Grade of Cataract and Its Influence on Measurement of Macular Pigment Optical Density Using Autofluorescence Imaging

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Purpose. To evaluate the influence of cataracts on measuring macular pigment optical density (MPOD) using a dual-wavelength confocal scanning autofluorescence imaging technique and to establish methods to compensate for the influence of cataracts.

Methods. This prospective case series comprised 100 eyes that underwent cataract surgery. Cataracts were graded based on the World Health Organization classification. MPOD levels were measured with the MPOD module of the Spectralis MultiColor instrument (Spectralis-MP), pre- and postoperatively. We investigated the relationship between change in MPOD values and age, cataract grade, and quality of autofluorescence images. Local MPOD levels were evaluated for four strategically chosen eccentricities within the macular region, and the total MPOD volume was evaluated within 8.98° eccentricity from the center.

Results. MPOD levels could be obtained in 67 eyes before surgery. Local and volume MPOD levels were higher postoperatively relative to preoperatively in all eyes. The mean ratio of local MPOD levels after and before surgery (correction factor, CF) ranged from 1.42 to 1.77, with larger CFs required for eccentricities closer to the foveal center. The CF for the MPOD volume was 1.31. Age, grade of nuclear cataract (NUC), posterior subcapsular opacity, and image quality index (IQI) significantly contributed to CFs. For example, regression equation for CF at $0.23° = 0.17 + 0.16 \times \text{IQI} + 0.29 \times \text{NUC grade} + 0.01 \times \text{age}$ ($P < 0.001$).

Conclusions. Cataracts affected MPOD measurements with the Spectralis-MP, but corrected MPOD results could be obtained via regression equations.

Keywords: macular pigment, dual wavelength autofluorescence technique, nuclear cataract, regression equation

The human macula has a yellow pigment that consists of three carotenoids: lutein ([3R, 3′R, 6′R]-lutein), zeaxanthin ([3R, 3′R]-zeaxanthin), and meso-zeaxanthin ([3R, 3′S meso]-zeaxanthin).[1,2] The macular pigment (MP) absorbs short-wavelength visible light and works to filter blue light. Blue light is highly scattered in the retina, and absorption of blue light by MP improves contrast sensitivity and reduces glare disability.[3] Blue light also causes oxidative damage to photoreceptor cells and retinal pigment epithelial cells through photochemical reactions.[4–6] Oxidative damage by blue light is considered one of the important factors that cause age-related macular degeneration (AMD).[7–11] The absorption of blue light and reduction of oxygen radicals caused by MP attenuate light-induced oxidative damage in the retina and may help prevent AMD.

Correct assessment of MP levels in human eyes is important for understanding visual function and vulnerability to AMD. There are several methods for measuring macular pigment optical density (MPOD) levels in human eyes, such as heterochromatic flicker photometry (HFP), fundus reflectometry, fundus autofluorescence (AF) spectroscopy, and resonance Raman spectroscopy. Among these, HFP has the longest history of use in studies[12]; however, HFP is a subjective method that requires proper understanding and cooperation of the subjects, and the examination time is rather lengthy. HFP is difficult to perform in approximately 10% of subjects, especially in older people.[13–16] Fundus AF spectroscopy is an objective way of measuring MPOD levels and can be performed quickly. The measurements are possible in an imaging modality, allowing one to obtain MPOD levels and their spatial distributions by evaluating how much AF fluorescence from the lipofuscin of the retinal pigment epithelium is locally blocked by the MP.[17,18] Using this concept, an MPOD-measuring module was developed for the confocal scanning laser ophthalmoscope platform of the Spectralis (Heidelberg Engineering, Inc., Heidelberg, Germany). The accuracy of this MPOD module was validated by comparing MPOD levels with levels determined via HFP.[19,20] Furthermore, good intra-

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examiner repeatability and interexaminer reproducibility for the Spectralis MPOD module were found in Caucasians.[21,22] We also confirmed the reliability, intra-examiner repeatability, and interexaminer reproducibility of this device in Japanese.[16]

Sasamoto et al.[23] reported cataract attenuation effects on the MPOD levels with a first-generation Heidelberg Spectralis scanning laser autofluorescence imaging configuration. While cataracts absorb and scatter the excitation and emission light in general, it can be expected that the confocal detection methodology employed in the Heidelberg Spectralis device could overcome this problem to some degree. For clinical use of the autofluoresence detection concept, it is of interest to find out its quantitative detection limits. Sharifzadeh et al.[24] developed a correction method for opacity-induced MPOD level attenuations in a nonmydriatic, single-wavelength autofluorescence imaging method based on a modified fundus camera with blue LED excitation and quantification of the ocular media opacities via fluorescence image pixel intensity histograms. Akuffo et al.[25] measured the MPOD levels of 36 subjects before and after cataract surgery with a confocal scanning Heidelberg Spectralis instrument and showed that the average MPOD levels increased significantly after surgery. Specifically, they investigated a correlation between the MPOD correction factor (CF), defined as the MPOD level after surgery divided by the MPOD level before surgery, and cataract status parameters such as nuclear opalescence (NO), nuclear color (NC), and central (C) and posterior (P) subcapsular opacities. Also, they investigated variables such as age, sex, and preoperative MPOD levels, and they developed a set of equations quantifying the effect of cataracts on measured MPOD levels. One of the key equations relates the CF at 0.23° eccentricity to the presurgery MPOD level at 1.72° eccentricity according to $\text{CF}\ (0.23°) = 0.222 + 0.184 \times \text{NC} + 2.193 \times \text{MPOD}$ level at 1.72°.

In these previous studies, the grading of cataracts was performed by physicians using a slit-lamp microscope and by classifying the cataracts according to the Lens Opacities Classification System III (LOCS III).[26] However, this subjective method makes it difficult to determine the extent of NO and NC in some cases. Also, it is not possible to quantify the C and P opacities with this method.

In the present study, we used an anterior segment tri-camera system to grade the cataract more objectively, and we investigated the influence of cataracts on MPOD levels in a larger number of subjects.

Subjects and Methods

Subjects

One hundred patients without ocular disorders, with the exception of age-related cataracts, were enrolled in this study. Patients with mature or brown cataracts were excluded from enrollment because AF images with sufficient clarity were not obtainable. All patients underwent cataract surgery at Seirei Hamamatsu General Hospital between October 2016 and October 2017. Patients were 41 to 86 years old, with a mean age of 73.1 ± 8.8 (standard deviation, SD) years. Forty patients were male, and 60 were female. In 53 patients who underwent bilateral cataract surgery, the particular eye was selected as the subject eye that met the following conditions: higher-quality AF images, lighter cataract, and higher preoperative visual acuity, with these three conditions listed in order of their importance. The current study ultimately included 100 eyes (52 right eyes, 48 left eyes) for analyses.

Patients underwent visual acuity testing, measurement of intraocular pressure, fundus examination by ophthalmoscopy, fundus photography, and optical coherence tomography (OCT), with mydriasis-induced using 2.5% phenylephrine hydrochloride and 1% tropicamide.

These prospective case series were approved by the institutional review board of Seirei Hamamatsu General Hospital (IRB No. 2251). The protocol followed the tenets of the Declaration of Helsinki. All patients provided written informed consent before cataract surgery.

Grading of the Cataract

Cataracts were graded with a Konan Anterior Segment Tri-Camera System 1000 (KATS 1000; Konan Medical, Hyogo, Japan). The advantage of this camera system is that Scheimpflug images and diaphanoscopic images can be obtained at the same time. Scheimpflug imaging was used to grade the lens nucleus, and the diaphanoscopic image was used to grade cortical opacity and posterior subcapsular cataracts. The examination was performed with pupil dilation. The grading using this device was based on the World Health Organization (WHO) classification system.[27] Further details concerning this device have been presented elsewhere.[28]

As for the grading of nuclear cataracts (NUC), the horizontal and sagittal Scheimpflug images of the patient's lens obtained with slit-illumination were shown on a monitor along with standard reference pictures of the lens nucleus, ordered according to the WHO classification. The evaluator determined the grade of NUC by referring to the standard reference pictures. The lens nucleus opacifications were categorized into one of the four grades (NUC 0, 1, 2, 3) of the WHO classification. While the determination was still subjective, personal bias may be reduced compared to previous experience-based determinations that were based just on slit-lamp images. Standard photographs of the Emery-Little (E-L) classification[29] were also shown on the monitor, and NUC were divided into five categories (E-L 0, 1, 2, 3, 4) using the E-L classification as a reference, although the E-L classification is not included in the WHO classification. As for grading cortical cataracts (COR), the evaluator judged the area of cortical opacity on the diaphanoscopic image and then used a computer to calculate the total area of the opacity; the four grades (COR 0, 1, 2, 3) of the WHO classification were determined automatically. An area surrounded by a circle of 3-mm diameter was shown at the center of the diaphanoscopic image, and the evaluator judged the involvement of cortical opacity within this circle. When the cortical opacity involved the central optical zone (CEN), it was determined to be CEN positive. When the opacity did not involve the CEN, it was determined to be CEN negative. The evaluator judged the upper and lower borders of the posterior opacity. The computer measured the vertical diameter, and any posterior subcapsular cataract (PSC) was categorized as one of four grades (PSC 0, 1, 2, 3) automatically. Two masked ophthalmologists (YG, HS) evaluated the first 20 eyes preliminarily, and the results were identical. Then, one ophthalmologist (YG) evaluated the subsequent eyes.

Measurement of Macular Pigment Optical Density

MPOD levels and spatial distributions were measured with a prototype MPOD module installed on a Heidelberg Spectralis MultiColor platform ("Spectralis-MP"). This device uses 486- and 517-nm excitation wavelengths. The basic functionality and handling of this instrument are described in more detail elsewhere.[22,25] The beam diameter at the subject's pupil was 3 mm; AF images of the 30° central area of the retina were recorded for both wavelengths with pupil dilation. All AF

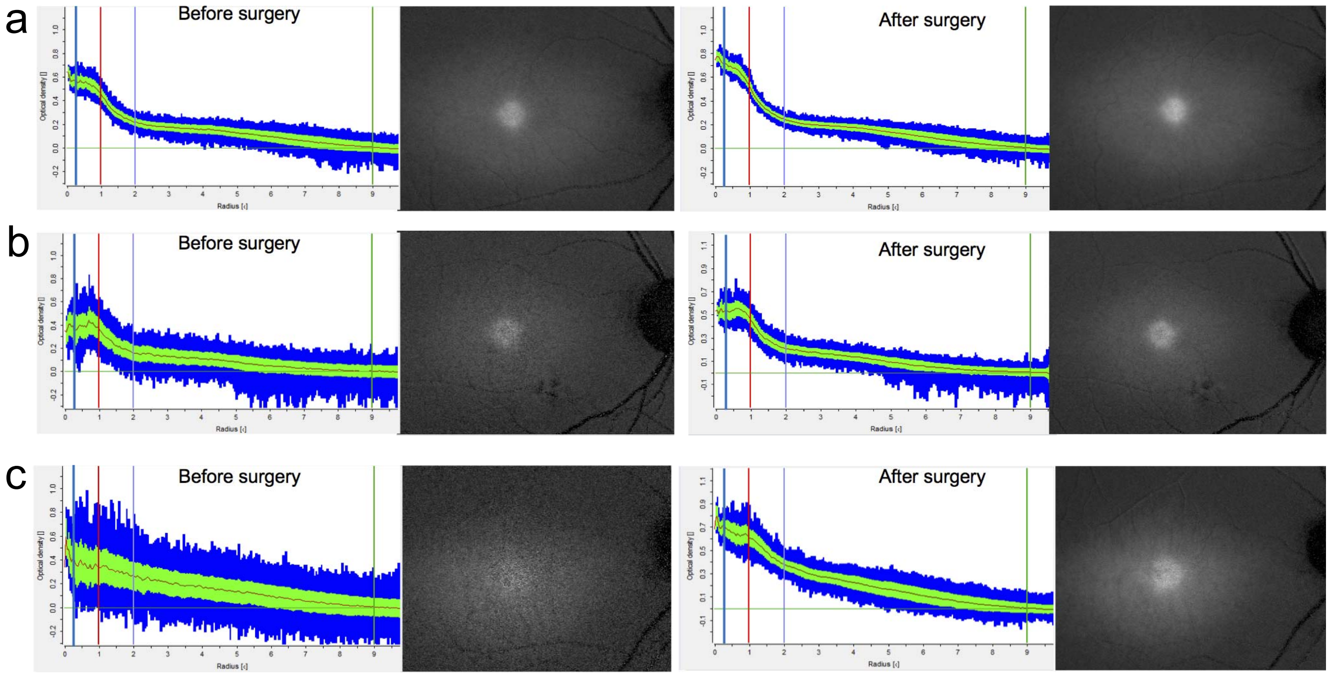

Figure 1. Examples of AF images and associated macular pigment profiles obtained before and after surgery, respectively, for three groups: (**a**) case of relatively high-quality AF image before surgery, as evidenced by a relatively bright circular area surrounded by a black background; (**b**) case of moderate-quality image with reduced brightness; (**c**) case of poor-quality image with barely discernable intensity differences. The quality of AF images after surgery is high in all groups. Each profile shows mean optical density with standard deviation (*green bars*) and maximum and minimum values (*blue bars*). The SD levels can be readily read off at selectable radius/eccentricity intercepts (*blue, red, purple vertical lines*), which for this study were chosen at 0.23° (*blue*), 0.98° (*red*), and 1.99° (*purple line*) eccentricity, respectively. The *green vertical line* at 8.98 eccentricity chosen as a zero MPOD cutoff.

images are displayed as grayscale images and were processed automatically with the device's software to generate MPOD profiles. The cutoff eccentricity was set to 8.98°, above which the plotted MPOD levels were no longer discernable from the noise. The derived MPOD levels were corrected as shown in a previous report.[30] The average optical densities at 0.23°, 0.51°, 0.98°, and 1.99° eccentricities (local MPODs) and the optical density volume within the eccentricity boundary of 8.98° (MPOD volume) were used for analyses. The ratios of MPOD levels measured after and before surgery (i.e., MPODs after surgery / MPODs before surgery) were chosen as CF for the local MPOD levels. Similarly, the ratio of the MPOD volume after and before surgery was chosen as the CF for the MPOD volume. Measurements were performed within 2 weeks before surgery and 4 or 5 days after surgery in all cases.

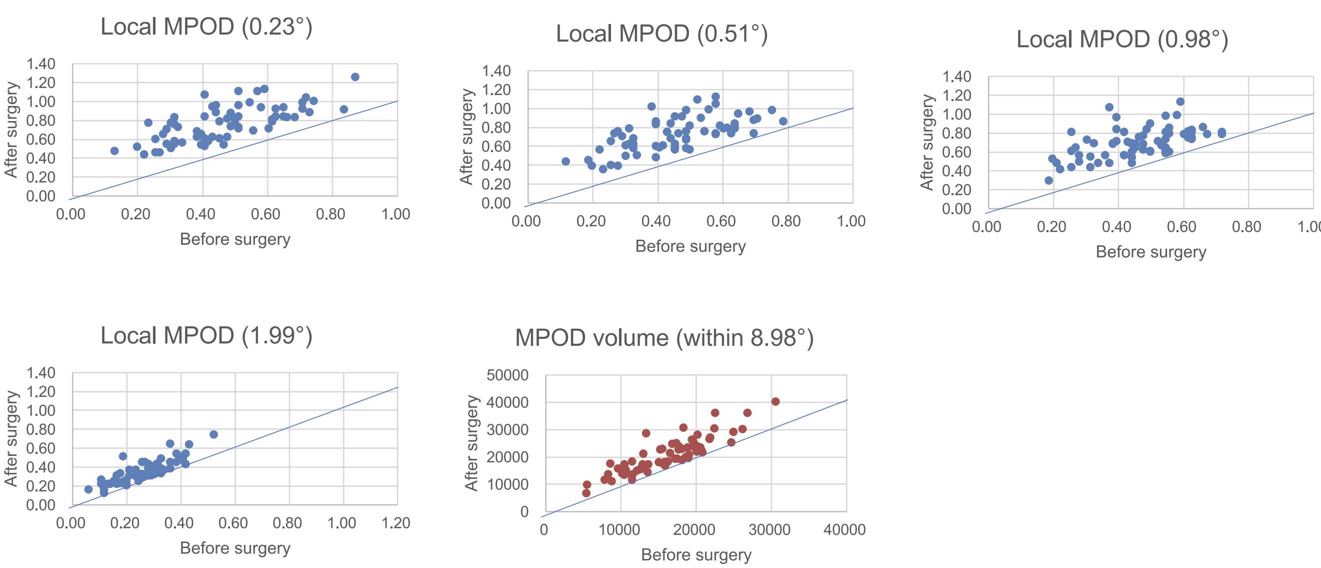

Figure 2. Scatter plots for the local MPOD levels and MPOD volume before and after surgery, showing that MPOD levels are higher after surgery in all cases, and correspondingly are positioned above a $y = x$ line.

Table 1. Demographic Data of the Subjects

Parameters	Subjects With Acceptable AF Images Recorded Before Surgery, 67	Subjects With Nonacceptable AF Images Recorded Before Surgery, 33	*P*
Age, y, mean ± SD	52 to 85, 73.5 ± 7.6	41 to 86, 72.1 ± 11.0	0.470, *t*-test
Sex	M 31, F 36	M 9, F 24	0.68, χ^2
LogMAR visual acuity before surgery, median	−0.08 to 0.52, 0.10	−0.08 to 1.30, 0.22	0.001, *t*-test
LogMAR visual acuity after surgery, median	−0.08 to 0.10, −0.08	−0.08 to 0.10, −0.08	0.734, *t*-test
Body mass index, kg/m^2; mean ± SD	15.1 to 29.0, 22.5 ± 3.1	12.5 to 29.0, 22.2 ± 3.2	0.099, *t*-test
Comorbid diabetes	Yes 11, no 56	Yes 5, no 28	0.871, χ^2
Tobacco smoking	No 43, past 20, current 4	No 26, past 6, current 1	0.329, χ^2
Taking lutein/zeaxanthin supplement	No 59, yes 8	No 31, yes 2	0.471, χ^2
The extent of the cataract			
NUC			
0	5	5	0.001, χ^2
1	57	14	
2	5	14	
3	0	0	
COR			
0	10	3	0.430, χ^2
1	6	3	
2	15	4	
3	36	23	
PSC			
0	38	7	0.001, χ^2
1	17	12	
2	11	5	
3	1	9	
CEN			
Negative	15	6	0.627, χ^2
Positive	52	27	
E-L			
1	51	19	0.001, χ^2
2	15	5	
3	1	9	
4	0	0	

CEN, existence of PSC in the central optical zone.

Derivation of Corrected MPOD Levels Based on Presurgery AF Images

The particular AF image quality used to calculate MPOD levels varied from relatively high quality to poor depending on the degree of the lens opacity, with the corresponding CF expected to increase accordingly. We subjectively classified preoperative AF images into three groups: relatively high quality (Fig. 1a), moderate quality (Fig. 1b), and poor quality (Fig. 1c). Typically, the SDs of the MPOD levels (widths of plots) were smaller for the relatively high-quality images and larger for the degraded images. In the case of Figure 1a, the relative SDs of the local MPOD levels at 0.23°, 0.98°, and 1.99° eccentricity are 0.12, 0.13, and 0.23, respectively, before surgery, and 0.08, 0.07, and 0.12 after surgery. Before surgery, the eye lens had a grade 1 NUC, grade 3 COR, grade 0 PSC, and a cortical opacity involving the CEN. The E-L classification was

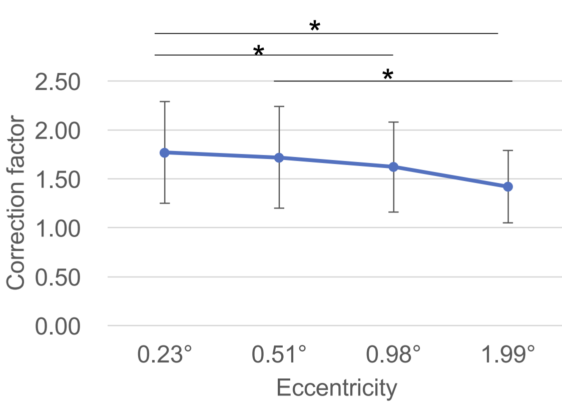

Figure 3. Mean correction factor as a function of eccentricity. Significant differences occur between three eccentricity pairs, connected by *black lines above the plot*, that is, between 0.23° and 0.98°, 0.23° and 1.99°, and 0.51° and 1.99° eccentricities ($P < 0.05$, Bonferroni).

Table 2. Local MPOD Levels at Four Eccentricities and MPOD Volume Before and After Surgery

Time Point	Local MPOD Levels				MPOD Volume
Eccentricity	0.23°	0.51°	0.98°	1.99°	8.98°
Before surgery, $n = 67$					
Mean	0.47	0.46	0.45	0.26	16,158
SD	0.16	0.15	0.13	0.09	5,224
Minimum	0.13	0.12	0.19	0.06	5,411
Maximum	0.87	0.79	0.72	0.52	30,564
After surgery, $n = 100$					
Mean	0.77	0.72	0.69	0.35	20,610
SD	0.18	0.18	0.17	0.12	6,761
Minimum	0.31	0.34	0.30	0.13	6,756
Maximum	1.26	1.12	1.13	0.74	42,810

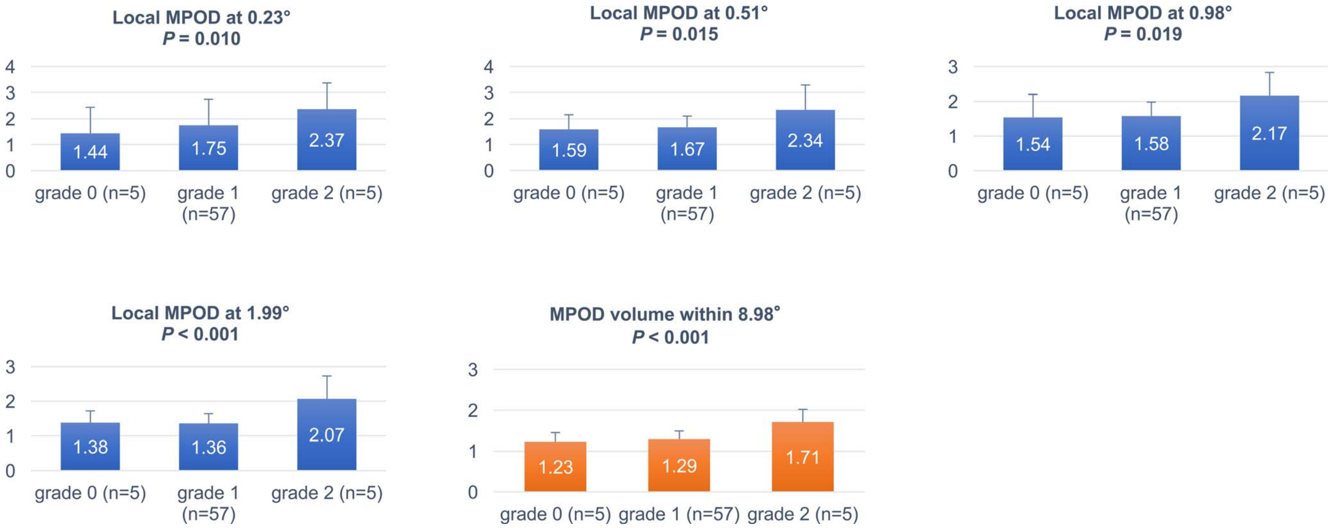

FIGURE 4. Correction factors for the local MPOD levels and MPOD volume versus nuclear cataract grade, revealing required increasing corrections with increasing grades.

grade 1. The CFs for the local MPOD levels at these three eccentricities were 1.26, 1.15, and 1.14, respectively. In the case of Figure 1b, the relative SDs were 0.33, 0.26, and 0.38 before surgery and 0.15, 0.13, and 0.16 after surgery. This eye lens had a grade 0 NUC, grade 3 COR, grade 2 PSC, and cortical opacity involving the CEN. The E-L classification was grade 1. The CFs for the local MPOD levels were 1.36, 1.24, and 1.31, respectively. In the case of Figure 1c, the relative SDs were 0.44, 0.44, and 0.48 before surgery and 0.11, 0.13, and 0.13 after surgery. This eye had a grade 1 NUC, grade 3 COR, grade 0 PSC, and cortical opacity involving the CEN. The E-L classification was grade 1. The CFs for the local MPOD levels were 1.73, 1.82, and 1.41, respectively. Therefore, we utilized the SDs of the MPOD levels as a quantitative measure for image quality.

In this process, we first recorded the SDs of the MPOD levels at 0.23°, 0.98°, and 1.99° eccentricity before and after surgery, respectively, and then calculated the ratio between SD and MPOD level for each eccentricity, that is, the relative SD. The average of relative SD after surgery at 0.23°, 0.98°, and 1.99° eccentricities in 100 eyes was 0.12 ± 0.05 (SD), 0.10 ± 0.04, and 0.17 ± 0.06, respectively. Next, we calculated the following three values for each eye: relative SD before surgery at 0.23° / 0.12, relative SD before surgery at 0.98° / 0.10, and relative SD before surgery at 1.99° / 0.17. Finally, we calculated the mean of these three values, and defined this value as the IQI.

Statistical Analyses

For statistical calculation purposes, the decimal visual acuity was converted to the logarithm of the minimum angle of resolution (logMAR). The correlations between the local and volume MPOD CFs, respectively, and the parameters of patient age, preoperative visual acuity, body mass index (BMI), and the relative SDs at three selected eccentricities were all assessed using Spearman's correlation coefficient by rank test. The differences in the CF with different grades of NUC, COR, PSC,

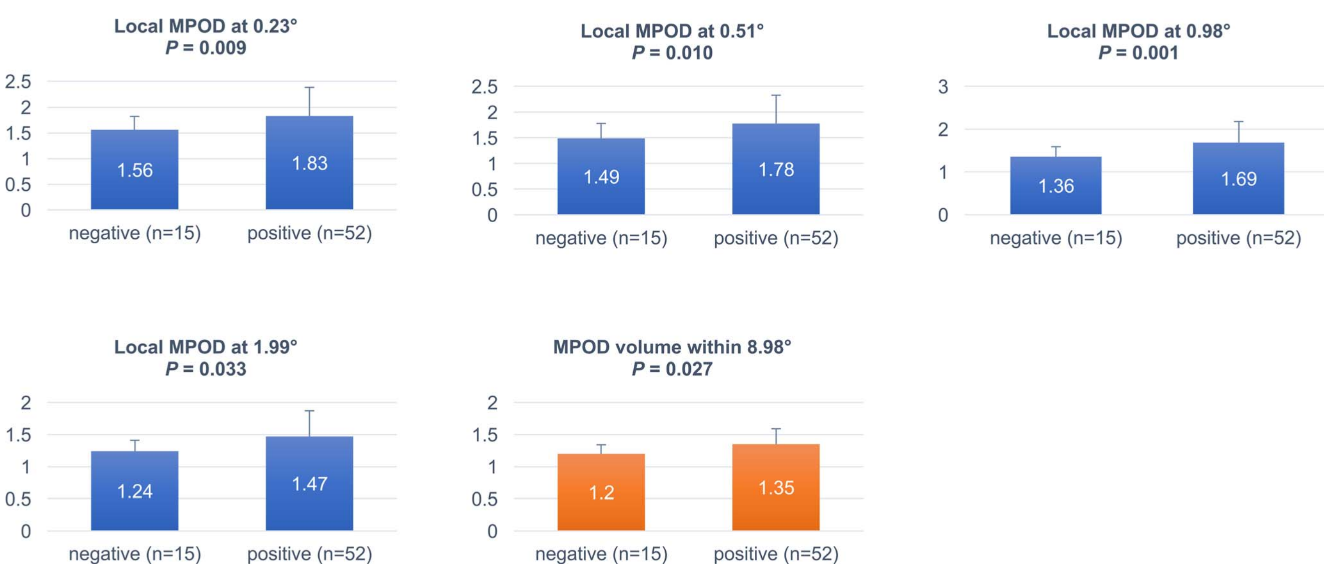

FIGURE 5. Correction factors for the local MPOD levels and MPOD volume with and without cortical opacity in the central optical zone, showing significantly different factors for the two categories.

TABLE 3. Correction Factors for the Local MPOD Levels and MPOD Volume

	Correction Factors for Local MPOD Levels				*P*	Correction Factor for MPOD Volume
Eccentricity	0.23°	0.51°	0.98°	1.99°		8.98°
Mean	1.77	1.72	1.62	1.42	<0.001	1.31
SD	0.52	0.52	0.46	0.37		0.23
95% confidence interval	1.65–1.90	1.59–1.84	1.51–1.73	1.33–1.51		1.26–1.37
Minimum	1.10	1.07	1.08	1.03		1.02
Maximum	3.73	3.80	3.18	3.16		2.15

Repeated ANOVA.

and smoking habit were analyzed with 1-way ANOVA. Using a *t*-test, the respective CFs were compared between sexes, for patient lutein supplementation use, and for the presence of a posterior opacity in the central 3-mm diameter. The differences in the CFs at selected four eccentricities were analyzed using a 1-way repeated ANOVA. The factors that affected the CFs were examined using a multiple regression analysis with stepwise methods to select independent variables, resulting in multiple regression equations correcting for influence of cataracts.

RESULTS

Three experienced surgeons (AO, YG, HS) performed all cataract surgeries with the same technical methods. Surgeries were performed without any complications, and intraocular lenses (IOLs) were fixed in the lens capsule in all eyes. Yellow-tinted IOLs (Acrysof SN60WF, Alcon Japan Ltd., Tokyo, Japan; Vivinex iSert XY1, Hoya Corporation, Tokyo, Japan; Avansee Preload1P YP2.2, Kowa Company Ltd., Nagoya, Japan; TECNIS PCB00V, Amo Japan K.K., Tokyo, Japan) were used in all cases except for one. A clear IOL (SA60AT, Alcon Japan Ltd.) was implanted in one eye. Visual acuity was improved in all eyes (preoperative mean logMAR was 0.18; postoperative mean logMAR was –0.05; $P < 0.001$; paired *t*-test). No severe intraocular inflammations, corneal disorders, or increased intraocular pressures were observed at the time of measurements of MPOD levels after surgery.

Differences Between Subjects With Acceptable and Nonacceptable AF Images Recorded Before Surgery

Among all subject eyes, AF images with acceptable quality for mapping of the MP distribution and calculation of MPOD levels before surgery could be obtained in 67 eyes of 67 subjects. In 33 eyes of the remaining 33 subjects, the image quality was too low due to the presence of a cataract. In contrast, MPOD mapping and level determination could be achieved in all 100 eyes after surgery. The demographic data of the two respective subject groups are shown in Table 1. No significant differences existed in age, sex, BMI, proportion of patients taking lutein supplements, smoking history, or postoperative visual acuity between the two groups. However, there was a significant difference in the preoperative visual acuity. Subjects with unusable AF images had lower visual acuities compared to subjects with acceptable AF images ($P = 0.001$, *t*-test). Also, significant differences were evident in the two categories of WHO-classified cataracts (NUC and PSC). For many of the subjects with acceptable AF images, the NUC was graded 0 or 1, and for most subjects with unacceptable AF images the NUC grade was 1 or 2. Furthermore, subjects with acceptable AF images had a PSC grade of less than 2, and only one subject had a PSC of grade 3. There was a significant difference also in the E-L classification, with most subjects with acceptable AF images in the E-L 1 category.

MPOD Levels Before and After Surgery

The local MPOD levels at the four selected eccentricities and the related MPOD volumes are summarized in Table 2. Figure 2 shows scatter plots of the local MPOD levels at each eccentricity and the MPOD volume before and after surgery in 67 eyes. All local MPOD levels and MPOD volumes were higher after surgery.

Correction Factors for Local MPOD Levels and MPOD Volume

The CFs for the local MPOD levels at each of the four selected eccentricities and for the MPOD volume are shown in Table 3. There was a significant difference in the CFs for the four local MPOD levels, revealing an increase of the required CF with decreasing eccentricity ($P < 0.001$, repeated ANOVA). The results of multiple comparisons are shown in Figure 3. A significant difference was observed between 0.23° and 0.98°, 0.23° and 1.99°, and 0.51° and 1.99° eccentricities ($P < 0.05$, Bonferroni).

Age and IQI were significantly associated with the CFs for the local MPOD level and MPOD volume (Spearman's correlation coefficient by rank test, Table 4), but there were no significant correlations between CF and sex, preoperative visual acuity, BMI, lutein supplementation, or smoking habit. Table 5 shows IQIs and CFs in three subjectively classified groups, with respective significant differences in IQIs. The average IQI was low in the relatively high-quality image group and high in the poor image quality group. Also, the average CF for the local MPOD levels at the four eccentricities and the MPOD volume differed significantly, with a smaller CF required for the group with high image quality relative to the groups with moderate and poor image quality, respectively. Required CFs were highest at lowest eccentricity from the foveal center in all groups.

There were significant differences in the CFs for the local MPOD levels at all selected eccentricities or the MPOD volume depending on the NUC grade (ANOVA, Fig. 4); also, the CFs differed significantly between CEN positive and CEN negative

TABLE 4. The Relationship Between Age or Image Quality Index and the Correction Factor for Local MPOD Levels or MPOD Volume (Spearman's Correlation Coefficient by Rank Test)

	Local MPOD Levels				MPOD Volume
Eccentricity	0.23°	0.51°	0.98°	1.99°	8.98°
Age					
r_s	0.386	0.359	0.408	0.279	0.409
P	0.001	0.003	0.001	0.022	0.001
IQI					
r_s	0.744	0.771	0.785	0.758	0.833
P	0.000	0.000	0.000	0.000	0.000

Table 5. Average (SD) Image Quality Indices and Correction Factors for MPOD Levels Before and After Surgery in Subjectively Classified Three Groups

IQI, CF	Eccentricity	Relatively High Quality, 25	Moderate Quality, 23	Poor Quality, 19	ANOVA
IQI		1.45, 0.40*	2.19, 0.72	5.09, 2.52	$P < 0.001$
		95% CI, 0.58–2.22	95% CI, 1.53–4.22	95% CI, 1.96–11.24	
CF for local MPOD levels	0.23°	1.44, 0.25*	1.77, 0.36†	2.21, 0.61	$P < 0.001$
	0.51°	1.40, 0.25*	1.66, 0.32	2.20, 0.61	$P < 0.001$
	0.98°	1.32, 0.04*	1.56, 0.27	2.08, 0.54	$P < 0.001$
	1.99°	1.21, 0.14*	1.34, 0.17	1.78, 0.48	$P < 0.001$
CF for MPOD volume	8.98°	1.17, 0.11*	1.29, 0.13	1.54, 0.28	$P < 0.001$

* Average value of relatively high-quality images was significantly lower than that of moderate- and poor-quality images ($P < 0.001$, Scheffe's test).
† Average value of moderate-quality images was significantly lower than that of poor-quality images ($P = 0.034$, Scheffe's test).

(Welch *t*-test, Fig. 5). There were no significant differences in the CFs for the COR or PSC grades. In terms of E-L classification, only one eye was categorized as grade 3. Therefore, we compared the CFs between grades 1 and 2 only. The CFs for the same local MPOD levels were significantly higher in grade 2 eyes compared to grade 1 eyes. Also, the CFs for the MPOD volume were significantly higher in grade 2 eyes compared to grade 1 eyes ($P = 0.007$, Welch *t*-test).

Multiple Regression Analysis and Multiple Regression Equation for the Correction of Cataract-Related MPOD Level Attenuation

Multiple regression analyses were performed with either the CFs of the local MPOD levels at the selected four eccentricities or the MPOD volume chosen as dependent variable. All factors that had a significant influence on the CFs were chosen as independent variables, including age, NUC, CEN, E-L classification, and IQI. The NUC and E-L grade were significantly correlated, but the value of the correlation coefficient was relatively low ($r = 0.467$, $P < 0.001$ Spearman). Nevertheless, both were included. The results of multiple regression analyses using a stepwise method are shown in Table 6. The obtained coefficients allow one to calculate CFs for the local MPOD levels and for the MPOD volume with respective equations. The decision factor for the multiple regression analysis was 0.44 to 0.65.

Discussion

The local MPOD levels and MPOD volume derived from the Spectralis-MP autofluorescence images are all underestimated at all eccentricities with respect to their true values in eyes with cataracts. The quality of a particular AF image, which can be quantified with a suitable index, IQI, depends on the severity of the cataract. Multiple regression equations, which include independent correction terms for IQI, age, and NUC grades, allow one to correct for the influence of cataracts if the latter are below NUC grade 2, PSC grade 2 or 3, or E-L 3 or 4.

Regarding differences between subjects with acceptable versus nonacceptable AF images obtainable before cataract surgery, the parameters age, BMI, smoking history, and lutein supplementation did not play a role. However, significant parameters were NUC and PSC grades and E-L classification (Table 1). No clear threshold for NUC and PSC grades or E-L classification could be determined to predict adequate image quality. But eyes with NUC grade 2, PSC grade 2 or 3, or E-L 3 or 4 had little chance to get acceptable AF images with Spectralis-MP.

The local MPOD levels at the selected four eccentricities and the MPOD volume after surgery were higher than those before surgery in all eyes in which MPOD levels could be measured before surgery (Fig. 2). This suggests that the MPOD levels of eyes with cataractous lenses were underestimated from their true value. The mean CFs for the focal MPOD levels at the four eccentricities ranged from 1.42 to 1.77 (Table 3), with the required CFs increasing toward the center of the fovea. This tendency was observed independent of image quality (Table 5). The same finding was reported by Akuffo et al.[25] Possible reasons for this finding could be that nuclear opacity is intense and thick at the center of the lens and PSC usually involved CEN.

We found that higher age, NUC grade, existence of CEN, E-L classification grade, and IQI all influenced the CFs for the local MPOD and MPOD volume measured with the Spectralis-MP. As a consequence, MPOD levels measured in the presence of cataracts need to be corrected accordingly in those subjects. The average CF varied significantly among three groups subjectively classified according to respective AF image quality, with average CF = 1.44 for local MPOD level at 0.23° eccentricity in the group with relatively high image quality, CF = 1.77 in the group with moderate image quality, and CF = 2.21 in the group with poor image quality (Table 5).

The true MPOD levels for eyes with cataracts can be roughly estimated with these CFs according to the following procedure. First, determine the image quality group for the

Table 6. Multiple Regression Analyses in the Correction Factors of the Local MPOD Levels and MPOD Volume in 67 Eyes

					Age		NUC		CEN		E-L		IQI	
MPOD	Eccentricity	R^2	SEE	Constant	B	β	B	β	B	β	B	β	B	β
Local MPOD levels	0.23°	0.56	0.35	0.17	0.01	0.18	0.29	0.22	–	–	–	–	0.16	0.63
	0.51°	0.65	0.31	0.19	0.01	0.20	–	–	–	–	–	–	0.19	0.76
	0.98°	0.44	0.35	−0.07	0.02	0.30	–	–	–	–	–	–	0.13	0.57
	1.99°	0.52	0.26	0.86	–	–	0.25	0.26	–	–	–	–	0.11	0.64
MPOD volume	8.98°	0.53	0.16	0.06	0.01	0.25	0.16	0.27	–	–	–	–	0.06	0.55

ANOVA, $P < 0.001$. R^2, coefficient of determination; SEE, standard error of estimate; CEN, existence of central optical zone opacity; E-L, B: nonstandardized regression coefficient; β; standardized regression coefficient.

recorded AF image (relatively high, moderate, or poor) with the help of Table 5, where image IQIs are listed. Second, look up the corresponding CF in the same table. Third, multiply the nominal MPOD level or MPOD volume with the CF for the desired eccentricity or volume, respectively (multiply nominal MPOD levels for relatively high-quality images at 0.23° eccentricity with CF = 1.44, for moderate to poor images with CF = 1.77, and for poor images with 2.21).

The true MPOD levels can be more accurately derived with established regression equations, using the correction terms of Table 6 for age, NUC, and IQI. Among these, the IQI term is the most important one, as revealed by the corresponding β values. As an example, the CF for local MPOD level at 0.23° is calculated as $0.17 + 0.16 \times$ IQI $+ 0.29 \times$ NUC grade $+ 0.01 \times$ age. The contribution rate of this formula was 0.56 (i.e., 56% of the CF could be determined through the IQI, age, and NUC grade). The actual CF was 1.77 according to Table 3. Then, the relative standard error (standard error divided by true MPOD level) is 0.198 (= 0.35 / 1.77) in this case. Similarly, ratios for 0.51°, 0.98° and 1.99° eccentricities are 0.180, 0.216, and 0.183, respectively. These results suggest that the possible error for the estimated MPOD level at 0.23°, 0.51°, 0.98°, and 1.99° is roughly 20% of the actual level. The CF for the MPOD volume can be derived from the regression equations as CF = $0.06 + 0.06 \times$ IQI $+ 0.16 \times$ NUC grade $+ 0.01 \times$ age. The relative standard error is 0.122, resulting in a possible error for the estimated MPOD volume of 12%. Since the subjects' age range for the example was 41 years and older, the equation applies to all subjects with age 41 and higher.

The limitations of this study are as follows. As a metric for the image quality, we used standard deviations of the MPOD levels automatically generated by the instrument software from its displayed AF image intensities. Since raw images were not available, it is not known to us whether the pixel intensities in the displayed AF images were processed with any image-enhancing software algorithms. This could have a direct bearing on the displayed MPOD plots and derived SD levels, and the image intensities could potentially differ between different instruments, so our developed methodology may be applicable only to the particular device used in our study. Also, even when using several strategically chosen discrete eccentricities toward the perimeter of the macula for our analyses, this approach has a principal problem in that the standard deviations are influenced not only by pixel intensity noise, but also by spatial irregularities of the particular MP distribution, which can often deviate from an idealized 360° rotational symmetry. In other words, if the pigment distribution varies already naturally along the selected eccentricity circumference, these variations along the circumference would cause an artifactual contribution to the standard deviation of the derived local MPOD levels, and this effect could significantly exceed the noise-induced deviation levels in cases of fragmented pigment distributions.

While cataracts clearly affect MPOD measurements with the Spectralis-MP, the present study revealed, nevertheless, that all MPOD results can be corrected relatively accurately, even in a large fraction of elderly subjects, either by suitable CFs derived from the quality of the recorded images or by use of suitable regression equations.

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