137, 486–495. doi:10.1016/j.ajo.2003.11.069

Kuroda, T., Fujikado, T., Maeda, N., Oshika, T., Hirohara, Y., Mihashi, T., 2002. Wavefront analysis in eyes with nuclear or cortical cataract. Am. J. Ophthalmol. 134, 1–9. doi:10.1016/S0002-9394(02)01402-2

Lai, J.C., Lapolice, D.J., Stinnett, S.S., Meyer, C.H., Arieu, L.M., Keller, M.A., Toth, C.A., 2002. Visual outcomes following macular translocation with 360° peripheral retinectomy. Arch. Ophthalmol. 120, 1317–1324.

Lakshminarayana, R., Aruna, G., Sangeetha, R.K., Bhaskar, N., Divakar, S., Baskaran, V., 2008. Possible degradation/biotransformation of lutein in vitro and in vivo: isolation and structural elucidation of lutein metabolites by HPLC and LC-MS (atmospheric pressure chemical ionization). Free Radic. Biol. Med. 45, 982–993. doi:10.1016/j.freeradbiomed.2008.06.011

Lamb, T.D., Collin, S.P., Pugh, E.N., 2007. Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. Nat. Rev. Neurosci. 8, 960–976. doi:10.1038/nrn2283

Landrum, J.T., Bone, R.A., 2001. Lutein, Zeaxanthin, and the Macular Pigment. Arch. Biochem. Biophys. 385, 28–40. doi:10.1006/abbi.2000.2171

Landrum, J.T., bone, R.A., Joa, H., Kilburn, M.D., Moore, L.L., Sprague, K.E., 1997. A One Year Study of the Macular Pigment: The Effect of 140 Days of a Lutein Supplement. Exp. Eye Res. 65, 57–62. doi:10.1006/exer.1997.0309

Lee, J., Taylor, H., 1989. The lens opacity meter: a method of quantification of lens opacity by measurement of scattering of incident light. Lens Eye Toxic. Res. 7, 31–38.

Lee, M., Yoo, H., Ahn, J., 2013. Comparison of Disc Analysis Algorithms Provided by Cirrus OCT and Stereo Optic-disc Photography in Normal and Open Angle Glaucoma Patients. Curr. Eye Res. 38, 605–613. doi:10.3109/02713683.2013.769059

Lengyel, I., Tufail, A., Hosaini, H.A., Luthert, P., Bird, A.C., Jeffery, G., 2004. Association of Drusen Deposition with Choroidal Intercapillary Pillars in the Aging Human Eye. Invest. Ophthalmol. Vis. Sci. 45, 2886–2892. doi:10.1167/iovs.03-1083

Lerman, S., Borkman, R., 1976. Spectroscopic Evaluation and Classification of the Normal, Aging, and Cataractous Lens. (With 1 color plate). Ophthalmic Res. 8, 335–353. doi:10.1159/000264841

Lerman, S., Yamanashi, B.S., Palmer, R.A., Roark, J.C., Borkman, R., 1978. Photoacoustic, Fluorescence and Light Transmission Spectra of Normal, Aging and Cataractous Lenses. Ophthalmic Res. 10, 168–176. doi:10.1159/000264951

Leung, C.K.-S., Weinreb, R.N., 2011. Anterior chamber angle imaging with optical coherence tomography. Eye 25, 261–267. doi:10.1038/eye.2010.201

Li, B., Vachali, P., Bernstein, P.S., 2010. Human ocular carotenoid-binding proteins. Photochem. Photobiol. Sci. 9, 1418. doi:10.1039/c0pp00126k

Li, P., An, L., Lan, G., Johnstone, M., Malchow, D., Wang, R.K., 2013. Extended imaging depth to 12 mm for 1050-nm spectral domain optical coherence tomography for imaging the whole anterior segment of the human eye at 120-kHz A-scan rate. J. Biomed. Opt. 18, 016012–016012. doi:10.1117/1.JBO.18.1.016012

Li, Y., Shekhar, R., Huang, D., 2006. Corneal Pachymetry Mapping with High-speed Optical Coherence Tomography. Ophthalmology 113, 792–799.e2. doi:10.1016/j.ophtha.2006.01.048

Liew, S.H.M., Gilbert, C.E., Spector, T.D., Mellerio, J., Kuijk, F.J.V., Beatty, S., Fitzke, F., Marshall, J., Hammond, C.J., 2006. Central retinal thickness is positively correlated with macular pigment optical density. Exp. Eye Res. 82, 915–920. doi:10.1016/j.exer.2005.10.014

Linetsky, M., James, H.-L, Ortwerth, B.J., 1996. The generation of superoxide anion by the UVA irradiation of human lens proteins. Exp. Eye Res. 63, 67–74. doi:10.1006/exer.1996.0092

Liu, T., Hu, A.Y., Kaines, A., Yu, F., Schwartz, S.D., Hubschman, J.-P., 2011. A PILOT STUDY OF NORMATIVE DATA FOR MACULAR THICKNESS AND VOLUME MEASUREMENTS USING CIRRUS HIGH-DEFINITION OPTICAL COHERENCE TOMOGRAPHY: Retina 31, 1944–1950. doi:10.1097/IAE.0b013e31820d3f13

Loffler, K.U., Lee, W.R., 1986. Basal linear deposit in the human macula. Graefes Arch. Clin. Exp. Ophthalmol. 224, 493–501. doi:10.1007/BF02154735

Loughman, J., 2013. Visual Performance in Patients with Neovascular Age Related Macular Degeneration Undergoing Treatment With Intravitreal Ranibizumab. Articles.

Loughman, J., Scanlon, G., Nolan, J.M., O'Dwyer, V., Beatty, S., 2012. An evaluation of a novel instrument for measuring macular pigment optical density: the MPS 9000. Acta Ophthalmol. (Copenh.) 90, e90–e97. doi:10.1111/j.1755-3768.2011.02294.x

Ma, L., Dou, H.-L., Wu, Y.-Q., Huang, Y.-M., Huang, Y.-B., Xu, X.-R., Zou, Z.-Y., Lin, X.-M., 2012. Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. Br. J. Nutr. 107, 350–359. doi:10.1017/S0007114511004260

Maclean, H., Taylor, C.J., 1981. An objective staging for cortical cataract in vivo aided by pattern-analysis computer. Exp. Eye Res. 33, 597–602. doi:10.1016/S0014-4835(81)80099-1

Mainster, M.A., Sparrow, J.R., 2003. How much blue light should an IOL transmit? Br. J. Ophthalmol. 87, 1523–1529. doi:10.1136/bjo.87.12.1523

Mainster MA, 2005. Intraocular lenses should block uv radiation and violet but not blue light. Arch. Ophthalmol. 123, 550–555. doi:10.1001/archopht.123.4.550

Makridaki, M., Carden, D., Murray, I.J., 2009. Macular pigment measurement in clinics: controlling the effect of the ageing media. Ophthalmic Physiol. Opt. 29, 338–344. doi:10.1111/j.1475-1313.2009.00667.x

Malinow, M.R., Feeney-Burns, L., Peterson, L.H., Klein, M.L., Neuringer, M., 1980. Diet-related macular anomalies in monkeys. Invest. Ophthalmol. Vis. Sci. 19, 857–863.

Mares-Perlman, J.A., Brady, W.E., Klein, R., Klein, B.E.K., Bowen, P., Stacewicz- Sapuntzakis, M., Palta, M., 1995. Serum antioxidants and age-related macular degeneration in a population- based case-control study. Arch. Ophthalmol. 113, 1518–1523.

Mares-Perlman, J.A., Fisher, A.I., Klein, R., Palta, M., Block, G., Millen, A.E., Wright, J.D., 2001. Lutein and Zeaxanthin in the Diet and Serum and Their Relation to Age-related Maculopathy in the Third National Health and Nutrition Examination Survey. Am. J. Epidemiol. 153, 424–432. doi:10.1093/aje/153.5.424

Mares-Perlman, J.A., Klein, R., Klein, B.E.K., Greger, J.L., Brady, W.E., Palta, M., Ritter, L.L., 1996. Association of zinc and antioxidant nutrients with Age-related maculopathy. Arch. Ophthalmol. 114, 991–997.

Margrain, T.., Boulton, M., Marshall, J., Sliney, D.., 2004. Do blue light filters confer protection against age-related macular degeneration? Prog. Retin. Eye Res. 23, 523–531. doi:10.1016/j.preteyeres.2004.05.001

Marmor MF, C.S., 2008. Visual insignificance of the foveal pit: Reassessment of foveal hypoplasia as fovea plana. Arch. Ophthalmol. 126, 907–913. doi:10.1001/archopht.126.7.907

Masters, B.R., 1996. Optical tomography of the in vivo human lens: three-dimensional visualization of cataracts. J. Biomed. Opt. 1, 289–295. doi:10.1117/12.241366

McCarty, C.A., Keeffe, J.E., Taylor, H.R., 1999. The need for cataract surgery: projections based on lens opacity, visual acuity, and personal concern. Br. J. Ophthalmol. 83, 62–65. doi:10.1136/bjo.83.1.62

McGinty, S.J., Truscott, R.J.W., 2006. Presbyopia: The First Stage of Nuclear Cataract? Ophthalmic Res. 38, 137–148. doi:10.1159/000090645

Mellerio, J., 1987. Yellowing of the human lens: Nuclear and cortical contributions. Vision Res. 27, 1581–1587. doi:10.1016/0042-6989(87)90166-0

Meyers, S.M., 1994. A twin study on age-related macular degeneration. Trans. Am. Ophthalmol. Soc. 92, 775–843.

Michael, R., Bron, A.J., 2011. The ageing lens and cataract: a model of normal and pathological ageing. Philos. Trans. R. Soc. B Biol. Sci. 366, 1278–1292. doi:10.1098/rstb.2010.0300

Michels, M., Sternberg Jr., P., 1990. Operating microscope-induced retinal phototoxicity: pathophysiology, clinical manifestations and prevention. Surv. Ophthalmol. 34, 237–252. doi:10.1016/0039-6257(90)90025-Q

Mitchell, P., Smith, W., Attebo, K., Wang, J., 1995. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. Ophthalmology 102, 1450–1460.

Mori, K., Gehlbach, P., Ando, A., McVey, D., Wei, L., Campochiaro, P.A., 2002. Regression of Ocular Neovascularization in Response to Increased Expression of Pigment Epithelium–Derived Factor. Invest. Ophthalmol. Vis. Sci. 43, 2428–2434.

Mortensen, A., Skibsted, L.H., 1997. Free radical transients in photobleaching of xanthophylls and carotenes. Free Radic. Res. 26, 549–563.

Munch, I.C., Ek, J., Kessel, L., Sander, B., Almind, G.J., Brøndum-Nielsen, K., Linneberg, A., Larsen, M., 2010. Small, Hard Macular Drusen and Peripheral Drusen: Associations with AMD Genotypes in the Inter99 Eye Study. Invest. Ophthalmol. Vis. Sci. 51, 2317–2321. doi:10.1167/iovs.09-4482

Mwanza, J.-C., Oakley, J.D., Budenz, D.L., Anderson, D.R., 2011. Ability of Cirrus HD-OCT Optic Nerve Head Parameters to Discriminate Normal from Glaucomatous Eyes. Ophthalmology 118, 241–248.e1. doi:10.1016/j.ophtha.2010.06.036

Neelam, K., Nolan, J., Loane, E., Stack, J., O'Donovan, O., Au Eong, K.G., Beatty, S., 2006. Macular pigment and ocular biometry. Vision Res. 46, 2149–2156. doi:10.1016/j.visres.2005.12.012

Ni, W., Li, X., Ao, M., Zhang, H., Hou, Z., Si, S., Wang, W., 2012. Using the real-life vision test to assess the functional vision of age-related cataract patients. Eye 26, 1402–1411. doi:10.1038/eye.2012.168

Nolan, J., Kenny, R., O'Regan, C., Cronin, H., Loughman, J., Connolly, E., Kearney, P., Beatty, S., 2010. Macular Pigment Optical Density in an Aging Irish Population: the Irish Longitudinal Study on Ageing. Articles.

Nolan, J.M., Loughman, J., Akkali, M.C., Stack, J., Scanlon, G., Davison, P., Beatty, S., 2011. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. Vision Res. 51, 459–469. doi:10.1016/j.visres.2010.12.016

Nolan, J.M., Meagher, K., Kashani, S., Beatty, S., 2013. What is meso-zeaxanthin, and where does it come from? Eye 27, 899–905. doi:10.1038/eye.2013.98

Nolan, J.M., O'Reilly, P., Loughman, J., Stack, J., Loane, E., Connolly, E., Beatty, S., 2009. Augmentation of Macular Pigment following Implantation of Blue Light–Filtering Intraocular Lenses at the Time of Cataract Surgery. Invest. Ophthalmol. Vis. Sci. 50, 4777–4785. doi:10.1167/iovs.08-3277

Nolan, J.M., Stack, J., O' Donovan, O., Loane, E., Beatty, S., 2007. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. Exp. Eye Res. 84, 61–74. doi:10.1016/j.exer.2006.08.016

Nolan, J.M., Stringham, J.M., Beatty, S., Snodderly, D.M., 2008. Spatial Profile of Macular Pigment and Its Relationship to Foveal Architecture. Invest. Ophthalmol. Vis. Sci. 49, 2134–2142. doi:10.1167/iovs.07-0933

Novella, S., 2008. Suboptimal Optics: Vision Problems as Scars of Evolutionary History. Evol. Educ. Outreach 1, 493–497. doi:10.1007/s12052-008-0092-1

Obana, A., Hiramitsu, T., Gohto, Y., Ohira, A., Mizuno, S., Hirano, T., Bernstein, P.S., Fujii, H., Iseki, K., Tanito, M., Hotta, Y., 2008. Macular Carotenoid Levels of Normal Subjects and Age-Related Maculopathy Patients in a Japanese Population. Ophthalmology 115, 147–157. doi:10.1016/j.ophtha.2007.02.028

Obana, A., Tanito, M., Gohto, Y., Gellermann, W., Okazaki, S., Ohira, A., 2011. Macular Pigment Changes in Pseudophakic Eyes Quantified with Resonance Raman Spectroscopy. Ophthalmology 118, 1852–1858. doi:10.1016/j.ophtha.2011.02.017

Ooto, S., Hangai, M., Tomidokoro, A., Saito, H., Araie, M., Otani, T., Kishi, S., Matsushita, K., Maeda, N., Shirakashi, M., Abe, H., Ohkubo, S., Sugiyama, K., Iwase, A., Yoshimura, N., 2011. Effects of Age, Sex, and Axial Length on the Three-Dimensional Profile of Normal Macular Layer Structures. Invest. Ophthalmol. Vis. Sci. 52, 8769–8779. doi:10.1167/iovs.11-8388

Ortiz, D., Alió, J.L., Ruiz-Colechá, J., Oser, U., 2008. Grading nuclear cataract opacity by densitometry and objective optical analysis. J. Cataract Refract. Surg. 34, 1345–1352. doi:10.1016/j.jcrs.2008.04.022

Otis, L.L., Everett, M.J., Sathyam, U.S., Colston, B.W., 2000. Optical Coherence Tomography: A New Imaging Technology for Dentistry. J. Am. Dent. Assoc. 131, 511–514.

Palomo-Alvarez, C., Puell, M., Gomez-Sanz, F., Barrio, A., Clement-Corral, A., Perez-Carrasco, M., 2010. Is foveal thickness correlated with macular pigment optical density and with Intraocular light scatter in early age-related maculopathy? Acta Ophthalmol. (Copenh.) 88, 0–0. doi:10.1111/j.1755-3768.2010.244.x

Pauleikhoff, D., Chen, J.C., Chisholm, I.H., Bird, A.C., 1990. Choroidal perfusion abnormality with age-related Bruch's membrane change. Am. J. Ophthalmol. 109, 211–217.

Peyman, G.A., Blinder, K.J., Paris, C.L., Alturki, W., Nelson Jr., N.C., Desai, U., 1991. A technique for retinal pigment epithelium transplantation for age-related macular degeneration secondary to extensive subfoveal scarring. Ophthalmic Surg. 22, 102–108.

Pollack, A., Marcovich, A., Bukelman, A., Oliver, M., 1996. Age-related macular degeneration after extracapsular cataract extraction with intraocular lens implantation. Ophthalmology 103, 1546–1554.

Postel, E.A., Agarwal, A., Schmidt, S., Fan, Y.-T.R., Scott, W.K., Gilbert, J.R., Haines, J.L, Pericak-Vance, M.A., 2005. Comparing Age-related Macular Degeneration Phenotype in Probands From Singleton and Multiplex Families. Am. J. Ophthalmol. 139, 820–825. doi:10.1016/j.ajo.2004.12.029

Provis, J.M., Dubis, A.M., Maddess, T., Carroll, J., 2013. Adaptation of the central retina for high acuity vision: Cones, the fovea and the avascular zone. Prog. Retin. Eye Res. 35, 63–81. doi:10.1016/j.preteyeres.2013.01.005

Rahi, J.S., Logan, S., Timms, C., Russell-Eggitt, I., Taylor, D., 2002. Risk, causes, and outcomes of visual impairment after loss of vision in the non-amblyopic eye: a population-based study. The Lancet 360, 597–602. doi:10.1016/S0140-6736(02)09782-9

Raman, R., Biswas, S., Gupta, A., Kulothungan, V., Sharma, T., 2012. Association of macular pigment optical density with risk factors for wet age-related macular degeneration in the Indian population. Eye Lond. Engl. 26, 950–957. doi:10.1038/eye.2012.69

Rathi, A., Sharma, R., Jha, B., Bhartiya, S., Panda, A., 2011. Role of Lens Extraction in Primary Angle Closure Disease. J. Curr. Glaucoma Pract. 5, 20–25.

Ravin JG, 1985. Monet's cataracts. JAMA 254, 394–399. doi:10.1001/jama.1985.03360030084028

Rezai, K.A., Gasyna, E., Seagle, B.-L.L., Jr, J.R.N., Rezaei, K.A., 2008. AcrySof Natural filter decreases blue light-induced apoptosis in human retinal pigment epithelium. Graefes Arch. Clin. Exp. Ophthalmol. 246, 671–676. doi:10.1007/s00417-006-0484-2

Richer, S., Cho, J., Stiles, W., Levin, M., Wrobel, J.S., Sinai, M., Thomas, C., 2012. Retinal Spectral Domain Optical Coherence Tomography in Early Atrophic Age-Related Macular Degeneration (AMD) and a New Metric for Objective Evaluation of the Efficacy of Ocular Nutrition. Nutrients 4, 1812–1827. doi:10.3390/nu4121812

Richer, S., Stiles, W., Statkute, L., Pulido, J., Frankowski, J., Rudy, D., Pei, K., Tsipursky, M., Nyland, J., 2004. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). Optom. - J. Am. Optom. Assoc. 75, 216–229. doi:10.1016/S1529-1839(04)70049-4

Romano, G., Mercatelli, L., Fusi, F., Guasti, A., Favuzza, E., Monici, M., Mencucci, R., 2011. Colorimetric comparison of light-filtering intraocular lenses and human crystalline lenses at various ages. J. Cataract Refract. Surg. 37, 758–762. doi:10.1016/j.jcrs.2010.10.053

Ruffolo Jr., J.J., Ham Jr., W.T., Mueller, H.A., Millen, J.E., 1984. Photochemical lesions in the primate retina under conditions of elevated blood oxygen. Invest. Ophthalmol. Vis. Sci. 25, 893–898.

Sabour-Pickett, S., Nolan, J.M., Loughman, J., Beatty, S., 2012. A review of the evidence germane to the putative protective role of the macular carotenoids for age-related macular degeneration. Mol. Nutr. Food Res. 56, 270–286. doi:10.1002/mnfr.201100219

Sample, P.A., Esterson, F.D., Weinreb, R.N., Boynton, R.M., 1988. The aging lens: in vivo assessment of light absorption in 84 human eyes. Invest. Ophthalmol. Vis. Sci. 29, 1306–1311.

Sarks, S.H., 1980. Council Lecture. Drusen and their relationship to senile macular degeneration. Aust. J. Ophthalmol. 8, 117–130.

Sasaki, M., Yuki, K., Kurihara, T., Miyake, S., Noda, K., Kobayashi, S., Ishida, S., Tsubota, K., Ozawa, Y., 2012. Biological role of lutein in the light-induced retinal degeneration. J. Nutr. Biochem. 23, 423–429. doi:10.1016/j.jnutbio.2011.01.006

Sasamoto, Y., Gomi, F., Sawa, M., Sakaguchi, H., Tsujikawa, M., Nishida, K., 2011. Effect of cataract in evaluation of macular pigment optical density by autofluorescence spectrometry. Invest. Ophthalmol. Vis. Sci. 52, 927–932. doi:10.1167/iovs.10-5664

Schaft, T.L. van der, Mooy, C.M., Bruijn, W.C. de, Mulder, P.G., Pameyer, J.H., Jong, P.T. de, 1994. Increased prevalence of disciform macular degeneration after cataract extraction with implantation of an intraocular lens. Br. J. Ophthalmol. 78, 441–445. doi:10.1136/bjo.78.6.441

Schlatterer, J., Breithaupt, D.E., 2006. Xanthophylls in Commercial Egg Yolks:  Quantification and Identification by HPLC and LC-(APCI)MS Using a C30 Phase. J. Agric. Food Chem. 54, 2267–2273. doi:10.1021/jf053204d

Schraermeyer, U., Heimann, K., 1999. Current Understanding on the Role of Retinal Pigment Epithelium and its Pigmentation. Pigment Cell Res. 12, 219–236. doi:10.1111/j.1600-0749.1999.tb00755.x

Schütt, F., Davies, S., Kopitz, J., Holz, F.G., Boulton, M.E., 2000. Photodamage to Human RPE Cells by A2-E, a Retinoid Component of Lipofuscin. Invest. Ophthalmol. Vis. Sci. 41, 2303–2308.

Sharifzadeh, M., Zhao, D.-Y., Bernstein, P.S., Gellermann, W., 2008. Resonance Raman imaging of macular pigment distributions in the human retina. J. Opt. Soc. Am. A 25, 947–957. doi:10.1364/JOSAA.25.000947

Siems, W.G., Sommerburg, O., van Kuijk, F.J.G.M., 1999. Lycopene and \beta-carotene decompose more rapidly than lutein and zeaxanthin upon exposure to various pro-oxidants in vitro. BioFactors 10, 105–113.

Silvestri, G., Johnston, P.B., Hughes, A.E., 1994. Is genetic predisposition an important risk factor in age-related macular degeneration? Eye 8, 564–568. doi:10.1038/eye.1994.138

Simons, K., 1993. Artificial light and early-life exposure in age-related macular degeneration and in cataractogenic phototoxicity. Arch. Ophthalmol. 111, 297–298.

Snodderly, D.M., Auran, J.D., Delori, F.C., 1984. The macular pigment. II. Spatial distribution in primate retinas. Invest. Ophthalmol. Vis. Sci. 25, 674–685.

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

Sommerburg, O., Keunen, J.E.E., Bird, A.C., Kuijk, F.J.G.M. van, 1998. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Br. J. Ophthalmol. 82, 907–910. doi:10.1136/bjo.82.8.907

Song, W.K., Lee, S.C., Lee, E.S., Kim, C.Y., Kim, S.S., 2010. Macular Thickness Variations with Sex, Age, and Axial Length in Healthy Subjects: A Spectral Domain–Optical Coherence Tomography Study [WWW Document]. URL http://www.iovs.org (accessed 9.16.13).

Spaide, R.F., Koizumi, H., Pozonni, M.C., 2008. Enhanced Depth Imaging Spectral-Domain Optical Coherence Tomography. Am. J. Ophthalmol. 146, 496–500. doi:10.1016/j.ajo.2008.05.032

Sparrow, J.M., Brown, N.A.P., Shun-Shin, G.A., Bron, A.J., 1990. The Oxford modular cataract image analysis system. Eye 4, 638–648. doi:10.1038/eye.1990.89

Sparrow, J.R., Miller, A.S., Zhou, J., 2004. Blue light-absorbing intraocular lens and retinal pigment epithelium protection in vitro. J. Cataract Refract. Surg. 30, 873–878. doi:10.1016/j.jcrs.2004.01.031

Sparrow, J.R., Nakanishi, K., Parish, C.A., 2000. The Lipofuscin Fluorophore A2E Mediates Blue Light–Induced Damage to Retinal Pigmented Epithelial Cells. Invest. Ophthalmol. Vis. Sci. 41, 1981–1989.

Stefánsson, E., Geirsdóttir, Á., Sigurdsson, H., 2011. Metabolic physiology in age related macular degeneration. Prog. Retin. Eye Res. 30, 72–80. doi:10.1016/j.preteyeres.2010.09.003

Sui, G.-Y., Liu, G.-C., Liu, G.-Y., Gao, Y.-Y., Deng, Y., Wang, W.-Y., Tong, S.-H., Wang, L., 2013. Is sunlight exposure a risk factor for age-related macular degeneration? A systematic review and meta-analysis. Br. J. Ophthalmol. 97, 389–394. doi:10.1136/bjophthalmol-2012-302281

Sujak, A., Okulski, W., Gruszecki, W.I., 2000. Organisation of xanthophyll pigments lutein and zeaxanthin in lipid membranes formed with dipalmitoylphosphatidylcholine. Biochim. Biophys. Acta 1509, 255–263.

Sun, M., Finnemann, S.C., Febbraio, M., Shan, L., Annangudi, S.P., Podrez, E.A., Hoppe, G., Darrow, R., Organisciak, D.T., Salomon, R.G., Silverstein, R.L., Hazen, S.L., 2006. Light-induced oxidation of photoreceptor outer segment phospholipids generates ligands for CD36-mediated phagocytosis by retinal pigment epithelium: A potential mechanism for modulating outer segment phagocytosis under oxidant stress conditions. J. Biol. Chem. 281, 4222–4230.

Sunness, J.S., Rubin, G.S., Zuckerbrod, A., Applegate, C.A., 2008. Foveal-Sparing Scotomas in Advanced Dry Age-Related Macular Degeneration. J. Vis. Impair. Blind. 102, 600–610.

Sunness JS, Massof RW, Johnson MA, Finkelstein D, Fine SL, 1985. PEripheral retinal function in age-related macular degeneration. Arch. Ophthalmol. 103, 811–816. doi:10.1001/archopht.1985.01050060071029

Suzuki, M., Kamei, M., Itabe, H., Yoneda, K., Bando, H., Kume, N., Tano, Y., 2007. Oxidized phospholipids in the macula increase with age and in eyes with age-related macular degeneration. Mol. Vis. 13, 772–778.

Swanson, E.A., Izatt, J.A., Lin, C.P., Fujimoto, J.G., Schuman, J.S., Hee, M.R., Huang, D., Puliafito, C.A., 1993. In vivo retinal imaging by optical coherence tomography. Opt. Lett. 18, 1864–1866. doi:10.1364/OL.18.001864

Tanito, M., Obana, A., Gohto, Y., Okazaki, S., Gellermann, W., Ohira, A., 2012. Macular pigment density changes in Japanese individuals supplemented with lutein or zeaxanthin: quantification via resonance Raman spectrophotometry and autofluorescence imaging. Jpn. J. Ophthalmol. 56, 488–496. doi:10.1007/s10384-012-0157-0

Tanvetyanon, T., Bepler, G., 2008. Beta-carotene in multivitamins and the possible risk of lung cancer among smokers versus former smokers. Cancer 113, 150–157. doi:10.1002/cncr.23527

Taylor, H., West, S., Rosenthal, F., Muñoz, B., Newland, H., Abbey, H., Emmett, E., 1988. Effect of ultraviolet radiation on cataract formation. N. Engl. J. Med. 319, 1429–1433.

Taylor, H.R., 1999. Epidemiology of age-related cataract. Eye 13, 445–448.

The Age-Related Eye Disease Study 2 (AREDS2) Research Group, 2013. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: The age-related eye disease study 2 (areds2) randomized clinical trial. JAMA 309, 2005–2015. doi:10.1001/jama.2013.4997

Thibos, L.N., 1987. Calculation of the influence of lateral chromatic aberration on image quality across the visual field. J. Opt. Soc. Am. A 4, 1673–1680. doi:10.1364/JOSAA.4.001673

Thornton, J., Edwards, R., Mitchell, P., Harrison, R.A., Buchan, I., Kelly, S.P., 2005. Smoking and age-related macular degeneration: a review of association. Eye 19, 935–944. doi:10.1038/sj.eye.6701978

Threlfall, T.J., English, D.R., 1999. Sun exposure and pterygium of the eye: a dose-response curve. Am. J. Ophthalmol. 128, 280–287. doi:10.1016/S0002-9394(99)00161-0

Tong, N., Zhang, W., Zhang, Z., Gong, Y., Wooten, B., Wu, X., 2013. Inverse relationship between macular pigment optical density and axial length in Chinese subjects with myopia. Graefes Arch. Clin. Exp. Ophthalmol. 251, 1495–1500. doi:10.1007/s00417-012-2225-z

Trieschmann, M., Heimes, B., Hense, H.W., Pauleikhoff, D., 2006. Macular pigment optical density measurement in autofluorescence imaging: comparison of one- and two-wavelength methods. Graefes Arch. Clin. Exp. Ophthalmol. Albrecht Von Graefes Arch. Für Klin. Exp. Ophthalmol. 244, 1565–1574. doi:10.1007/s00417-006-0289-3

Trieschmann, M., van Kuijk, F.J.G.M., Alexander, R., Hermans, P., Luthert, P., Bird, A.C., Pauleikhoff, D., 2007. Macular pigment in the human retina: histological evaluation of localization and distribution. Eye 22, 132–137. doi:10.1038/sj.eye.6702780

Trokel, S., 1962. The Physical Basis for Transparency of the Crystalline Lens. Invest. Ophthalmol. Vis. Sci. 1, 493–501.

Truscott, R.J.W., 2005. Age-related nuclear cataract—oxidation is the key. Exp. Eye Res. 80, 709–725. doi:10.1016/j.exer.2004.12.007

Ueda, T., Nawa, Y., Hara, Y., 2006. Relationship between the retinal thickness of the macula and the difference in axial length. Graefes Arch. Clin. Exp. Ophthalmol. 244, 498–501. doi:10.1007/s00417-005-1146-5

Van Best, J.A., Putting, B.J., Oosterhuis, J.A., Zweypfenning, R.C.V.J., Vrensen, G.F.J.M., 1997. Function and morphology of the retinal pigment epithelium after light-induced damage. Microsc. Res. Tech. 36, 77–88. doi:10.1002/(SICI)1097-0029(19970115)36:2<77::AID-JEMT1>3.0.CO;2-S

Van Der Veen, R.L.P., Berendschot, T.T.J.M., Hendrikse, F., Carden, D., Makridaki, M., Murray, I.J., 2009. A new desktop instrument for measuring macular pigment optical density based on a novel technique for setting flicker thresholds. Ophthalmic Physiol. Opt. 29, 127–137. doi:10.1111/j.1475-1313.2008.00618.x

Van der Veen, R.L.P., Ostendorf, S., Hendrikse, F., Berendschot, T.T.J.M., 2009. Macular pigment optical density relates to foveal thickness. Eur. J. Ophthalmol. 19, 836–841.

Wakitani, Y., Sasoh, M., Sugimoto, M., Ito, Y., Ido, M., Uji, Y., 2003. Macular thickness measurements in healthy subjects with different axial lengths using optical coherence tomography. Retina Phila. Pa 23, 177–182.

Waldstein, S.M., Hickey, D., Mahmud, I., Kiire, C.A., Charbel Issa, P., Chong, N.V., 2012. Two-wavelength fundus autofluorescence and macular pigment optical density imaging in diabetic macular oedema. Eye 26, 1078–1085. doi:10.1038/eye.2012.100

Wang J, F.S., 2003. Risk of age-related macular degeneration in eyes with macular drusen or hyperpigmentation: The blue mountains eye study cohort. Arch. Ophthalmol. 121, 658–663. doi:10.1001/archopht.121.5.658

Wang, R.J., 1975. Lethal Effect of "daylight" Fluorescent Light on Human Cells in Tissue-Culture Medium. Photochem. Photobiol. 21, 373–375. doi:10.1111/j.1751-1097.1975.tb06688.x

Wang, Y., Nelson, J., Chen, Z., Reiser, B., Chuck, R., Windeler, R., 2003. Optimal wavelength for ultrahigh-resolution optical coherence tomography. Opt. Express 11, 1411–1417.

Weber-Krause, B., Eckardt, U., 1996. Incidence of posterior vitreous detachment in eyes with and without age-related macular degeneration. An ultrasonic study. Ophthalmol. Z. Dtsch. Ophthalmol. Ges. 93, 660–665.

Weigert, G., Kaya, S., Pemp, B., Sacu, S., Lasta, M., Werkmeister, R.M., Dragostinoff, N., Simader, C., Garhöfer, G., Schmidt-Erfurth, U., Schmetterer, L., 2011. Effects of Lutein Supplementation on Macular Pigment Optical Density and Visual Acuity in Patients with Age-Related Macular Degeneration. Invest. Ophthalmol. Vis. Sci. 52, 8174–8178. doi:10.1167/iovs.11-7522

Weiter, J., Delori, F., Wing, G., Fitch, K., 1985. Relationship of senile macular degeneration to ocular pigmentation. Am. J. Ophthalmol. 99, 185–187.

Weiter, J.J., Delori, F.C., Wing, G.L., Fitch, K.A., 1986. Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. Invest. Ophthalmol. Vis. Sci. 27, 145–152.

Wenzel, A.J., Fuld, K., Stringham, J.M., 2003. Light Exposure and Macular Pigment Optical Density. Invest. Ophthalmol. Vis. Sci. 44, 306–309. doi:10.1167/iovs.01-1191

Wenzel, A.J., Fuld, K., Stringham, J.M., Curran-Celentano, J., 2006. Macular pigment optical density and photophobia light threshold. Vision Res. 46, 4615–4622. doi:10.1016/j.visres.2006.09.019

West SK, R.F., 1989. Exposure to sunlight and other risk factors for age-related macular degeneration. Arch. Ophthalmol. 107, 875–879. doi:10.1001/archopht.1989.01070010897038

Westrup, V.K.M. zu, Dietzel, M., Pauleikhoff, D., Hense, H.W., 2014. The association of retinal structure and macular pigment distribution. Invest. Ophthalmol. Vis. Sci. IOVS–13–12903. doi:10.1167/iovs.13-12903

Whitehead A, M.J., 2006. Macular pigment: A review of current knowledge. Arch. Ophthalmol. 124, 1038–1045. doi:10.1001/archopht.124.7.1038

Wolffsohn, J.S., Cochrane, A.L, Khoo, H., Yoshimitsu, Y., Wu, S., 2000. Contrast is enhanced by yellow lenses because of selective reduction of short-wavelength light. Optom. Vis. Sci. Off. Publ. Am. Acad. Optom. 77, 73–81.

Wolffsohn, J.S., Eperjesi, F., 2005. The effect of relative distance enlargement on visual acuity in the visually impaired. Clin. Exp. Optom. 88, 97–102. doi:10.1111/j.1444-0938.2005.tb06674.x

Wolffsohn, J.S., Eperjesi, F., Bartlett, H., Sheppard, A., Howells, O., Drew, T., Sulley, A., Lorenz, K.O., 2012. Does blocking ultra-violet light with contact lenses benefit eye health? Contact Lens Anterior Eye 35, Supplement 1, e40. doi:10.1016/j.clae.2012.10.027

Wong, A.C.M., Chan, C.W.N., Hui, S.P., 2004. Relationship of Gender, Body Mass Index, and Axial Length with Central Retinal Thickness Using Optical Coherence Tomography. Eye 19, 292–297. doi:10.1038/sj.eye.6701466

Wong, A.L, Leung, C.K.-S., Weinreb, R.N., Cheng, A.K.C., Cheung, C.Y.L., Lam, P.T.-H., Pang, C.P., Lam, D.S.C., 2009. Quantitative assessment of lens opacities with anterior segment optical coherence tomography. Br. J. Ophthalmol. 93, 61–65. doi:10.1136/bjo.2008.137653

Wooten, B.R., Hammond, B.R., 2002. Macular pigment: influences on visual acuity and visibility. Prog. Retin. Eye Res. 21, 225–240. doi:10.1016/S1350-9462(02)00003-4

Wooten, B.R., Hammond, B.R., Land, R.I., Snodderly, D.M., 1999. A Practical Method for Measuring Macular Pigment Optical Density. Invest. Ophthalmol. Vis. Sci. 40, 2481–2489.

Yehoshua, Z., Wang, F., Rosenfeld, P.J., Penha, F.M., Feuer, W.J., Gregori, G., 2011. Natural History of Drusen Morphology in Age-Related Macular Degeneration Using Spectral Domain Optical Coherence Tomography. Ophthalmology 118, 2434–2441. doi:10.1016/j.ophtha.2011.05.008

Zeng, Y., Liu, Y., Liu, X., Chen, C., Xia, Y., Lu, M., He, M., 2009. Comparison of Lens Thickness Measurements Using the Anterior Segment Optical Coherence Tomography and A-scan Ultrasonography. Invest. Ophthalmol. Vis. Sci. 50, 290–294. doi:10.1167/iovs.07-1216

Zheng, W., Zhang, Z., Jiang, K., Zhu, J., He, G., Ke, B., 2013. Macular pigment optical density and its relationship with refractive status and foveal thickness in Chinese school-aged children. Curr. Eye Res. 38, 168–173. doi:10.3109/02713683.2012.713150
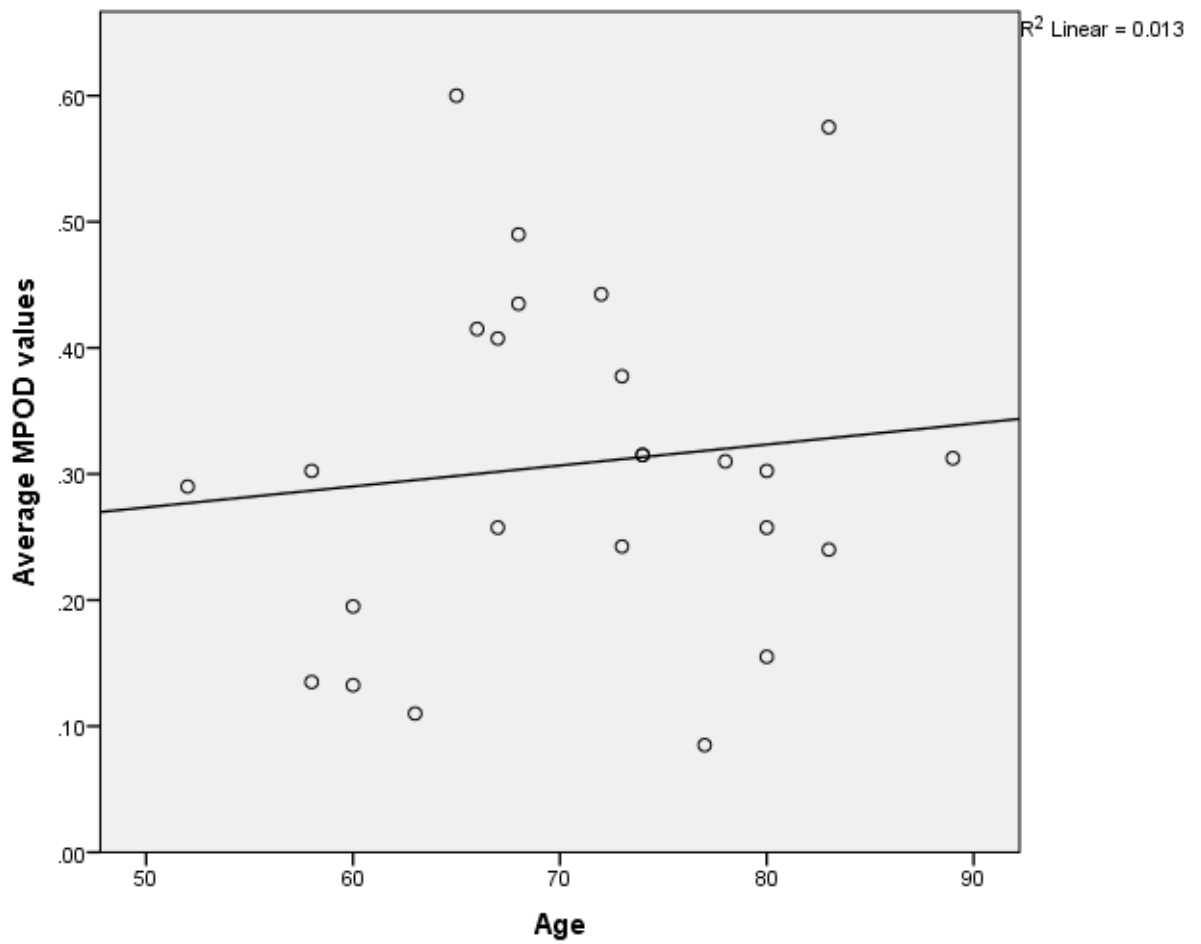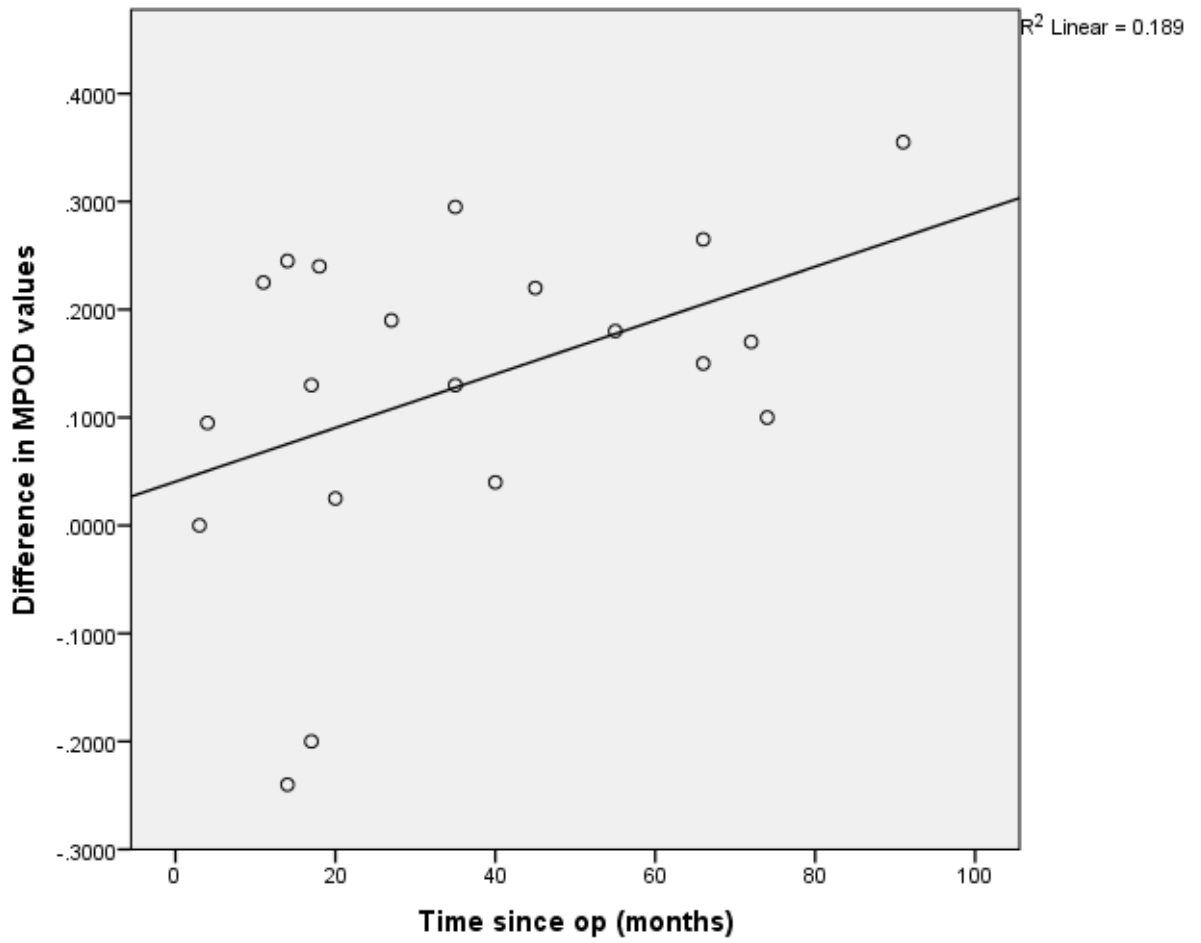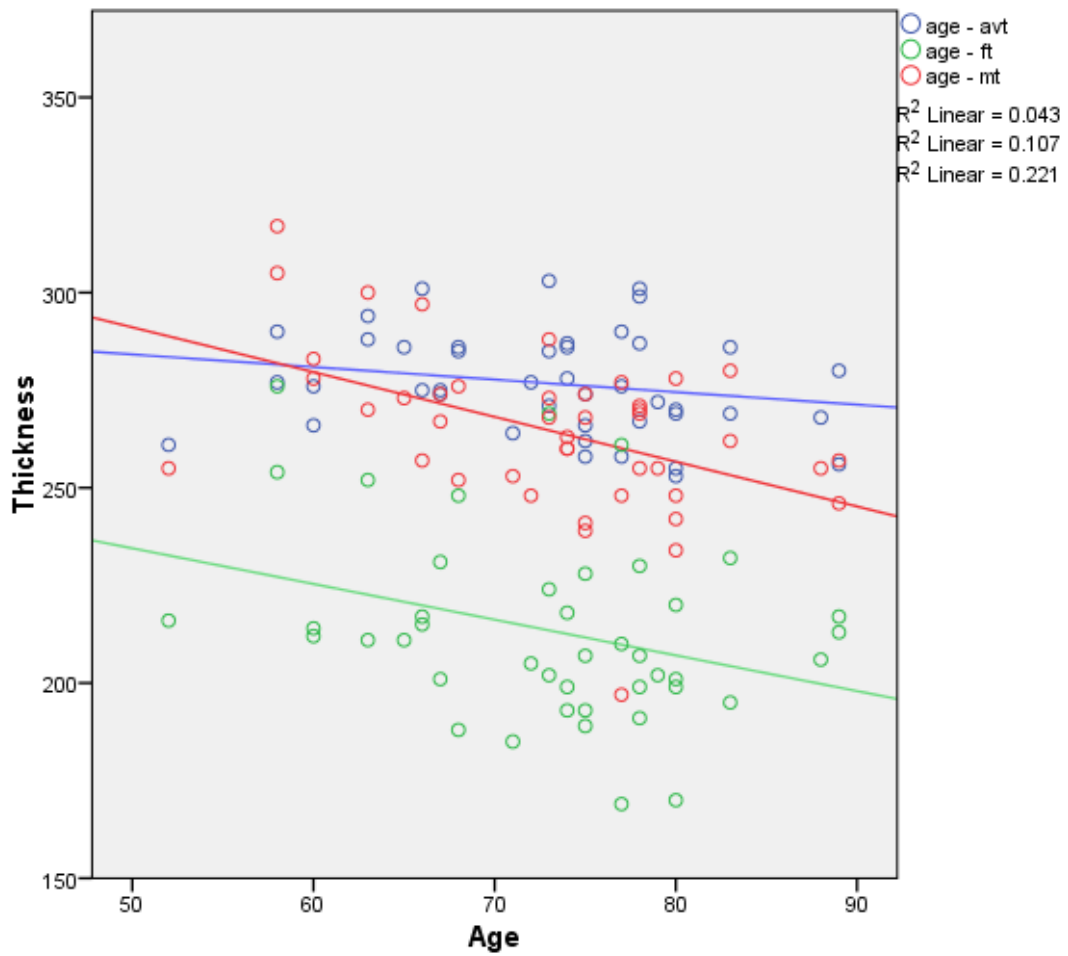
Appendix 1.1: Average MPOD values plotted against age. N=25
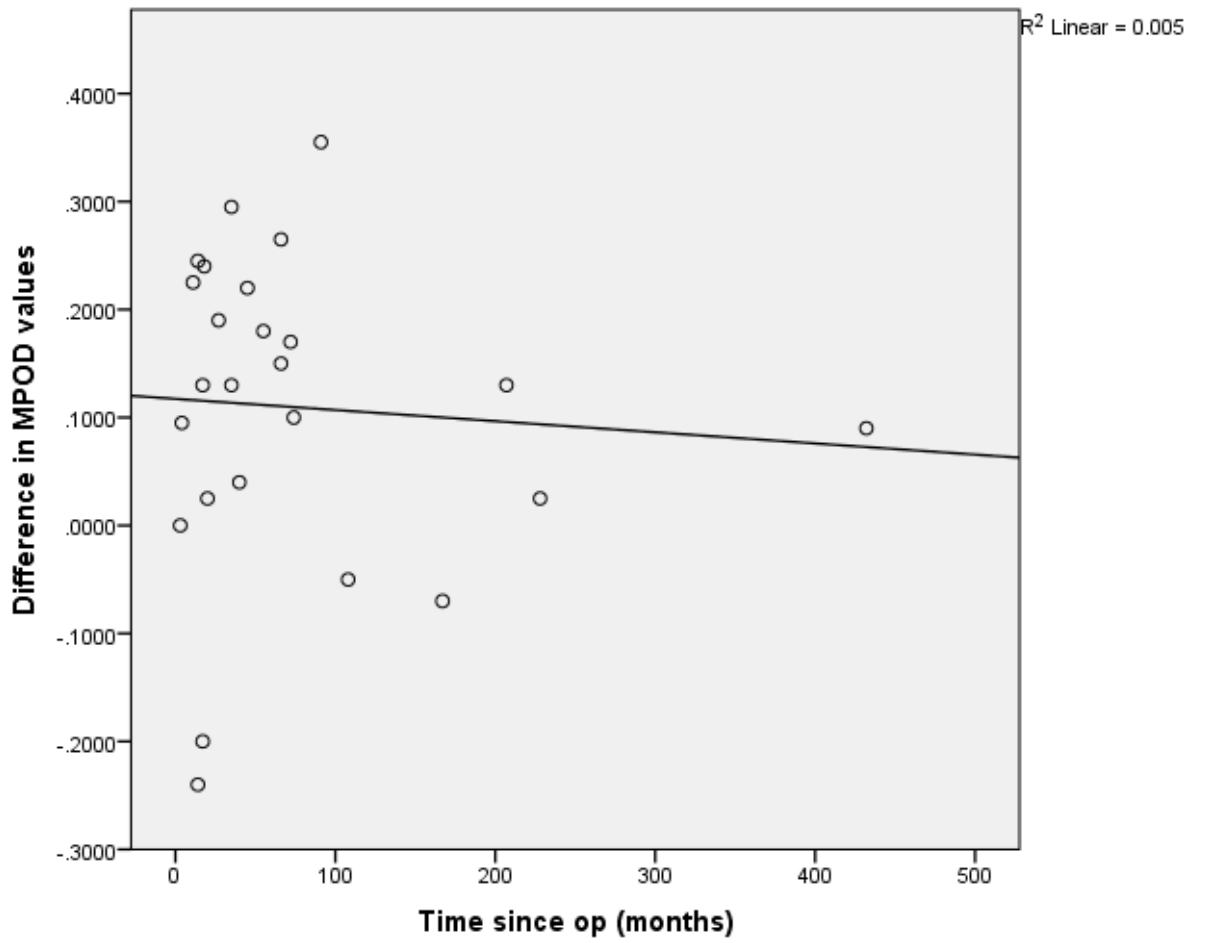
Appendix 1.2: The difference in MPOD values between pseudophakic and phakic eyes plotted against the time since operation. N=25

Appendix 1.3: The difference in MPOD values plotted against time since operation up to 100

months. N=20

Appendix 1.4: Macular thickness values plotted against age. avt is the thickness over the entire 6mm diameter area, mt is the thickness of the central 1mm diameter of the macula and ft is the central thickness of the fovea. N = 43 eyes.
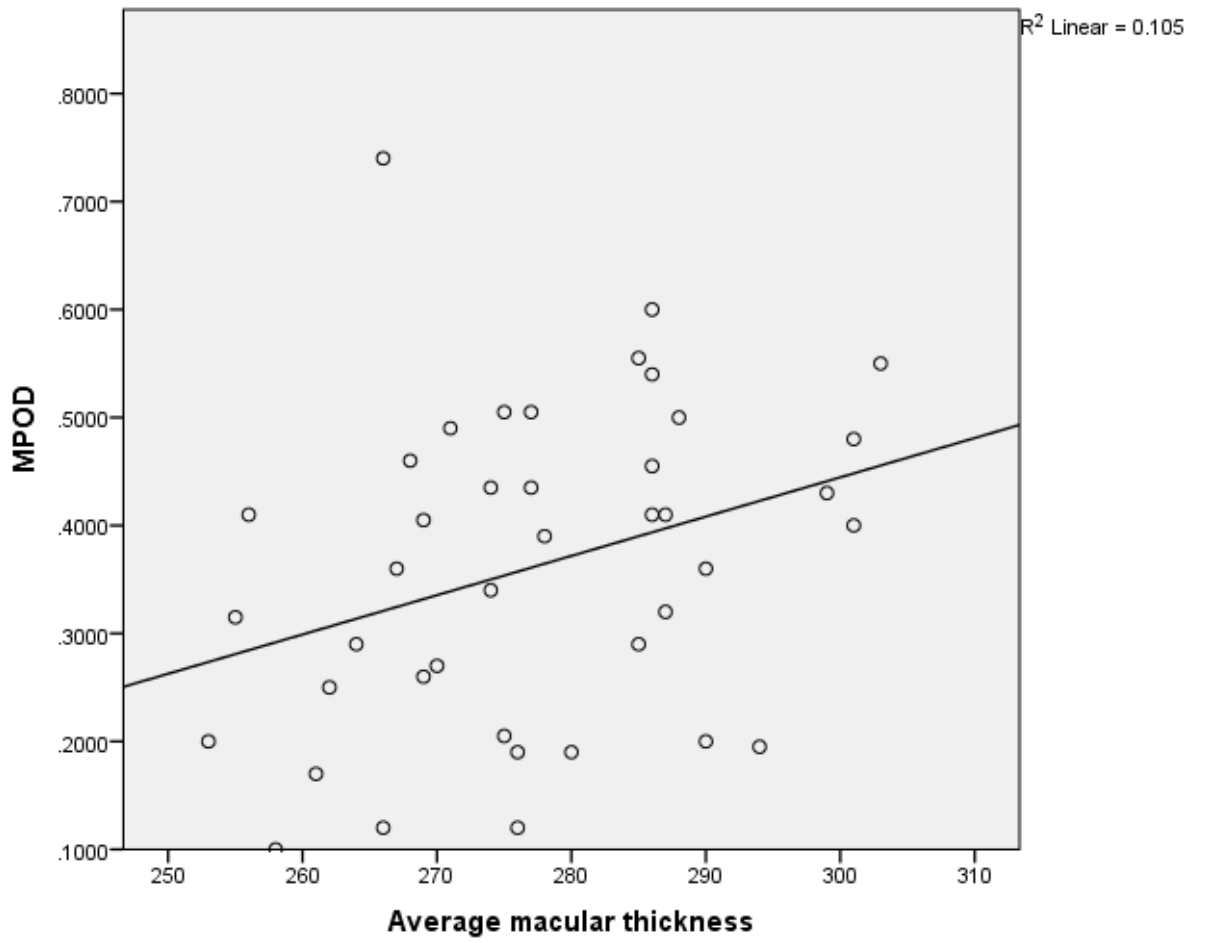
Appendix 1.5: MPOD plotted against average macular thickness over the 6mm diameter area N= 41 eyes.

**Aston University**
Life & Health Sciences

**Response from AOREC**

4<sup>th</sup> March 2011

**Our Ref:** AO2010.21

**Project title:** Assessment of the long term effect of cataract removal on the level of macular pigment as measured by heterochromatic flicker photometry and variations in macular thickness related to macular pigment levels

**Application Number:** AO2010.21.R2

**Researchers:**  Professor James Wolffsohn, Mr Martin Smith

Ophthalmic Research Group

Aston University, B4 7ET

Dear Professor Wolffsohn,

On behalf of the Audiology/ Optometry Research Ethics Committee (AOREC), I am pleased to inform you that the AOREC are happy to give approval to the above study and the following documents:

- Research Protocol macular pigment and OCT final revised after reviewer.docx (dated: 25$^{th}$ February 2011)
- Consent_form_martin_smith_revised after reviewer.docx (dated: 25$^{th}$ February 2011)

The details of the investigation will be placed on file. You should notify the Committee of any difficulties experienced by the volunteer subjects, and any significant changes which may be planned for this project in the future.

Yours sincerely,

Dr Leon N. Davies

Chair AOREC

Research workers, school and subject area responsible

Mr. Martin Smith, School of Life & Health Sciences, Optometry, Aston University, Birmingham.

Project Title

**Assessment of the long term effect of cataract removal on the level of macular pigment as measured by heterochromatic flicker photometry and variations in macular thickness related to macular pigment levels.**

Invitation

You are being invited to take part in a research study.  Before you decide it is important for you to understand why the research is being done and what it will involve.  Please take time to read the following information carefully.

What is the purpose of the study?

The purpose of this study is to determine whether cataract removal has any effect on the level of pigment in the macular region of the retina. This pigment is important for detailed vision. We also want to know if there is a relationship between macular pigment levels and retinal thickness. The levels of this pigment may be affected by the increase in light levels reaching the retina in an eye with an artificial lens which is implanted during a cataract operation. Macular pigment protects the retina from blue light damage and low levels may be harmful in the long term.

Why have I been chosen?

You have been chosen because you have an artificial lens in one eye and a natural lens in the other.

What will happen to me if I take part?

By volunteering to participate you will be invited to perform a quick, painless test similar to the visual field examination which you perform during your normal eye test called an MPOD test which determines how dense the pigment in the macular region of your eyes is. The test will take about around 10 minutes and will be performed twice on each eye with a short gap in between. The test involves detecting flickering lights on a screen. The measurement of macular thickness involves a very quick, painless test called Optical Coherence Tomography which scans the back of the eye with red light. You will only need to do these tests on a single visit to the practice which will usually be during your routine sight test or at an alternative time to your convenience if you prefer. You will also be giving everybody in the research team consent to analyse your results and compare them other people. If the tests show any deficiency in macular health or pigment levels appropriate advice will be offered on how to you.

Are there any potential risks in talking part in the study ?

There is a risk of breaching privacy and confidentiality in relation to your test data. This will be minimised by keeping your data anonymous at all times. As a member of practice staff, Mr. Smith has access to your patient records. He will be responsible for putting your results onto a database and maintaining your privacy and confidentiality. Other members of the research team will only be given access to the database after your identity has been removed from it. The procedures themselves are painless and cannot cause any harm.

Do I have to take part?

No, you do not have to participate if you do not wish to do so. You are free to withdraw at any time from the project without reason or explanation.

Expenses and payments:

There are no expenses or payments for participation in this project.

Will my taking part in this study be kept confidential?

Yes, your participation in the study will be fully confidential. There will be no way to link any research data to any individual participant. Research data will be destroyed after 7 years.

What will happen to the results of the research study?

We aim to publish the results of this research. However, there will be no reference to any individual's results in any publication. Copies of the published research will be available on request to all patients.

Who is organising and funding the research ?

Martin Smith Opticians Ltd.

Who has reviewed the study?

The research has been approved by the Audiology and Optometry Research Ethics Committee at Aston University.

Who do I contact if something goes wrong or I need further information?

Please feel free to contact Martin Smith on 01522 521100 or martin@martinsmithopticians.co.uk or Prof James Wolffsohn on j.s.w.wolffsohn@aston.ac.uk

Who do I contact if I wish to make a complaint about the way in which the research is conducted?

If you have any concerns about the way in which the study has been conducted, then you should contact Secretary of the University Research Ethics Committee on j.g.walter@aston.ac.uk or telephone 0121 204 4665.

**Assessment of the long term effect of cataract removal on the level of macular pigment as measured by heterochromatic flicker photometry.**

Name of Chief Researcher: Mr. Martin Smith

|   |   | Tick Box |
|---|---|---|
| 1 | I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. |   |
| 2 | I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. |   |
| 3 | I agree to take part in the above study. |   |

_____    _____         _____

Name of volunteer                                Date                              Signature