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# Macular pigment optical density measurements by one-wavelength reflection photometry – Influence of cataract surgery on the measurement results


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***Dedicated to my family***

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## I Bibliographical description

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**Review**:

The current study was designed with the purpose to determine the possible influence of lens opacification on macular pigment optical density (MPOD) measurements.

In 86 eyes of 64 patients with planned cataract surgery the MPOD was prospectively measured before and after the implantation of a blue-light filtering intraocular lens (AlconSN60WF). For MPOD measurements the one-wavelength reflection method by modified fundus camera Visucam 500 Carl Zeiss Meditec AG was used. The median of the maximum optical density (MaxOD) and the median of the mean optical density (MeanOD) measurements of macular pigment across the subject group were evaluated.

Statistically significant deviations were established between pre-operative and post-operative MPOD measurements, the absolute values were generally lower after cataract extraction. Larger changes were observed in elderly patients and in patients with progressed stage of cataract.

In conclusion, cataract presented a strong effect on MPOD measured by one-wavelength reflection method. Particular care should therefore be taken when evaluating MPOD using this method in elderly patients with progressed stage of cataract. Future optimization of correcting parameters of scattered light and consideration of cataract influence may allow more precise evaluation of MPOD.

## II List of abbreviations

| | |
|---|---|
| AMD | age-related macular degeneration |
| i.e. (id est) | that is |
| e.g. (exempli gratia) | for example |
| et.al. (et alii) | and others |
| L | lutein |
| MaxOD | maximum of the optical density |
| MeanOD | mean of the optical density |
| Meso-Z | meso-zeaxanthin |
| MP | macular pigment |
| MPOD | macular pigment optical density |
| n | number of (eyes) |
| nm | nanometer |
| ODU | optical density units |
| RPE | retinal pigment epithelium |
| SD | standard deviation |
| VEGF | vascular endothelial growth factor |
| vs | versus |
| Q1 | lower quartiles |
| Q3 | upper quartiles |
| Z | zeaxanthin |

# 1. Introduction

## 1.1. Age-related macular degeneration

Age-related macular degeneration (AMD) is known to be the one of the leading causes for reading disability and irreversible blindness in the elderly population of the western world [1]. At the initial stages, in particular central vision is affected, which is responsible for important daily tasks allowing a person to recognize faces, read a book or newspaper and/or drive a car.

### 1.1.1 Risk factors

For development and progression of AMD several risk factors have been introduced. Thus, for example, advanced age has been shown to be associated with growing prevalence, incidence and progression of this disease [2-7]. Systemic risk factors of AMD development include, among others, history of cigarette smoking [8-9], obesity [10] and cardiovascular diseases [9, 11-12]. Also several genetic factors influencing biological pathways e.g. complement processes, angiogenesis and lipoprotein pathways as well as family history of AMD have been shown to be related to AMD [11, 13-27]. Hyperopic refraction was shown to be an ocular risk factor for developing of the AMD [28]. Previous cataract surgery, iris color, ethnicity and gender have been inconsistently reported to be further risk factors [9, 29-30].

### 1.1.2 Classification and pathomechanisms of AMD

Although currently multiple classification schemes and grading systems for AMD have been developed [31-39], at present no universally accepted precise staging scale exists. Therefore and considering the fact that the classification proposed by the Age-Related Eye Disease Study is now increasingly used [40-41], in present report this classification is presented to emphasize the growing importance of AMD and diagnostic of this disorder. According to this classification the stages of AMD can be categorized in general as early, intermediate and late. The late, which is also named advanced stage, is divided into “dry” and “wet” forms.

In the pathomechanisms of AMD the transport processes of retinal pigment epithelium (RPE) have been assumed to play an important role [42]. RPE is a part of the blood-ocular barrier and lies posterior to the photoreceptors. The main functions of the RPE are cytokine secretion, photoreceptor phagocytosis and nutrient transport of fluids and ions between photoreceptors and the choriocapillaris [43].

Thus in the early and intermediate stages of AMD the transport of nutrients and wastes slows down and this results in increasing accumulation of wastes under the RPE. There they form yellow deposits, which are called drusen [44-45]. In order to carry on with pathogenesis of AMD the term of drusen should be depicted. Drusen are classic initial lesions of early stages of dry AMD and can be categorized as hard and soft drusen on the basis of the appearance of their borders. Hard drusen are sharply demarcated small lesions and have discrete margins [46]. They are deposits of cholesterol and other materials below the RPE. Soft drusen have indefinite borders, they are large and confluent [46] and are commonly assumed to be precursors of advanced AMD [47]. Besides, strong correlation was found between the presence of reticular pseudodrusen and geographic atrophy in dry AMD. The association between these lesions suggests that reticular pseudodrusen are an early manifestation of the process which is leading to progression of geographic atrophy in dry AMD [48].

The early stage of AMD is characterized by the presence of few small or medium sized drusen or retinal pigmentary abnormalities. Additionally, areas with hyperpigmentation and hypopigmentation can be presented in RPE. With further slowing down in RPE transport the overlying photoreceptors become damaged leading over time to blindness of the eye. To sum up, such drusen, hyperpigmentations or small hypopigmentations, without visible choroidal vessels can be characterized as early stage of AMD. Patients with early AMD are usually asymptomatic and have generally mild visual loss.

The intermediate stage represents at least one large druse or large number of medium sized druses with pigmentation disorders. While during the early stages of AMD visual symptoms do not attract attention, in following stages severe loss of vision is usual [49].

The late, also known as advanced stage of AMD, is divided into non-neovascular ("dry", atrophic, or nonexudative) and neovascular ("wet" or exudative) AMD.

Advanced non-neovascular ("dry") AMD shows drusen or "geographic atrophy", which is extending to the center of the macula. Such "geographic atrophy" starts with sharply demarcated hypopigmented area with large visible choroidal vessels, which shows progressive atrophy of the RPE, photoreceptors and choriocapillaris [50]. Patients get clinical disorders very slowly, thus gradual visual loss over the course of months to years is not rare. Such slow progressive loss of the vision over many years tends to cause gaps in an image, which are mostly remarked by reading the text [42]. The fovea can be spared until late in the disease.

Advanced neovascular ("wet") AMD is stage of AMD which shows the proliferation of new vessels, either under the RPE or breaking through the RPE named choroidal neovascularization, or within the neural retina known as retinal angiomatous proliferation. This proliferation of new vessels results in different stage in exudates like fluid, lipids and blood, fibrous scarring and detachment of the RPE from the choroid, which on their part can lead to death of the photoreceptors [51-58].

The damage of the RPE and chronic inflammatory response are shown by de Jong to be responsible for retinal atrophy and expression of angiogenic cytokines such as vascular endothelial growth factor (VEGF) [42, 43]. In recent years the importance of VEGF in the pathomechanism of AMD has been developed. VEGF is a key regulator of angiogenesis and can lead to determining of vascular growth and neovascular regression [59]. Thus, it is believed to be substantial for choroidal neovascularisation and development of retinal angiomatous proliferation [60].

As a result, patients develop rapid visual loss describing in particular worsening of central vision. Typically the objects in that portion of the visual field appear distorted or wavy, which is clinically known as metamorphopsia [61-62]. Additionally patients complain about dark patch in central vision named scotoma. If untreated, progression can be fast, resulting in very poor visual acuity. Also sudden visual loss within couple of days to week is typically. The reason for such fast vision loss is usually fluid accumulation secondary to choroidal neovascularization or subretinal hemorrhage.

### 1.1.3 Treatment:

Summarizing the above mentioned, it can be emphasized that underlying mechanisms of pathogenesis of AMD remain until today incompletely understood [81]. On the other side, it is well known that photoreceptor cells cannot regenerate [82] and therefore the promising therapy of AMD is possible only for certain stages.

Lifestyle and dietary modifications including inter alia control of blood pressure and body-mass index as well as smoking determination are important treatment strategies for all stages of AMD. Besides increased dietary intake of antioxidants, beta-carotene vitamine C and E and zink n−3 long-chain polyunsaturated fatty were shown to decrease the risk of the development of neovascular AMD [63-64].

Supplemetation

High dietary intake or supplementation of lutein, zeaxanthin and antioxidants results in relevant increase in macular pigment, which appears to lower the risk of AMD and improve human visual function [66-69]. Furthermore a positive influence of supplementation of lutein and zeaxanthin on drusen morphology as well could be shown [65].

Also the Lutein Antioxidant Supplementation Trial study showed that an enhancement of the protective MP layer leads to improvement in vision acuity in elderly man patients for the period

of 12 months. Furthermore the greatest changes in MPOD appeared in subjects with lowest initial MPOD [70].

Intravitreal antiangiogenic therapy is the primary treatment for neovascular AMD. During this procedure anti-VEGF agents ranibizumab (Lucentis, Novartis), aflibercept (Eylea, Bayer) and bevacizumab (Avastin, Roche) are injected directly into the vitreous. In recent years such antiangiogenetic agents become increasingly well-established therapies and have almost replaced earlier treatment options such as Ocular Photodynamic Therapy or Laser Photocoagulation [91].

Ocular Photodynamic Therapy is characterized by intravenous infusion of photosensitive drug, named verteporfin, which accumulates preferentially in neovascular membranes and its activation with the use of a 689-nm laser beam focused over the macula, followed by dye activation with infrared light. The activation results in the formation of cytotoxic oxygen species [71], which in their part damage the cellular structures of endothelium with following platet activation, causing localized choroidal neovascular thrombosis within the treated area [72]. In neovascular age-related macular degeneration such described photodynamic therapy is assumed to limit the visual loss [73].

Argon-Laser Photocoagulation Therapy was once the most common therapy for neovascular age-related macular degeneration [74]. During the procedure, a laser is used to finely cauterize ocular blood vessels. Due to the fact of possible creation of large retinal cicatrice through the treatment itself, as well as the lack of vision gain and the high recurrence rates, today it is used only incidentally for discrete small extrafoveal choroidal neovascularisation lesions, which are localized distant from the fovea [75].

Vitreoretinal surgery including, for example, surgical extraction of choroidal neovascularization was researched before and was shown to have poor efficacy [76] Studies investigating subretinal injection of tissue plasminogen activator combined with intravitreal air injection [77-79] as well as those researching the effect of surgical relocation of the macula [80] showed improved visual outcomes after several months of follow up period. However, due to the potential for complications and rate of recurrence of choroidal neovascularisation as well as to the deficiency of long-term studies data, vitreoretinal surgery was relegated by others described treatment procedures.

Some benefit for retinal angiomatous proliferation by combinations of treatment processes e.g. anti-VEGF agents, focal laser and photodynamic treatment were found in case studies [75].

To sum up, incomplete comprehension of pathomechanisms of AMD and consequently a lack of the "gold-standard" therapy of this disorder are main reasons why treatment strategies of AMD focus on preventing the progression of the disease process as, for example, by supplementation or dietary intake of lutein and zeaxanthin. Furthermore, it has been considered that macular pigment (MP) is playing an important role in diagnostic and treatment methods of macular diseases. Considering this fact the following chapter concentrates on the constitution and characteristics of the MP.

## 1.2. Macular pigment

Macular pigment (MP) has become the focus of much research in recent years. It is a yellow carotenoid pigment located temporal to the optic disc on the fundus in the posterior pole of the retina [83-84]. The foveal region with MP is assumed to have influence on the central vision [85], because it contains the densest concentration of photoreceptors within the retina. MP consists of lipid-like molecules, which are named retinal carotenoids and include lutein (L), zeaxanthin (Z), and meso-zeaxanthin (meso-Z). Carotenoids are part of natural pigments which are synthesized de novo by plants and some microorganisms for the photoprotection and coloration [86]. Considering this and due to the fact that humans are not able to synthesize carotenoids, MP is completely of alimentary origin. L and Z have the ability to cross the blood-retinal barrier to accumulate in the central or macular retina [87]. As carotenoids contain oxygen they also are named xanthophylls.

According to previous studies, MP is located in receptor axons and in the inner plexiform layer [88]. The highest density of the MP is concentrated in the foveal region of the macula. Here they are commonly assumed to act as antioxidants and as optical filters for blue light, the most phototoxic region of the visible spectrum [89]. The MP decreases approximately exponentially toward the periphery [90]. Meso-Z is found only in the central macula, but neither in the diet nor in serum. Consequently it is assumed that the meso-Z is generated at the retina folllowing L isomerization [87].

## 1.3. The role of the macular pigment

The increased interest in MP in recent years has been justified by its characteristics as well as by its commonly assumed protective role in the fovea. The role of MP in various aspects of visual performance was evaluated and reported by several studies. Especially the role of color vision, contrast and glare sensitivity, and photostress recovery are attributed to MP [92-98].

Furthermore, the positive association of the MP with the best corrected visual acuity, which was reported in numerous studies [92, 94-95], suggests its possible role in optimization of visual acuity. The visual acuity for their part relates strongly to a quality of life [99], which emphasizes the importance of MP role.

As mentioned above, a widely shared assumption exists, that MP plays protective and preventive role in relation to the pathogenesis of AMD. Its putative protective role is based on a combination of its anatomical, chemical and physical properties. The first one is the commonly assumed ability to absorb energy-rich blue radiation of wavelengths of 390 until 540nm acting like an optical filter. Second one is working like a powerful antioxidant by scavenging of free radicals formed by oxidative stress [100-102]. Thus, Stahl and colleagues showed in their research that small concentration of carotenoids of the macular pigment, which are acting like natural radical scavenger, might be a further risk factor for increasing efficacy of free radicals [103].

Summing up macular pigment is a part of the retina, which is responsible for the high spatial resolution, sharpest visual acuity, colour vision and protection against macular diseases [92].

Since age is the major risk factor for the development of AMD, a following question to answer comes up. Is there a possible age-dependence of the MP, measured as macular pigment optical density (MPOD)? This question was the subject of research in numerous studies over the years [104-120], but no clear conclusion has been reached, primary because of different methods to measure MP employed across studies. For example, Berendshot and Van Norren declared after comparing values measured with different methodes (two setups for Fundus Reflectance Spectroscopy, Scanning Laser Ophthalmoscope for obtaining MP reflectance and Scanning Laser Ophthalmoscope for depiction of autofluorescence maps), that there was no significant age-effect influencing the optical density of the MP [104]. Only Heterochromatic Flicker Photometry (HFP) showed a slight decrease of measured MPOD with increasing age [104]. Similarly, Loan et al. found a small decrease of MP with increasing age when measuring MPOD by using HFP [105]. On the other hand, Ciulla reported that even when elderly subjects with cataracts and AMD are enclosed; MP measured with HFP did not change significantly with age when analyzed across the group [106].

A large number of other previous studies based on Autofluorescence [107], Fundus Reflectometry [108], psychophysical methods [109], or High-performance Liquid Chromatography [110] examined MPOD changes in relation to age. Although the results varied, the majority of the studies, which reported on the age effect on MPOD, presented with an age-related decline in MPOD [81, 111-118, 122, 121]. Some other studies, on the other hand, which measured MPOD using High-performance Liquid Chromatography, Fundus Reflectometry or

methods based on HFP did not detect any age-related difference in MPOD [104, 110, 119-120], even when elderly subjects with cataracts were considered [106].
To sum up, statistically significant change in MPOD was investigated by numerous studies in relation to the age, but there exist on the other hand also a lot of studies that have investigated no relationship between age and MPOD. The discrepancy in results of the different studies may be related to differences in subject selection, methods of measurement or size of the sample. Considering the fact, that the age-dependence remains unsettled topic until today, further investigation of this possible relation was among other things the subject of the present study.

## 1.4. Importance of the measurement of the macular pigment

Due to the importance of MP and its functions, the question arises, why MP measurements have been considered important? The size and distribution of MP, measured as MPOD, are believed to correlate with some risk factors for macular diseases in general. Less MP might result in an increased risk of developing the disease and vice versa. Thus, the longstanding suggestion, that MP makes a significant contribution to protection against the AMD, can be supported by following facts:

- Analysis of retinas of human donors with AMD and eyes in healthy people with high risk for AMD (like history of smoking or family predisposition) show lower levels of MPOD [81, 115, 122].
- Compared to the healthy subjects reduced MPOD levels were found by the patients with an early stage of AMD [123].
- Wüstemeyer detected in his study, using a modified Scanning Laser Ophthalmoscope, statistically significant higher MPOD values in healthy subjects in comparison with patients with dry AMD [124].

Considering these facts, MP may give information about the possible risk situation relating to macular diseases.

Furthermore, growing evidence exists that MPOD can be increased by a carotenoid-rich dietary modification [125-126] or by the ingestion of supplements like L and Z by healthy subjects and patients with beginning stages of AMD [67, 68, 125]. In this way, dietary intake or supplementation of lutein and zexanthin could give some degree of protection or could potentially slow the progression in subjects with family predisposition or early stage of disease. Thus, the longstanding suggestion of the importance of MP monitoring over the time can be confirmed by several studies, which identify the long term influence of supplementation on the course of the disease and on drusen morphology [65-69,127-128]. Considering these facts, the

monitoring of supplementation or dietary intake of L and Z by repeated measurements of MP over time might be important to track the effects of a particular treatment.

To sum up, the monitoring measurements of MP are potentially useful to uncover predictors of particularly early stage of AMD and to provide information about the disease progression and success of supplementation with L and/or Z or any other treatment.

## 1.5. Methodes for measurement of macular pigment

Since the importance of MP measurements became more understandable, various different methods to measure the MP were developed. Thus, MPOD can be measured in vitro or in vivo.
High-performance Liquid Chromatography [129] and Microdensitometry [130] are techniques to measure the MPOD in vitro. These techniques are not suitable to common use in everyday clinical practice. For this issue there are two categories for in vivo measurement of optical density of the MP. The first category includes psychophysical methods that require the active response from the subject. Those are Heterochromatic Flicker Photometry and Minimum Motion Photometry. These are also the most commonly used methods.
The second group contains objective methods, which require minimal input from the subject and includes Fundus Autofluorescence, Resonance Raman Spectroscopy, Fundus Reflectometry and other. At present there is no technique that could be characterized as a true "gold-standard" for the measurement of MPOD. Different studies employed different subjective or objective measurement techniques, which is one of the main reasons why values for measured MPOD vary across research reported. Also the macular carotenoid concentrations, which can be described by measuring of MPOD, vary widely among individuals. The following section concentrates on the short introduction of advantages and disadvantages of measure methods.

The Heterochromatic Flicker Photometry is the most commonly used psychophysical method to measure MP in clinical research. Heterochromatic Flicker Photometry is noninvasive, low-priced and there is no need for pupil dilation [126]. On the other side its main disadvantage is a dependence on the patient's ability to comprehend the task. Therefore examiner and patient need to learn the examination skill and procedure before the measurement [131]. Furthermore the experimental conditions, e.g. head or eye movement or flicker frequency may affect the accuracy and measured values of MPOD [132]. Considering these facts this method is unsuitable for some individuals like children or elderly with insufficient visual fields or people with insufficient visual acuity or difficulty to learn and / or to communicate [132]. Advantages of the

Motion photometry are similarly to those of HFP. No pupil dilation is needed. The main disadvantage is that a good comprehension of the task by the subjects is required.

To carry on with introduction it is important to explain also the characteristics of the objective methods.

A fast measurement can be obtained with Fundus Autofluorescence. This objective method uses the fluorescence of lipofuscin which is located in the RPE cells [133-135]. Lipofuscin has the ability to absorb short-wavelength radiation between 400-580nm. Advantage of Fundus Autofluorescence is no need of subject participation and for this reason it is applicable to many subjects' populations. Measurements of MP distribution are within a short period of time possible. Disadvantages of Fundus Autofluorescence are the need for pupil dilation, expensive equipment and unpleasant light levels during the measurement. Furthermore the measurements of this method might be influenced by lens opacities [132].

The main difference of Resonance Raman Spectroscopy in compare to other techniques is that it measures absolute levels of MP in a 1mm (3.5°) area, without any peripheral consideration. Advantages are rapid measurements that provide detailed spatial distribution of the MP and that are possible also in individuals with reduced visual acuity. Disadvantages are needed pupil dilation, questionable validity, expensive and highly specialized equipment, attenuation of the Raman signal by changes in ocular media. Furthermore, Raman counts are not simply convertible to MP optical density units (ODU), therefore the direct comparison with other techniques might be complex.

Fundus Reflectometry is another fast objective method that may be used also for elderly handicapped patients. The measurement of reflected light from the retina and choroid makes it possible to assess the MP by Fundus Reflectometry [136-138]. The distribution of the MP is shown on density maps. Disadvantages of Fundus Reflectometry are the need of pupil dilation and unpleasant light flash because of the necessity of photopigment bleaching [132].

In 2010 Schweitzer and co-workers introduced a new simple objective method based on one-wavelength reflection fundus imaging. Despite the fact that one-wavelength reflection method is a simplified one, significant correlation was found in comparison with two –wavelength-autofluorescence method for determination of the optical density of MP [139]. Considering this fact, the measurements in the current study were carried out based on using this objective method.

The principe of this method is based on one-wavelength reflection photometry, which employs the local and spectral selectivity of xanthophyll. The local selectivity indicates that xanthophyll is traceable only in a certain foveal region. Spectral selectivity means the ability of xanthophyll to

absorb blue light for wavelength shorter than 540nm. During the measurement the fundus is illuminated with blue light. Under illumination with the wavelength at 480 nm, which is near the absorption maximum of xanthophyll, the fundus can be determined as a uniform reflecting surface, where the increased absorption in the region of fovea can be considered. The logarithmic ratio of the virtual fundus reflection below the macular pigment compared to the macular reflection leads to the optical density of the MP. Hereby the fundus reflection and illumination inhomogeneity are accounted for by a shading function. The logarithmic ratio of every pixel results in spatial distribution of the optical density of MP. Area, in which the optical density exceeded a defined threshold, volume as a sum of the density of all pixels, maximal optical density and mean optical density over all pixels are four parameters, which describe the spatial distribution of MP.

## 1.6. Possible factors with influence on the measurement results

Macular pigment measurement is generally affected by the density of the media as the light must go through tissue. Such eye media include e.g. cornea, aqueous humor, lens and vitreous. As previously established, these optical media scatter the light to a certain degree due to their biological configuration [140]. The scattering and absorption of the light due to this described effect increase with rising patient age. It has long been recognized, that it is in particular the crystalline lens that tends to influence the transmittance most strongly [141-142]. The stray light of crystalline lens is also known to increase with the patient age [143]. A lot of studies reported the exponential change in the amount of scatter with age. There is an increase of insoluble and soluble material with age in the human lens, which is believed to be responsible for this age-dependent increase in scatter [144].

Moreover, in-vitro performed examinations of the lenses from donor eyes showed an increased scattering with increased degree of cataract [145]. Thus, the lens appears to be an important source of stray light and measurements of MPOD could be affected by, for instance the light loss due to reflection and scattering by the dense lens [146-147]. The human crystalline lens owns the property to yellow with increasing age. Because of this fact the older lens transmits less blue light than a younger one [148]. It might provide a part of the physiological protection against AMD at this stage of life [149]. In other words, the cataract absorbs the blue light, which may affect the accuracy of MPOD measurement. In this way measurements of MPOD in cataract patients might be influenced by lens opacities. So, when taking measurements of MPOD by the patients with dens lenses it is important to know the cataract influence on the results.

Previous investigations for MPOD measurements using Autofluorescence Spectrometry showed reduced signals in a 488-nm autofluorescence image by cataract presence [142, 150-153].

Also the analysis of MP by Raman-scattering method [122, 121, 154] seems to be dependent on accurate data of the transmission of ocular media, in particular by testing the elderly patients. On the other hand it is assumed that psychophysical techniques remain unaffected by individual differences in opacity of the crystalline lens and could be used to measure the MPOD also in elderly subjects, even in those with presence of cataract [155]. However, the results of different published reports are inconsistent. Therefore, present study aimed to compare MPOD measured by one-wavelength reflection method before and after implantation of blue-light filtering intraocular lens in cataract patients. A possible future correction of individual differences in pre-retinal light loss may be important to render MPOD more precisely.

## 1.7. Cataract and its role as a possible influence factor on measurement results of MPOD

Enormous importance for medicine and economy of cataract today is justified not only by the fact that lens opacity is a most common cause of vision decrease and blindness worldwide but also by the frequency of its surgical procedure.

To carry on with most important possible disrupting factor for the MP measurement, the pathomechanisms of cataract should be enlightened. The lens is cellular organ and its transparency is due to its protein formation and complex architecture. The pathogenesis of cataracts in humans crystallized to be a multifactorial process. Large number of different factors e.g. general diseases (diabetes, uraemia, metabolic disorders), nutritional deficiencies or disturbances, eye diseases (glaucoma, uveitis, intraocular infections), UV-irradiation, as well as a life style (smoking, alcohol consumption) and drug side effects especially from drugs taking as long-term therapy(e.g. corticosteroids, phenothiacines, diuretics) are supposed to be responsible for the formation of cataracts [156]. It is commonly assumed that the changes in proteins or macromolecules inside the crystalline lens are responsible for the changes in the opacity of the lenses as well as in their physical properties [157]. One of the numerous molecular processes, leading to age-related cataract, is a protein unfolding, that results in protein aggregation and changed interaction, the second one is association between native crystallins [158].

The interaction of denatured proteins with fiber cell membranes increases with the age, particularly in the fifth decade of life. This process can influence the proper functioning of membrane pores with decreased diffusion of small molecules within the lens. Furthermore, the agglutination of membrane fragments as well as occlusion of pores as a result of binding of

crystalline aggregates to the fiber cell membranes, appear to contribute to the development of cataract [159]. The Beaver Dam Eye Study showed that especially nuclear, cortical and posterior subcapsular cataract increase with the age. The total incidence of these three forms of age-related cataract account about 45% for people between the age 55-64 and about 75% for people between age 65 and 74 and about 88%for people older than 75 years of age [160]. The recent outcomes showed an increase in incidence of cataract surgery in persons older than 65 years over the past 20 years [161]. Simultaneously with growing incidence of cataract, increase the number of carried out cataract surgeries. Furthermore cataract surgery is a most frequently performed surgical procedure in medicine today not least due to the fact of its rapidness in the performance but also by the fact that it is the one and only treatment possibility. The clinical importance of cataract surgery is emphasized amongst others particularly by the strong relation between vision-related quality of life, cognitive impairment and mental status of patients. Cataract extraction significantly improves vision-related quality of life in elderly patients. The cognitive impairment and (depressive) mental status had been shown to improve in parallel [162].

To sum up, ageing of the lenses accompanies the balance between processes of molecular damage, cytoprotection and consumption of non-renewable resource [163]. Due to the facts of rising life expectancy and ageing of the population, the incidence of cataract and cataract surgeries is growing and is expected to increases in the near future. These are essential reasons, which justify closer inspection of cataract influence on the MP measurements taken over longer period of time.

## 1.8. Main objective of the present study

To sum up, the effect of possible light absorption, reflection and scattering by the dens lens and therefore the influence of cataract on the measured MPOD values have not been adequately determined. Thus, the main research questions of current study were concerned with the investigation of the influence of different grade of lens opacity and patient age on MPOD measurements, carried out using one-wavelength reflection method . Furthermore also the possible effect of cataract surgery on values of MPOD was evaluated. Thereby MPOD of the same subjects were measured before and after the implantation of blue-light filtering intraocular lens. A future correction of individual differences in preretinal light loss can be important to render MPOD more precisely.

## 2. Publication

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RETINAL DISORDERS

# Macular pigment optical density measurements by one-wavelength reflection photometry—influence of cataract surgery on the measurement results

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**Abstract**
*Purpose* The main objective of the present study was the investigation of possible influence of lens opacification on macular pigment optical density (MPOD) measurements.
*Methods* Eighty-six eyes of 64 patients (mean age 73.4 ± 8.3 years) were included in the study. MPOD was prospectively measured using the one-wavelength reflection method (Visucam500, Carl Zeiss Meditec AG) before and after cataract extraction, with implantation of a blue-light filtering intraocular lens (AlconSN60WF). The median of the maximum optical density (MaxOD) and the median of the mean optical density (MeanOD) measurements of macular pigment across the subject group were evaluated.
*Results* Statistically significant differences were noticed between pre-operative and post-operative measurements, the absolute values were generally lower after cataract extraction. The following median (lower/upper quartile) differences across the group were determined: MaxOD −33.8 % (−46.2 to −19.1 %), MeanOD −44.0 % (−54.6 to −26.6 %). Larger changes were observed in elderly patients [<70 years of age ($n$=25 eyes): MaxOD −13.4 % (−20.5 to 3.6 %), MeanOD −23.6 % (−30.5 to −15.3 %) versus patients ≥70 years ($n$=61 eyes) MaxOD −40.5 % (−53.2 to −30.1 %), MeanOD −47.2 % (−57.8 to −40.1 %)] and in patients with progressed stage of cataract. MaxOD for lens opacification grade 1 ($n$=9 eyes): −27.4 % (−42.1 to −19.6 %), grade 2 ($n$=26 eyes): −35.0 % (−44.2 to −25.3 %), grade 3 ($n$=21 eyes): −34.4 % (−45.4 to −11.4 %), grade 4 ($n$=25 eyes): −32.6 % (−53.2 to −6.4 %), and grade 5 ($n$=5 eyes): −53.5 % (−61.7 to −38.7 %) and MeanOD for cataract stage 1 ($n$=9 eyes): −42.6 % (−46.0 to −26.0 %), stage 2 ($n$=26 eyes): −44.1 % (−51.8 to −26.2 %), stage 3 ($n$=21 eyes): −45.7 % (−54.7 to −24.7 %), stage 4 ($n$=25 eyes): −39.5 % (−59.4 to −26.1 %), and stage 5 ($n$=5 eyes): −57.0 % (−66.1 to −51.4 %).
*Conclusions* As established by comparison of pre- to post-operative measurements, cataract presented a strong effect on MPOD measured by one-wavelength reflection method. Particular care should therefore be taken when evaluating MPOD using this method in elderly patients with progressed stage of cataract. Future optimization of correcting parameters of scattered light and consideration of cataract influence may allow more precise evaluation of MPOD.

**Keywords** Cataract · Macular pigment · One-wavelength reflection method · Lutein · Zeaxanthin · Age-related macular degeneration

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## Introduction

Macular pigment (MP) has become the focus of much research in recent years. The interest is focused on the protective role of MP as well as on the ability to measure the macular pigment optical density (MPOD) with different methods in vivo.

MP is a yellow carotenoid pigment which is located in receptor axons and in the inner plexiform layer of the retina [1–3]. The highest density of the MP is concentrated in the foveal region of the macula, and decreases approximately exponentially towards the periphery [4]. MP consists of retinal carotenoids lutein, zeaxanthin, and meso-zeaxanthin. Carotenoids are part of natural pigments which are synthesized de novo by plants and some microorganisms for photoprotection and coloration [5]. Due to the fact that humans are not able to synthesize carotenoids, MP is entirely of alimentary origin.

The role of MP in various aspects of visual performance has been evaluated and reported by several studies. In particular, the role of visual acuity, contrast and glare sensitivity, photostress recovery, and color vision are attributed to MP [6–12]. However, the increased interest in MP in recent years has been justified not only by these characteristics but also by its protective role in the fovea, where MP is commonly assumed to act as an antioxidant and as an optical filter for blue light, the most phototoxic region of the visible spectrum of the light [13–15].

Although underlying pathomechanisms of various macular diseases (e.g., central serous chorioretinopathy [16], age-related macular degeneration (AMD) [17] and idiopathic macular telangiectasia [18]) are until today not completely understood, it has been considered that MP plays an important role in diagnostic and treatment methods of such conditions.

Reduced MPOD levels were shown by patients with early stage of AMD or a family predisposition for this disease in comparison with healthy subjects [17, 19–21]. Also Wüstemeyer detected in his 2002 study statistically significant higher MPOD values in healthy subjects in comparison with patients with dry AMD [23].

Furthermore, growing evidence exists that MPOD can be increased by a carotenoid-rich dietary modification [24, 25] or by the ingestion of supplements such as lutein and zeaxanthin in healthy subjects or patients with beginning stages of AMD [26–28].

The longstanding suggestion of the importance of MP monitoring over time can be confirmed by several other studies which identify the long-term influence of supplementation on the course of the disease [26, 27, 29–32]. Furthermore, it was possible to show a positive influence of supplementation of lutein and zeaxanthin on drusen morphology [33].

To sum up, the longitudinal measurements of MP are potentially useful for uncovering predictors of particularly early stages of AMD, and for providing information about disease progression and success of supplementation with lutein and/or zeaxanthin or any another treatment.

Since the importance of MP measurements became more understandable, various different methods to measure the MP have been developed. In-vivo measurement techniques of the optical density of MP can be divided into two categories: subjective and objective methods. The first category includes psychophysical methods which require active response from the patient [25, 34, 35]. These are also the most commonly used methods. The second group contains objective methods which require only minimal input from the patient [35–41]. At present there is no technique that could be characterized as a true "gold-standard" for the measurement of MPOD. Different studies have employed different subjective or objective measurement techniques, which is one of the main reasons why values for measured MPOD vary across research reported.

In 2010 Schweitzer and co-workers introduced a new simple objective method based on one-wavelength reflection fundus imaging. Despite the fact that the one-wavelength reflection method is a simplified one, significant correlation was found in comparison with the two-wavelength autofluorescence method for determination of the optical density of MP [42]. Considering this fact, the measurements in the current study were carried out based on using this objective method. The reproducibility of the one-wavelength reflection method has been tested before [42].

MP measurement is generally affected by the density of the media as the light must travel through tissue, e.g., cornea, aqueous humor, lens, and vitreous. As previously established, these optical media scatter the light to a certain degree due to their biological configuration [43]. The scattering and absorption of the light due to this described effect increases with a rising patient age. It has long been recognized that it is in particular the crystalline lens that tends to influence the transmittance most strongly [44, 45]. Stray light of the crystalline lens is also known to increase with the patient age [46], e.g., in-vitro performed examinations of lenses from donor eyes showed larger scattering with increased degree of cataract [47]. Thus, the lens appears to be an important source of stray light and measurements of MPOD could be affected by, for instance, the light loss due to reflection and scattering by the dense lens [48, 49]. In addition, cataract absorbs the blue light which may affect the accuracy of MPOD measurement. For this reason, measurements of MPOD in cataract patients might be influenced by lens opacities. Previous investigations for MPOD measurements using autofluorescence spectrometry showed reduced signals in a 488-nm autofluorescence image by cataract presence [45, 50–53]. Also the analysis of MP by Raman-scattering method [21, 54, 55] appears to be dependent on accurate data of the transmission of ocular media, in particular when older patients are tested. On the other hand, it is assumed that psychophysical techniques remain unaffected by individual differences in opacity of the crystalline lens, and could be used to measure the MPOD also in elderly patients, even in those with presence of cataract [56]. However, the results of different published reports are inconsistent. Therefore, this study aimed to compare MPOD measured by one-wavelength reflection method before and after implantation of blue-light filtering intraocular lens. A possible future correction of individual differences in pre-retinal light loss may be important to render MPOD more precisely.

To sum up, the main research questions of the study were concerned with the investigation of the influence of different grade of lens opacity and patient age on MPOD measurements, as well as the evaluation of the possible effect of cataract surgery on values of MPOD, by measuring MPOD of the same subjects before and after cataract surgery.

## Methods

### Patient characteristics

All subjects were recruited from a cohort of patients with a clinical indication for future cataract extraction. The subjects were invited to participate in the study while they were attending a consultation in the eye hospital. Prior to examination, written consent was obtained from each patient after careful explanation of the nature and consequences of the study, its voluntariness, as well as demonstration of the test procedure. As part of the study, all patients underwent the measurements described during their dilated fundus examination before and after cataract extraction.

The study was approved by clinical ethics committee of the Medical Faculty of Leipzig University, and adhered to the tenets of the Declaration of Helsinki. The recruitment of patients was conducted at the University Hospital Leipzig, Department of Ophthalmology from July 2011 to April 2012.

Inclusion criteria for the investigation comprised lens opacity with planned cataract surgery, alongside with the mandatory additional criterion of possible visualization of the retina and therefore permission of MP measurement, and good general health of the subject. During analysis, eyes with hereditary corneal dystrophy or central geographic atrophy, as well as those with choroidal neovascularisation and exsudative forms of AMD, were excluded from the study results. Patients who had no possibility to come to post-operative measurement were excluded as well.

In total, 86 eyes of 64 patients were included in the current analysis. Age ranged from 51.9 to 89.5 years, with a mean age (± SD) of 73.4 (±8.3 years). The male to female ratio was 28: 36, i.e., 35 male and 51 female eyes were enrolled in the study. In 22 patients, both eyes were considered cataractous, and MPOD was assessed in both.

### Measurement of MPOD

All measurements were carried out in mydryasis (0.5 % tropicamide) in the examined eye. Subjects were asked to position themselves in front of the instrument, and then were instructed to remain steady, look straight ahead and fixate the green cross inside the camera with their tested eye. If the patients had low visual acuity and could not fixate, the outside fixation target was used for their non-tested eye. This is a standard procedure in ophthalmology. A sequence of three single measurements was taken, and for MPOD analysis an average of those was calculated for all parameters. MPOD was first measured once before cataract surgery, then the measurement was repeated 6–8 weeks after surgery. All patients participating in this study were examined by the same person.

For the current study, an objective one-wavelength reflection method measuring the MPOD by Visucam 500, Carl Zeiss Meditec AG was used. This method was previously described by Schweitzer et al. [42]. The main focus of attention in the current report is set on the maximal optical density (MaxOD) and the mean optical density (MeanOD) across all pixels, both measured in optical density units (ODU). It is generally known that with age the lens increases in thickness; stray light increases [46] and the crystalline lens becomes more opaque, which can lead to cataract. These influences can affect MP measurement by the one-wavelength reflection method. For this reason, an automatic age-dependent correction factor for the greyness level of the lens is implemented in the software of the instrument. The post-operative measurements were not affected by these artifacts due to the newly implanted clear lens. Therefore, the post-operative measurements were carried out by setting the"intraocular lens mode" without correction for the opacification of the lens.

### Cataract surgery

All patients underwent planned standard phacoemulsification and implantation of a blue-light-filtering intraocular lens (Alcon SN60WF). All cataract extractions were performed by the same physician in the Department of Ophthalmology, University of Leipzig, on an outpatient basis. All patients had clear corneal incision, continuous curvilinear capsulorrhexis, phacoemulsification, and intraocular lens implantation in the bag. No complications were reported during the surgeries.

### Statistical analysis

Descriptive and statistical analyses were subsequently performed using Minitab statistical software (version 14). Patient age of determined groups, as presented, was expressed as the mean ± standard deviation (SD). To describe and compare the MPOD values the median, lower (Q1), and upper (Q3) quartiles were specified. Correlation between the MPOD and age was evaluated by calculating Spearman's rank correlation coefficient $\rho$. To denote the strength of the linear association between two variables the coefficient of determination $R^2$, expressed as a percentage, was calculated. To estimate the difference in MPOD levels between preoperative and postoperative measurements the non-parametric equivalent of a paired $t$-test, the Wilcoxon test for matched pairs, was carried out. Statistical significant level was set as $p$-values less than 0.05.

## Results

The current analysis included 86 eyes of 64 patients recruited for the purpose of investigation of the relationship between MPOD values measured before and after cataract extraction. Significant differences in MPOD were found between

preoperative and postoperative measurements analyzed. The results indicated a general tendency for lower MPOD measurement levels after cataract surgery. The main focus was placed on two parameters characterizing MP: the maximum optical density (MaxOD) and the mean of the optical density values (MeanOD).

Figures 1 and 2 illustrate the results of MPOD measurements plotted as a function of age in years for all 64 observers. The filled circles represent the absolute values for MPOD obtained for each of the eyes included in the present investigation. Pre-operative MPOD measurements are presented in Fig. 1, the post-operative data is depicted in Fig. 2.

Pre-operative MPOD data of all 86 eyes ranged from 0.15 to 0.53 ODU: median (Q1/Q3) 0.37 (0.32/0.41) ODU for MaxOD, and 0.07–0.24 ODU: median (Q1/Q3) 0.15 (0.13/0.17) ODU for MeanOD.

Post-operative data ranged from 0.12 to 0.39 ODU for MaxOD and 0.04–0.16 ODU for MeanOD. The median values for MPOD (Q1/Q3) were 0.23 (0.20/0.28) ODU for MaxOD and 0.09 (0.07/0.10) ODU for MeanOD.

As presented in Fig. 1, the pre-operative MPOD data contained a statistically significant age-effect. Spearman's correlation coefficient ($\rho = 0.79$) of pre-operative MaxOD and patient age indicated an association ($R^2 = 62.7$ %). Spearman's correlation coefficient of pre-operative MeanOD and patient age was 0.81, which again presented an age affect in the data ($R^2 = 66.3$ %).

No significant age-dependence of MPOD, on the other hand, was found for pseudophakic eyes presented by post-operative measurements (Fig. 2). Spearman's correlation co-efficient $\rho$ of post-operative MaxOD and patient age was −0.19 ($R^2 = 3.6$ %) and $\rho$ of post-operative MeanOD and patient age was −0.18 ($R^2 = 3.2$ %), i.e., no correlations were found.

Due to the fact of considerable individual variability of optical density values between subjects, the following analysis was carried out on the basis of a relative percentage change of optical density to allow cross-comparison. The main results of this analysis are represented in Table 1. The median differences of MP density before and after cataract extraction are shown overall as a group and subdivided for different patient groups. Negative values of relative difference represent hte fact that the measured MPOD values post-operatively are less than the pre-operative ones.

The difference between MPOD levels in patients measured before and after cataract surgery was analyzed by the Wilcoxon test for matched pairs, a statistically significant difference between pre- and post-operative MaxOD and MeanOD measurements across all patients was found ($p < 0.0001$). The post-operative decrease of optical density is represented by a median (Q1/Q3) decrease in MaxOD of 33.8 % (−46.2 to −19.1 %) and MeanOD of 44.0 % (−54.6 to −26.6 %).

In Fig. 3, the relative difference of MPOD values for each eye was plotted against the patient age. Variations in density change among all subjects ranged from −72.0 to 58.9 % for MaxOD and from −76.5 to 27.8 % for MeanOD. Elderly patients presented larger differences between pre-operative and post-operative values with a tendency to lower post-operative values. There was a small correlation between the relative difference of MPOD and patient age. According to Spearman's $\rho$-test, the correlation co-efficient was −0.65 ($R^2 = 41.6$ %) for Max OD and −0.65 ($R^2 = 42.8$ %) for MeanOD. In other words, with increasing patient age the relative difference of the MPOD also showed higher rates with a tendency to smaller MPOD values after cataract removal.

In order to investigate this relationship in more detail, patients were subdivided into groups by age (younger than 70 years of age and 70 years of age or older). Here, elderly

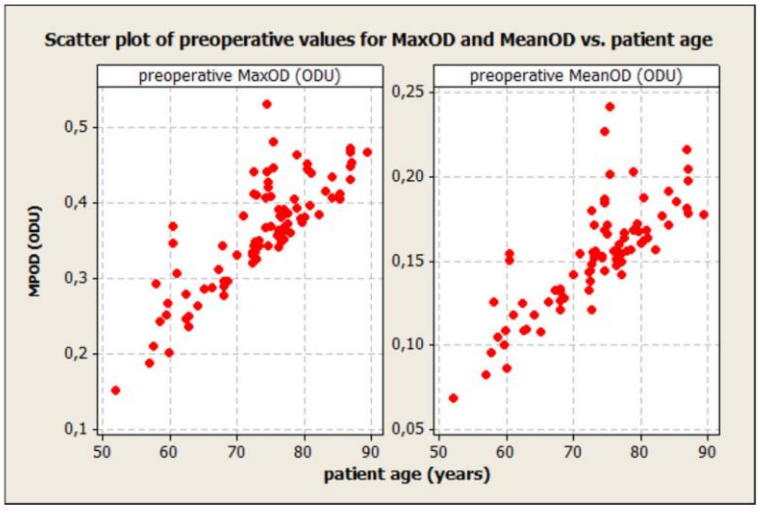


**Fig. 1** Scatter plots of pre-operative macular pigment optical density data as a function of age. Depicted are individual results of 86 eyes for MaxOD and MeanOD plotted versus age of the patient. Pre-operative measurements were obtained with a built-in correction factor of an age-dependent grey-level increase of a typical eye, which aims to account for the effect of cataract on the measurements obtained

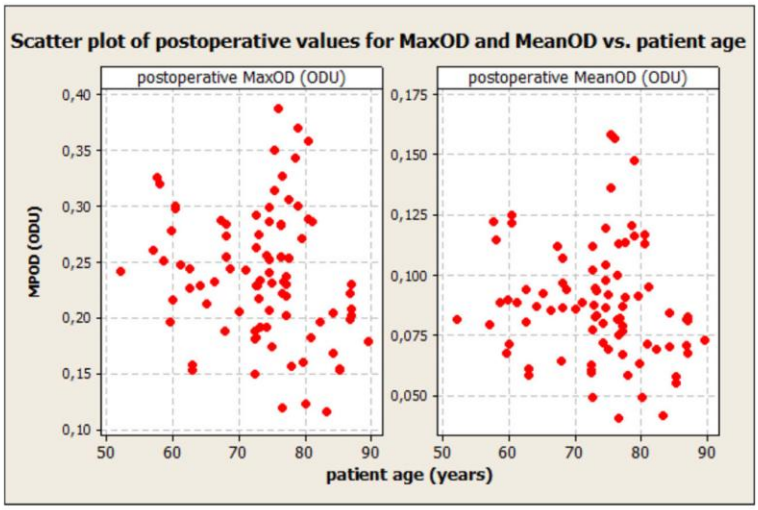


**Fig. 2** Scatter plots of post-operative macular pigment optical density data as a function of age. Depicted are individual results of 86 eyes for MaxOD and MeanOD plotted versus age of the patient. Post-operative measurements were obtained in intraocular lens mode, which accounted for the implantation of a clear lens

patients presented with larger differences between pre-operative and post-operative tests: patients <70 years: ($n$=25 eyes): median change of MaxOD −13.4 % (−20.5 to 3.6 %), MeanOD −23.6 % (−30.5 to −15.3 %) versus patients >70 years: ($n$=61 eyes): median change of MaxOD −40.5 % (−53.2 to −30.1 %), MeanOD −47.2 % (−57.8 to −40.1 %).

In addition to the effect of patient age on the measurements obtained, the influence of the grade of density of the crystalline lens on the MPOD measurements was analyzed. To examine this effect, all tested eyes were subdivided into five groups according to the quality of pre-operative images. In this way, the cataract classification was conducted on the basis of the best pre-operative MPOD images. The following Table 2 summarizes the classification criteria employed.

Furthermore, the influence of patient age and classified lens opacity as a measure for cataract density on the MPOD data was investigated, and is summarized in Figs. 4 and 5. As demonstrated in Fig. 4, elderly subjects (>70 years) showed a higher relative difference of MaxOD between pre-operative and post-operative data. In addition, the relative difference increased with increasing lens opacity. Similar relationships applied for all calculated parameters of MeanOD (Fig. 5).

In summary, it should be noted, that statistically significant differences were detected between pre-operative and post-operative measurements. Classification based on patient age and pre-operative cataract opacity indicated larger differences in measured data for the elderly patients with progressed stage of cataract.

## Discussion

To the best of our knowledge, the presented study is the first investigation on the influence of cataract surgery on MP measurement using the one-wavelength reflection method.

To evaluate the change of MPOD values, obtained before and after cataract extraction, the results of the Wilcoxon test provided the evidence that absolute pre-operative and post-operative MPOD differed significantly from each other. These findings do not reproduce the results of other related studies with heterochromatic flicker photometry [56, 74], which had registered no significant differences in MPOD measurements taken before and after cataract surgery (mean pre-operative MPOD was 0,206±0.13 in comparison to mean post-operative MPOD of 0.18±0.12 [56], and respectively 0.28±0.17 to 0.27±0.16 [75]). According to Ciulla et al., different types of cataract with varying opacity of the crystalline lens did not seem to have a measurable influence on MPOD [56]. Two further studies represented conflicting results. Demirel et al. found mean MPOD values of pseudophakic patients over 50 years of age to be in a similar range but statistically significantly lower than mean MPOD in either reference group of patients with clear lenses (younger and older than 50) when measured with heterochromatic flicker photometry [mean MPOD in patients with clear lenses was respectively 0.57±0.17 log units (younger than 50) and 0.528±0.203 log units (older than 50) in comparison to mean MPOD of 0.40±0.18 log units in pseudophakic patients over 50 years of age] [75]. On the other hand, Sasamoto et al. measured MPOD with two-wavelength autofluorescence spectrometry in a group of 41 subjects (mean age of 71.6, SD±6.7 years) and observed a significant increase in MPOD after cataract extraction in those patients [mean pre-operative MPOD was 0.35 (0.313–0.388) in comparison to mean post-operative data of 0.6 ODU (0.562–0.637), which potentially could be due to their detector sensitivity setting in their study] [49]. The main potential reasons for such inconsistencies could be the difference between measurement methods. The data of the present study suggest that cataract strongly affected the measurement of MPOD by one-wavelength reflection method.

**Table 1** Representation of comparison of macular pigment optical density measurements before and after cataract extraction. Measurements of macular pigment optical density were carried out before and after cataract extraction. The main focus of the analysis considered two parameters: the maximum optical density (MaxOD) and the mean of the optical density values (MeanOD). Results are represented as a median of the relative differences between each individual's pre- and post-operative measurements (relative difference of MaxOD and relative difference of MeanOD in %). The median differences of macular pigment optical density are shown overall as a group (in general for all included eyes) and subdivided for different patient selections (grouped by patient age, grouped by lens opacification grade, grouped by patient age and lens opacification grade). Negative values of relative differences represent the result of smaller post-operative MPOD values in comparison to the respective pre-operative dataset. The influence of age on the relative change in MPOD was investigated in two groups according to each patient's age (<70 and ≥70 years). Lens opacification grade is indicated numerically (1–5) according to the quality of pre-operative images. Number of eyes (*n*) represents the number of tested eyes. Patient age is expressed in years as mean (± standard deviation)

| | | Relative difference of the MPOD | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variable | In general for all included eyes | Grouped by patient age | | Grouped by lens opacification grade | | | | | Grouped by patient age and lens opacification grade | | | | | | | | | | | |
| | | | <70 years | ≥70 years | Lens opacification grade groups | | | | | <70 years | | | | | ≥70 years | | | | | | |
| | | | | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | | |
| Median | Relative difference of MaxOD (%) | −33.8 | −13.4 | −40.5 | −27.4 | −35.0 | −34.4 | −32.6 | −53.5 | −19.0 | −17.5 | 3.3 | −8.3 | * | −36.5 | −37.5 | −39.5 | −47.4 | −53.5 | | |
| | Relative difference of MeanOD (%) | −44.0 | −23.6 | −47.2 | −42.6 | −44.1 | −45.7 | −39.5 | −57.0 | −22.0 | −19.1 | −15.2 | −26.1 | * | −43.4 | −44.9 | −47.4 | −53.7 | −57.0 | | |
| Number of eyes | *N* | 86 | 25 | 61 | 9 | 26 | 21 | 25 | 5 | 2 | 7 | 5 | 11 | 0 | 7 | 19 | 16 | 14 | 5 | | |
| Patient age (years) | Mean | 73.4 | 62.7 | 77.8 | 71.9 | 73.5 | 74.1 | 71.9 | 80.7 | 61.4 | 63.2 | 60.9 | 63.5 | | 74.8 | 77.3 | 78.2 | 78.5 | 80.7 | | |
| | SD | 8.3 | 4.6 | 4.7 | 6.4 | 8.2 | 9.0 | 8.7 | 5.2 | 1.4 | 5.9 | 4.6 | 4.1 | * | 2.6 | 5.0 | 5.0 | 4.3 | 5.2 | | |

Further sub-classification of tested eyes into groups according to patient age and lens opacification grade of pre-operative images aided examination of other important factors which could affect the MPOD measurements. Significant correlation with age was found for pre-operative measurements of MPOD in patients with cataract (Fig. 1). Since age is the major risk factor for the development of AMD, a possible age-dependence of MPOD has been the subject of numerous studies over the years [57–73]. No clear conclusion has been reached on this question of age-dependence, primarily because of different methods employed across studies to measure MPOD. For example, Berendshot and Van Norren declared after comparing values measured with five different methods (heterochromatic flicker photometry, two setups for fundus reflectance spectroscopy, scanning laser ophthalmoscopy for obtaining MP reflectance, and scanning laser ophthalmoscopy for depiction of autofluorescence maps), that there was no significant age-effect influencing the optical density of the MP, only heterochromatic flicker photometry showed a slight decrease of MPOD with increasing age [57]. Similarly, Loan et al. found a small decrease of MPOD with increasing age when measuring MPOD by using heterochromatic flicker photometry [58]. On the other hand, Ciulla reported that even when elderly subjects with cataract and AMD are enclosed, MP measured with heterochromatic flicker photometry did not change significantly with age when analysed across the group [60]. A large number of other previous studies based on autofluorescence [60], fundus reflectometry [61], psychophysical methods [63], or high-performance liquid chromatography [64] examined MPOD changes in relation to age. Although the results varied, the majority of the studies which reported on the age effect on MPOD presented with an age-related decline in MPOD [17, 21, 55, 64–71]. Some other studies, on the other hand, which measured MPOD using high-performance liquid chromatography, fundus reflectometry or methods based on heterochromatic flicker photometry did not detect any age-related difference in MPOD [57, 63, 72, 73], even when elderly subjects with cataracts were considered [60]. The discrepancy in results of the different studies may be related to differences in subject selection, methods of measurement, or size of the sample. Interestingly, in the present study, individual values for MPOD — when measured before cataract surgery — seemed to depend on age: a clear tendency for higher pre-operative MPOD values with increasing patient age was shown (Fig. 1). However, MPOD data of the same patients after cataract extraction provided results without any dependency on age (Fig. 2). As described above, pre-operative measurements were carried out with a built-in correction factor accounting for increasing grayness level of the crystalline lens with increasing age, but post-operative measurements were performed by employing the so called "intraocular lens mode", i.e., without correction for opacification level of

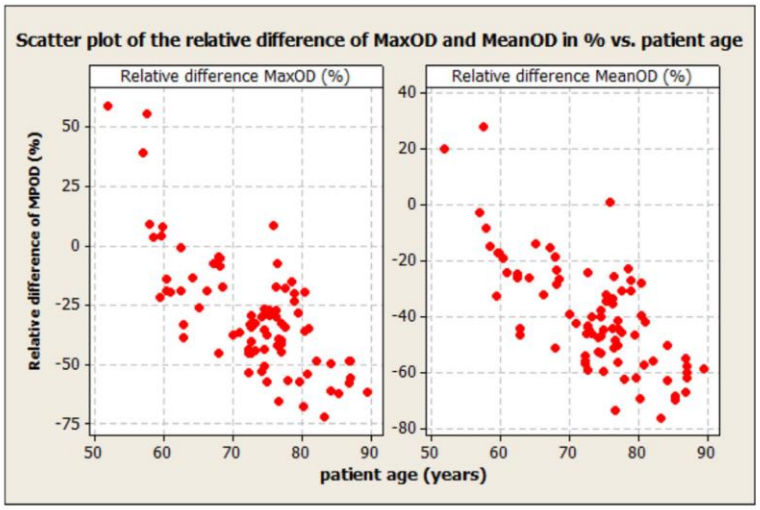


**Fig. 3** Scatter plot of the relative difference of MaxOD and MeanOD, expressed in percentage, as a function of age. A decrease from pre-operative to post-operative data is depicted by a negative relative change

the lens. According to the fact that MPOD data determined post-operatively may in fact be closer to the true MPOD level of the respective patient eye, because of the absence of the main disruptive factor, the dense lens, it should be assumed that there is no real correlation between MPOD and patient age. Increasing MPOD values with age obtained during pre-operative measurement procedure are therefore thought to be affected by the correction term incorporated into the built-in compensation mode. The corrective term integrated in the instrument employed in this study is based on an increase in grayness level of the lens with age, i.e., the patient's age determines the amount of correction employed [42]. The influence of this correction term therefore most likely resulted in the age-dependency of pre-operative measurements. Age dependency of relative MPOD differences from pre- to post-operative data (Fig. 4) and the larger change in elderly patients (≥70 years) which resulted when grouping by age (Fig. 5, Table 1) may most likely also be attributed to the apparent dependency of age on individual pre-operative absolute values. The corrective term for age-dependent grey levels of the lens incorporated into the function of the instrument could be an explanation also for this behavior.

Furthermore, similarly to results reported by Ciulla et al. [59], the data of the present study showed that elderly patients do not have uniformly low MPOD density but display a full range of MPOD (Fig. 2), a range that is similar to the younger population.

The lens is an important component of the optical pathway of the eye, and its density therefore plays a key role as it influences light transmittance [44, 45]. Due to this fact, the intensity of lens opacity became an important issue of consideration. Several previous studies have suggested that scattering of light in the lens increased with severity of cataract and rising patient age [46, 47, 76]. Furthermore, it is important to point out that also the degree and type of cataract were reported to play a significant role in the scattering of the light [47] and consequently possibly in the influence of lens opacity on MPOD measurements.

To determine the effect of different cataract density on MPOD measurements, the classification of grade of lens opacity could provide important insight. For this, pre-operative MPOD fundus images, were evaluated according to the quality criteria represented in Table 2. Thus, the present investigation showed that the relative differences of MPOD increased with declining quality of the pre-operative images and consequently with increasing lens opacity. Sasamoto et al. provided an assessment of the effect of cataract on the evaluation of MPOD in elderly patients based on autofluorescence spectrometry. They

**Table 2** Grading scale of cataract density according to the quality of pre-operative one-wavelength reflection fundus images

| Lens opacification grade | Full grading criteria | Number of eyes |
|---|---|---|
| 1 | Slight opacity. Easy visibility of: macular pigment, large and small vessels. | 9 |
| 2 | Opacity. However, visibility of macular pigment, large and small vessels is still obtained | 26 |
| 3 | Opacity. Visible: macular pigment, large vessels. Invisible: small vessels. | 21 |
| 4 | Opacity. Visible: macular pigment. Invisible: large and small vessels. | 25 |
| 5 | Strong opacity. Macular pigment cannot be detected automatically with adequate measurement certainty. Invisible: large and small vessels. | 5 |

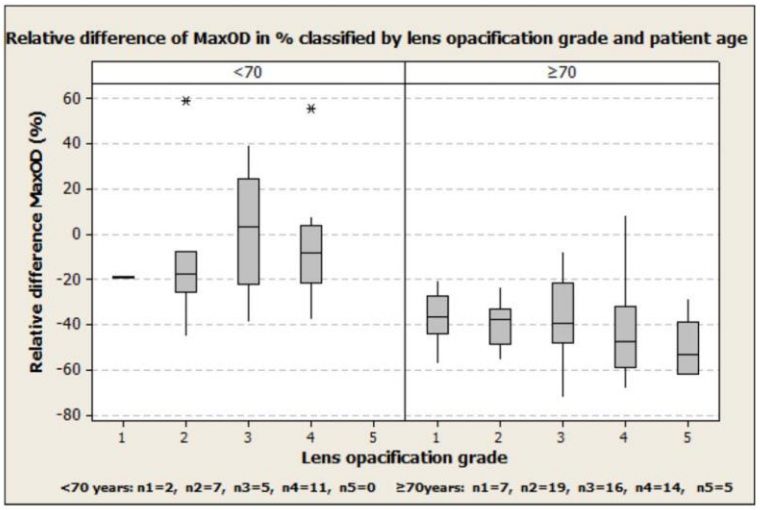


**Fig. 4** Boxplots of relative difference of MaxOD in % in relation to patient age and cataract opacity. Data is presented for each lens opacity group (1–5), see Table 2. A decrease from pre-operative to post-operative data is depicted by a negative relative change

employed the Lens Opacities Classification System III to grade the cataracts at baseline [49]. Their results showed that especially the nuclear component of the cataract affected the MPOD values. In agreement with their findings, the present study also established a correlation of greater opacification over the measured area (as outlined in Table 2) with larger differences in MPOD between pre-operative and post-operative measurements. Different grading schemes were applied in literature to grade lens opacification [78–82]. It has to be investigated which one provides the best correlation to MPOD measurements for future investigation to accurately account for the effect of the graying of the lens while taking MPOD measurements over time.

In conclusion, patient age and intensity of lens opacification were found to impact relative difference between pre-operative and post-operative measurements of maximum and mean optical density based on the one-wavelength reflection method. Elderly patients with progressed stage of cataract in particular showed increased changes in MPOD values. These deviations may present a problem for MPOD monitoring over time, when increasing lens opacification influences MPOD over time during follow-up measurements and therefore disguising real changes in macular pigment. MPOD has been postulated to be a marker for protection of AMD [17, 19–22], and furthermore dietary changes have been monitored using MPOD [26, 27, 29–33]. However, longitudinally measured MPOD data using the one-wavelength reflection method, cannot provide reliable information about AMD disease progression or supplement success in cataract patients. Considering the fact of improbability of such significant changes of MPOD in such a short period of time (6–8 weeks in the present investigation between pre- and post-operative measurements), the discrepancy between determined values is thought to be largely due to an assessment problem. The results of this study suggest that lens

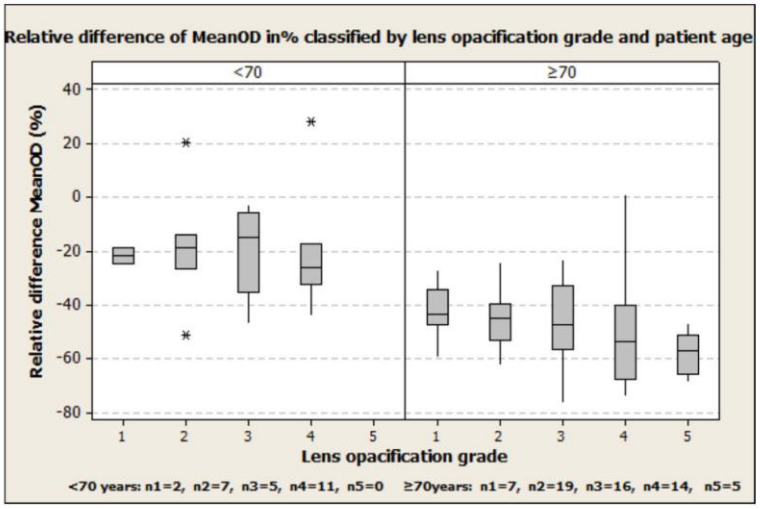


**Fig. 5** Boxplots of relative difference of MeanOD in % in relation to patient age and cataract opacity. Data is presented for each lens opacity group (1–5), see Table 2. A decrease from pre-operative to post-operative data is depicted by a negative relative change

opacification affected the measurement of MPOD by the one-wavelength reflection method in a clinically significant way. Particular care should therefore be taken when evaluating MPOD using this method in elderly patients with progressed stage of cataract. Future optimization of correction parameters for lens opacification, ideally as an individual corrective measurement, could contribute to the reduction of the measured variation errors and aid the understanding of the influence of cataract, allowing a more precise evaluation of MPOD by the one-wavelength reflection method in future.

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**Conflict of interest** None of the authors has any financial interest in the presented study.

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## 3. Additional data to section Results

The MPOD difference between pre- and post-operative MaxOD and MeanOD measurements for all patient eyes is summarized in Figure 6. Here post-operative decrease of optical density in both MaxOD and MeanOD is represented (a decrease from pre-operative to post-operative data is depicted by a negative relative change). MaxOD decreased by median (Q1/Q3) of 33.8% (-46.2% /-19.1%) and MeanOD by 44.0% (-54.6%/ -26.6%).

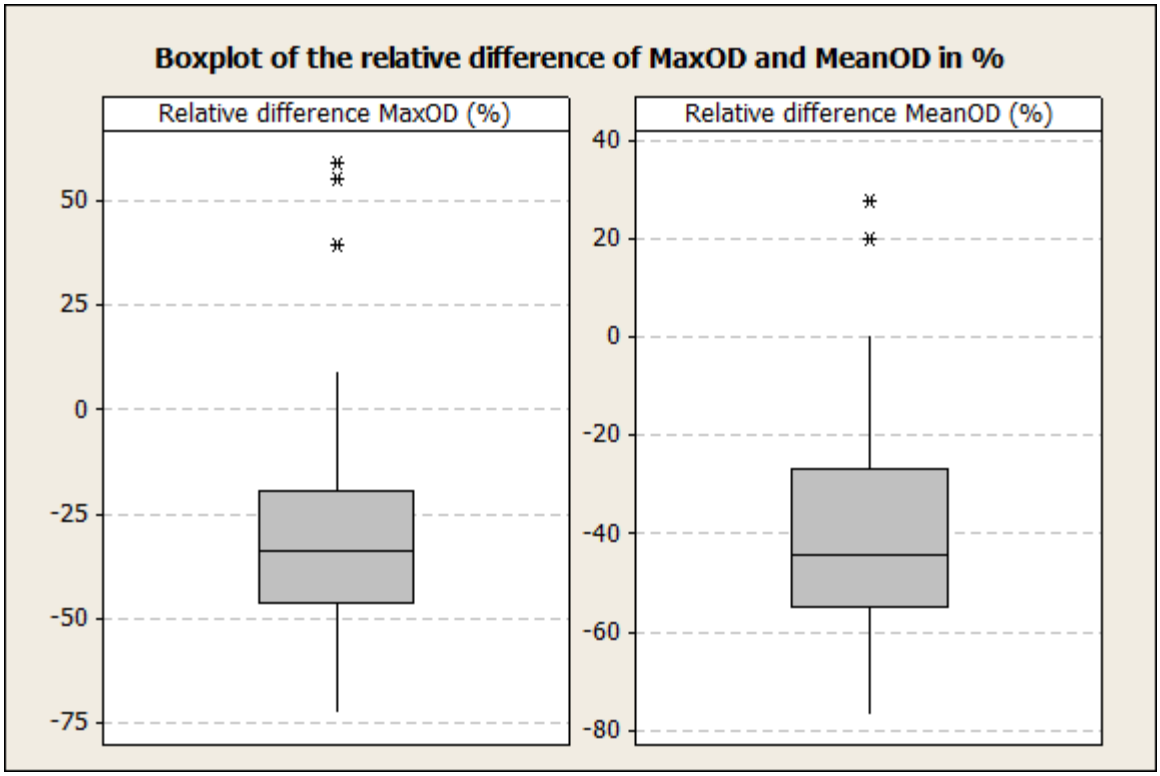


**Figure 6**: Representation of the relative difference of MaxOD and MeanOD in % between pre-operative and post-operative values in general for all patients' eyes. A decrease from pre-operative to post-operative data is depicted by a negative relative change.

The relative difference of the MPOD showed higher rates with increasing patient age (with a tendency to smaller MPOD values after cataract removal). In order to investigate this relationship in more detail, patients were subdivided into groups by age (Figure 7). Two groups were formed, patients who were younger than 70 years of age and those who were 70 years of age or older. Figure 5 illustrates clearly that elderly patients presented with larger differences between pre-operative and post-operative tests: patients <70 years: (n=25 eyes): median change of MaxOD -13.4% (-20.5% / 3.6%), MeanOD -23.6% (-30.5% / -15.3%) versus patients > 70 years: (n=61eyes): median change of MaxOD -40.5%(-53.2% / -30.1%), MeanOD -47.2%(-57.8% / -40.1%).

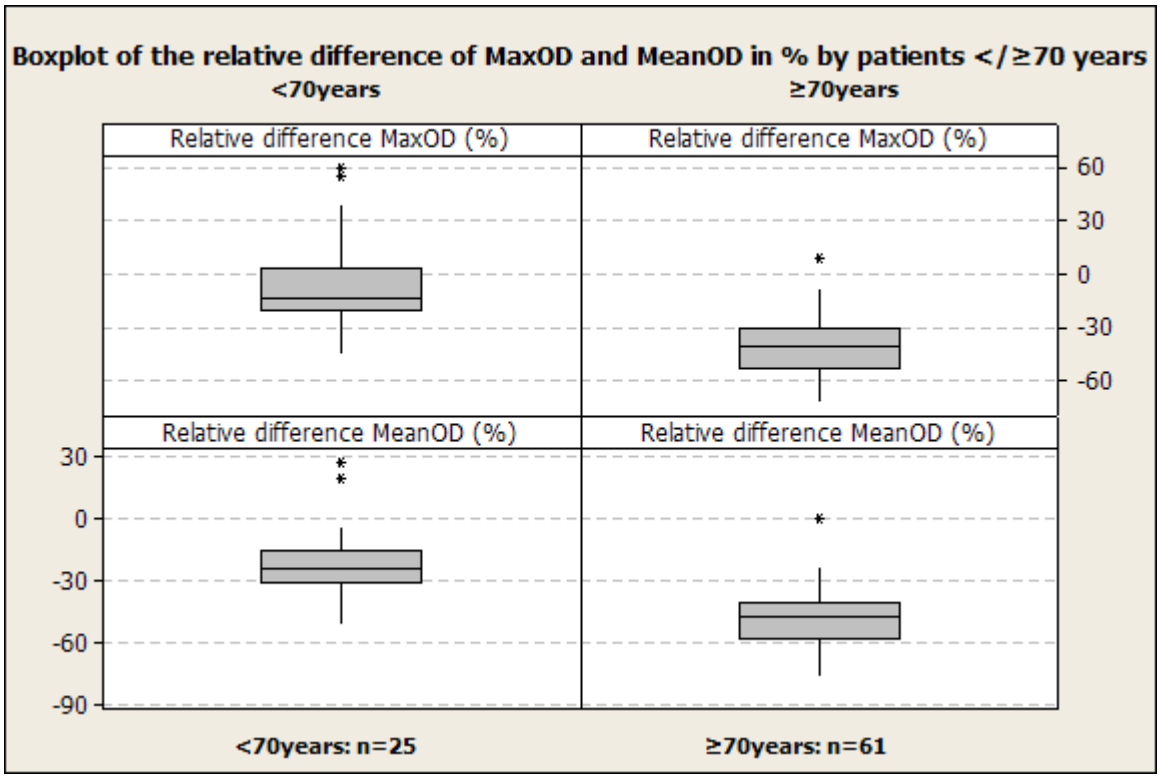


**Figure 7:** Boxplots of the relative difference from pre-operative to post-operative data of MaxOD and MeanOD in % divided into groups by patients age (<70years/≥70years). A decrease from pre-operative to post-operative data is depicted by a negative relative change.

To determine the effect of different cataract density on MPOD measurements, the grade of lens opacity was classified according to the pre-operative MPOD fundus image quality criteria represented in Table 2. This specification enabled classification of opacification severity. On the basis of this classification all examined eyes were divided into five groups and their relative difference of MPOD in each group were demonstrated in Figure 8 and 9.

Figure 8 highlights the percentage change of MaxOD in relation to the quality of the pre-operative images. On the basis of this diagram it can be concluded that the group with the smallest lens opacity (see Boxplot 1 in Figure 8) offers the smallest changes between pre-operative and post-operative data. The relative difference of MaxOD in percent increases with declining quality of the pre-operative images, which is linked with increasing lens opacity. A decrease from pre-operative to post-operative data is depicted by a negative relative change for MaxOD investigated. The following median differences of MaxOD were determined for patients grouped by the grading scale described in Table 2: grade 1: (n=9 eyes) -27.4% (-42.1%/-19.6%), grade 2: (n=26 eyes) -35.0% (-44.2% /-25.3%), grade 3:(n=21 eyes) -34.4% (-45.4%/-11.4%), grade 4:(n=25 eyes) -32.6% (-53.2%/-6.4%) and grade 5:(n=5 eyes) -53.5%(-61.7%/-38.7%).

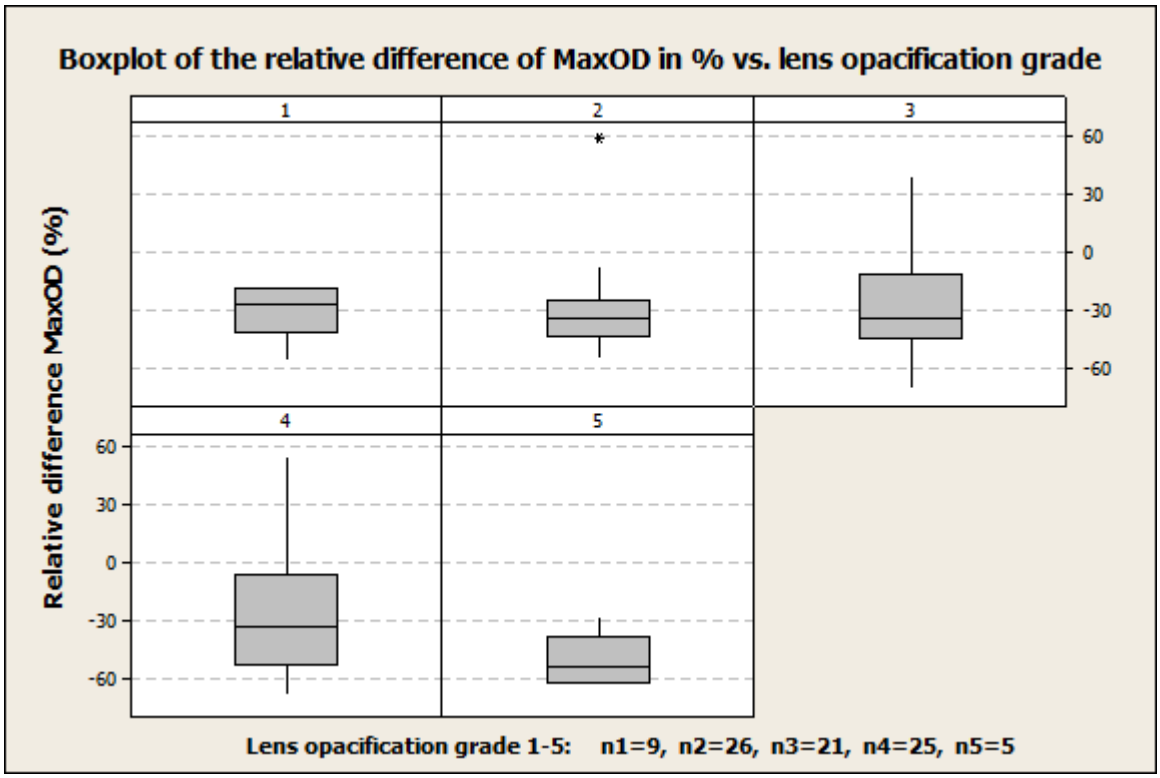


**Figure 8:** Boxplots of the relative change of MaxOD in % classified according to cataract density. Data is presented for each lens opacity group (1-5), see Table 2. A decrease from pre-operative to post-operative data is depicted by a negative relative change.

Similar to the representation of MaxOD, an increasing opacity of the lens as classified by the scheme represented in Table 2 resulted in a larger relative change of MeanOD. The following median differences of MeanOD were determined for patients grouped by the grading scale described in Table 2: grade 1:(n=9 eyes) -42.6% (-46.0%/-26.0%), grade 2: (n= 26 eyes) -44.1% (-51.8%/26.2%), grade 3: (n=21 eyes) -45.7%(-54.7%/-24.7%), grade 4: (n=25 eyes) -39.5%(-59.4%/-26.1%), grade 5: (n=5 eyes) -57.0%(-66.1%/-51.4%). In summary, an increase in lens density, classified as described above, resulted in a greater relative change of MPOD values between pre-operative and post-operative measurements

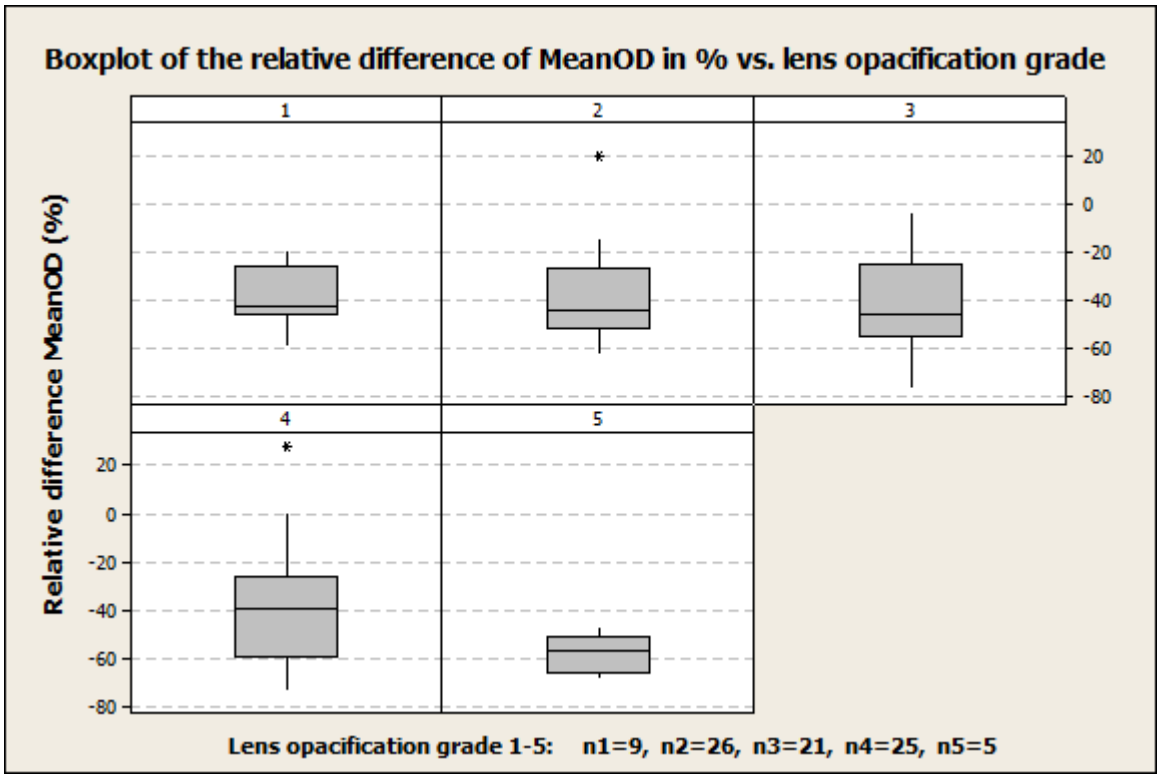


**Figure 9:** Boxplots of the relative change of MeanOD in % classified according to cataract density. Data is presented for each lens opacity group (1-5), see Table 2. A decrease from pre-operative to post-operative data is depicted by a negative relative change

## 4. Summary

**Dissertation**

**to obtain the academic degree**

**Dr.med.**

**Macular pigment optical density measurements by one-wavelength reflection photometry – Influence of cataract surgery on the measurement results**


submitted by: Bogdana Komar

made in: Medical Faculty of Leipzig University

University Hospital Leipzig,

Department of Ophthalmology

supervisor: Prof. Dr.med. Jens Dawczynski

Dr. Franziska G. Rauscher

Dr. med. Renate Wiedemann



Submitted on: 2015



**Introduction**

Macular pigment (MP) has become the focus of much research in recent years. The interest is focused on the protective role of MP as well as on the ability to measure the macular pigment optical density (MPOD) with different methods in vivo.

MP consists of retinal carotenoids including lutein, zeaxanthin and meso-zeaxanthin. These carotenoids are part of natural pigments synthesized de novo by plants and microorganisms [86]. Humans are not able to synthesize carotenoids and therefore are reliant on their alimentation.

The role of MP in visual acuity, contrast and glare sensitivity, photostress recovery and color vision was evaluated and reported by several studies [92-98]. Moreover MP is commonly assumed to act as an antioxidant and as an optical filter for blue light, the most phototoxic region

of the visible spectrum of the light [89, 164-165]. Not only its protective role in the fovea, but also its important role in diagnostic and treatment methods of macular diseases has made MP a point of interest in recent years [81,166-167]. The possibility to measure and consequently compare MP in patients carried out at various points of time emphasized the importance of MP acquisition. Thus, the size and distribution of MP, measured as MPOD, are believed to correlate with some risk factors for macular diseases in general. Less MP might result in an increased risk of developing the disease and vice versa. Thus reduced MPOD levels were shown by patients with early stage of age-related macular degeneration (AMD) or a family predisposition for this disease in comparison with healthy subjects [81,122,168]. Furthermore growing evidence exists that MPOD can be increased by a carotenoid-rich dietary modification [125-126] or by the ingestion of supplements like lutein and zeaxanthin in healthy subjects or patients with beginning stages of AMD [67-68]. An enhancement of the protective MP layer was shown to result in improvement in vision acuity in elderly patients for a period of supplementation [70]. In this way, dietary intake or supplementation of retinal carotenoids could give some degree of protection or could potentially slow the progression in subjects with early stage of disease. The supplementation or dietary intake of lutein and zeaxanthin in their turn can be monitored by repeated measurements of MP over time to track the effects of a particular treatment during the course of the disease [66-69, 127-128].

To sum up, the longitudinal measurements of MP are potentially useful to uncover predictors of particularly early stages of AMD and to provide information about the disease progression and success of supplementation with lutein and/or zeaxanthin or any another treatment.

**Methods**

Since the importance of MP measurements became clear, various principles to measure the MP were explored. MPOD can be measured in vitro or in vivo. In vitro techniques are not suitable to common use in everyday clinical practice. For this issue there are two categories for in vivo measurement of optical density of the MP: subjective and objective methods. The first category includes psychophysical methods which require the active response from the patient. The second group contains objective methods which require only minimal input from the patient. At present there is no technique that could be characterized as a true “gold-standard” for the measurement of MPOD. Different studies employed different subjective or objective measurement techniques, which is one of the main reasons why values for measured MPOD vary across research reported.

Considering the advantages of objective principles (e.g. ease, speed and the ability to yield a spatial profile of the MP ), the measurements in the current study were carried out using fast, objective principle, which was introduced in 2010 by Schweitzer and co-workers [139]. This is a new simple objective method based on one-wavelength reflection fundus imaging measuring the

MPOD by Visucam 500, Carl Zeiss Meditec AG. The use of this technique for measuring MPOD avoids the disadvantages of other objective methods e.g. unequal fundus illumination at the two wavelengths used, unequal relation of fluorescence at two excitation wavelength, sophisticated procedures. At the same time it provides significant correlation in comparison with two-wavelength-autofluorescence method for determination of the MPOD [139].

The principle of one-wavelength reflection method is based on using one-wavelength reflection photometry employing the local and spectral selectivity of xanthophyll. During the measurement the fundus is illuminated with blue light. Under illumination with the wavelength at 480 nm, which is near the absorption maximum of retinal carotenoids, the fundus can be determined as a uniform reflecting surface, where the increased absorption in the region of fovea can be considered. The logarithmic ratio of the virtual fundus reflection below the macular pigment compared to the macular reflection leads to the MPOD. Area, in which the optical density exceeded a defined threshold, volume as a sum of density of all pixels, maximal optical density and mean optical density over all pixels are four parameters, which describe the spatial distribution of MP. The main focus of attention in current report is set on the maximal optical density (MaxOD) and the mean optical density (MeanOD) across all pixels, both measured in optical density units (ODU).

MP measurement is generally affected by the density of the media as the light must go through tissue, which scatters the light to a certain degree due to their biological configuration [140]. It has long been recognized, that it is in particular the crystalline lens that tends to influence the transmittance most strongly [141-142]. It is generally known that with age the lens increases in thickness; it becomes more opaque, which can lead to cataract. Stray light of the crystalline lens additionally increases with patient age [143]. These influences can affect MP measurement by one-wavelength reflection method. For this reason an automatic age-dependent correction factor for the grayness level of the lens is introduced by the software of the instrument. The post-operative measurements were not affected by these artefacts due to the newly implanted clear lens. Therefore the post-operative measurements were carried out by setting the ”intraocular lens mode” without correction for the opacification of the lens.

**Main objective of present study**

To sum up, the main research questions of the study were concerned with the investigation of the influence of different grades of lens opacity and patient age on MPOD measurements as well as the evaluation of the possible effect of cataract surgery on values of MPOD by measuring MPOD of the same subjects before and after cataract surgery.

The study was approved by clinical ethics committee of University Hospital Leipzig and adhered to the tenets of the Declaration of Helsinki. The recruitment of subjects was facilitated by word

of mouth at the University Hospital Leipzig, department of Ophthalmology from July 2011 to April 2012.

**Patient characteristics**

All subjects were recruited from a cohort of patients who had cataract that was sufficiently symptomatic to warrant extraction. The subjects were invited to participate in the study while they were attending a consultation in the eye clinic. Prior to examination, written consent was obtained from each patient after careful explanation of the nature and consequences of the study, voluntariness as well as demonstration of the test procedure. All patients underwent dilated fundus examination once before and second time 6-8 weeks after completion of the cataract surgery. During fundus examination by using a fundus camera Visucam 500 (Carl Zeiss Meditec AG) fundus images were made. By means of these images macular pigment optical density was measured using an objective one-wavelength-reflection method. A sequence of three single measurements was taken and for the examinations of MPOD an average of those was calculated for four parameters characterizing MP with a main focus of attention on the MaxOD and MeanOD.

In total 86 eyes of 64 patients, who had clinical indication for cataract surgery, were included in current analysis. Age ranged from 51.9 to 89.5 years with the mean age (± SD) of 73.4 (+/- 8.3) years. The male to female ratio was 28: 36. All cataract extractions were performed by the same physician in the Department of Ophthalmology, University of Leipzig, on an outpatient basis. All patients underwent planned standard phacoemulsification and implantation of a blue-light-filtering intraocular lens (Alcon SN60WF). Descriptive and statistical analyses were subsequently performed using Minitab statistical software (version 14).

**Results and discussion**

The individual variability of optical density values between subjects was seen in our results as well as reported in comparable studies measuring MPOD using variable methods. Considering this fact, in following analysis the changes between MPOD values measured before and after cataract surgery were expressed as relative percentage.

Significant differences in MPOD were found between preoperative and postoperative measurements analyzed. The results indicated a general tendency for lower MPOD measurement levels after cataract surgery. Thus, the data of the present study suggests that cataract strongly affected the measurement of MPOD by one-wavelength reflectance method. To examine other important factors which could affect the MPOD measurements, further classification resulted in subdivision of tested eyes into groups according to patient age and lens opacification grade of preoperative images.

To estimate the behavior of relative difference of MPOD by patients with various age, tested eyes were subdivided into groups according to patient age. Two groups were formed, patients who were younger than 70 years of age and those who were 70 years of age or older. The results of this analysis represented larger differences between pre-operative and post-operative tests in elderly patients.

Individual absolute values for MPOD measured before cataract extraction showed significant correlation with age of the patients. A clear tendency for higher pre-operative MPOD values with increasing patient age was represented. On the other side, MPOD measurement of the same patients after cataract extraction provided results without any dependency on age. Considering the fact that postoperative values may in fact be closer to the true MPOD level of the respective patient eye, because of the absence of the main disruptive factor, the dense lens, it should be assumed that there is no real correlation between MPOD and patient age. Increasing MPOD values with age obtained during pre-operative measurement procedure are therefore thought to be affected by the correction term incorporated in the compensation mode. This built-in correction factor accounts for increasing grayness levels with increasing age. (Post-operative measurements were performed by employing the so called “intraocular lens mode”, i.e. without correction).

Due to the fact that the lens opacity is known to influence the light transmittance and to grow with the cataract severity the influence of this factor became a point of interest in following analyzing. All preoperative images of tested eyes were divided into five groups according to their lens opacification grade. Classification was carried out from slight (lens opacification grade 1) to strong opacity (lens opacification grade 5) by one person accordingly to the classification criteria scheme. Resulting data represented a greater relative change of MPOD values between pre-operative and post-operative measurements with an increase in lens density. Further classification based on patient age and pre-operative cataract opacity indicated larger differences in measured data for the elderly patients with progressed stage of cataract.

**Conclusion**

In conclusion, patient age and intensity of lens opacification were found to impact relative differences between pre-operative and post-operative measurements of maximum and mean optical density. Particularly elderly patients with progressed stage of cataract showed significant changes in MPOD values. MPOD is postulated to be a marker for protection of AMD [81,102,122 168--169] and its monitoring is potentially useful to provide information about the disease progression and success of supplementation [65-66, 67-68- 69, 127-128]. In this way such strong deviations as seen in current study might influence possible interpretation of MPOD over the time. Summarizing the above it can be pointed out, that longitudinally measured MPOD data

using one-wavelength reflectance method without further correction factors, may not provide accurate data about AMD disease progression or treatment success in cataract patients. Possible reason for such significant discrepancy between determined values is thought to be largely due to an assessment problem.

Future optimization of correction parameters for lens opacification, ideally as an individual corrective measurement, could contribute to the reduction of measured variation errors and add to the understanding of the influence of cataract on the measured values. In such way more precise evaluation of MPOD could be allowed in future.

## 5. List of references for Introduction and Summary sections


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## 6. List of figures

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## III Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren.

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Datum Unterschrift

## IV Publications List (Verzeichnis der wissenschaftlichen Veröffentlichungen)

| | |
|---|---|
| 09/2012 | Postervorstellung der Ergebnisse der klinischen Studie mit dem Thema „Makulapigmentdichtemessung – Einfluss der Kataraktoperation auf die Messergebnisse“ im Rahmen des 110. Kongresses der Deutschen Ophthalmologischen Gesellschaft 2012 in Berlin |
| 04/2014 | Publikation der Ergebnisse der klinischen Studie mit dem Thema „Makulapigmentdichtemessung – Einfluss der Kataraktoperation auf die Messergebnisse“ in „Graefe’s Archive for Clinical and Experimental Ophthalmology“ |
| | Komar B, Rauscher FG, Wiedemann R, Dawczynski J. Macular pigment optical density measurements by one-wavelength reflection photometry-influence of cataract surgery on the measurement results. Graefes Arch Clin Exp Ophthalmol. |
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