The Impact of Cataract, and Its Surgical Removal, on Measures of Macular Pigment Using the Heidelberg Spectralis HRA+OCT MultiColor Device

Kwadwo Owusu Akuffo,[1] John M. Nolan,[1] Jim Stack,[1] Rebecca Power,[1] Clare Kirwan,[1,2] Rachel Moran,[1] Laura Corcoran,[1] Niamh Owens,[1] and Stephen Beatty[1,2]

[1]Macular Pigment Research Group, Nutrition Research Centre Ireland, School of Health Sciences, Waterford Institute of Technology, Waterford, Ireland
[2]Institute of Eye Surgery and Institute of Vision Research, Whitfield Clinic, Waterford, Ireland

Correspondence: Kwadwo Owusu Akuffo, Macular Pigment Research Group, Nutrition Research Centre Ireland, Waterford Institute of Technology, West Campus, Carriganore, Waterford, X91 K236, Ireland; kakuffo@wit.ie.

Submitted: January 14, 2016
Accepted: March 27, 2016

Citation: Akuffo KO, Nolan JM, Stack J, et al. The impact of cataract, and its surgical removal, on measures of macular pigment using the Heidelberg Spectralis HRA+OCT MultiColor device. *Invest Ophthalmol Vis Sci*. 2016;57:2552–2563. DOI:10.1167/iovs.16-19141

Purpose. To investigate the effect of cataract (and cataract surgery) on macular pigment (MP) measurements using the Heidelberg Spectralis HRA+OCT MultiColor device.

Methods. Thirty-six patients (age, 54–87 years) scheduled for cataract surgery at the Institute of Eye Surgery, Ireland, were enrolled in this study. Cataracts were graded using the Lens Opacities Classification System (LOCS) III, and surgery was performed using standard phacoemulsification technique with implantation of a Tecnis ZCB00 or Tecnis ZCT intraocular lens. Macular pigment was measured before and after cataract surgery in the operated (study) eye and in the fellow (control) eye.

Results. In the study eye, there was statistically significant disagreement in measures of MP taken before and after surgery. At all eccentricities, and also for MP volume, the postsurgery measurements were significantly ($P < 0.05$) greater, ranging from an average 16% greater at 1.72° to an average 35% greater at 0.23° eccentricity. Eyes exhibiting large disagreement between pre- and postsurgery measurements at a given eccentricity also generally exhibited substantial disagreement at other eccentricities. Overall severity of cataract contributed to greater disagreement between pre- and postoperative measures of MP, as did grade of nuclear opalescence, nuclear color, and posterior subcapsular cataract. In control eyes, there was no statistically significant disagreement in terms of measures of MP taken before and after cataract surgery ($P > 0.05$ for all; 1-sample *t*-test).

Conclusions. Macular pigment measurements using the Spectralis are affected by cataract. Accordingly, we recommend that cataract be graded when measuring MP with a device that utilizes dual-wavelength fundus autofluorescence and propose the employment of a correction factor to compensate for cataract when measuring MP.

Keywords: macular pigment, macular pigment optical density, fundus autofluorescence, concordance correlation coefficient, agreement, spectralis, cataract, cataract surgery, phacoemulsification, Tecnis IOL, lutein, zeaxanthin, meso-zeaxanthin, visual acuity, optical coherence tomography

Macular pigment (MP) is composed of the carotenoids lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ). Macular pigment is found at the macula, the specialized part of the retina that mediates fine central and color vision.[1] Macular pigment's anatomic location,[2] short-wavelength (blue) light filtering properties,[3] and antioxidant[4–6] and anti-inflammatory properties[7–10] make this pigment important for vision in diseased[11–13] and nondiseased retinas.[14,15]

Given the importance of MP for vision and its role in reducing risk of age-related macular degeneration (AMD) progression,[16] there is clearly a need to measure this pigment accurately in vivo in the clinical and the research setting. Moreover, it is important to be able to measure changes in MP over time.

There are several techniques for measuring MP in vivo, and the most common include heterochromatic flicker photometry (HFP)[17] and fundus autofluorescence (AF).[18] Measurement of MP using either of these techniques rests on assumptions, and each has its own advantages and limitations. While HFP is most widely used, it requires the patient to fixate on the targets presented and follow operator instructions, rendering this method unsuitable for persons with advanced retinal disease (e.g., advanced AMD), dementia, learning difficulties, or memory problems. In addition, when measuring the MP spatial profile, this technique can take up to 30 minutes per eye and provides data only at specific points (retinal eccentricities) across the retina, and therefore does not yield a continuous profile of the pigment.

The Heidelberg Spectralis HRA+OCT MultiColor device (a new commercially available device) utilizes the dual-wavelength AF technique. The Spectralis does not require responses from the patient in order to measure MP. Limitations of this device include the need to pharmacologically dilate the pupil and the relatively bright lights required for photopigment bleaching.

Table 1. Study Procedures Conducted Before Cataract Surgery (V1) and After Cataract Surgery (V2)

Study Procedures	V1	V2
Informed consent	•	
Demographic and lifestyle questionnaire	•	
Dietary carotenoid assessment	•	•
Visual acuity	•	•
Pupillary dilation	•	•
Cataract grading	•	
MP measurement using the Heidelberg Spectralis	•	•
Optical coherence tomography	•	•
Blood sample collection	•	•
Serum carotenoid assessment	•	•

Concordance of MP measurements using the Spectralis and the Densitometer (an established and validated device[19,20] that utilizes customized HFP [cHFP]) has been examined in healthy eyes (i.e., free of retinal disease)[21] as well as in patients with early AMD.[22] In persons with no retinal disease, Dennison et al.[21] reported good concordance between MP readings using the Densitometer and the Spectralis. However, in patients with early AMD, Akuffo et al.[22] recently reported poor concordance between these two devices; they recommended that readings on these devices not be considered interchangeable in a given study in the clinical and research setting, but also concluded that each device yielded reliable measures of MP (and changes in MP) within subjects over time.

One important question with respect to MP measurements using the Spectralis relates to the impact of lens opacification (cataract) on the measurement. A cataract is any opacity of the crystalline lens and causes visual disturbance. Cataracts absorb blue light, and this blue light–absorbing property may affect measures of MP using AF devices. Of note, a previous study conducted by Sasamoto et al.[23] reported that cataracts (especially the nuclear component) affect AF-derived measures of MP. Although the Spectralis also utilizes dual-wavelength AF, it is mechanistically different from the device employed by Sasamoto et al.,[23] and therefore the effect of cataract on MP measurement using the Spectralis merits investigation.

This study was designed to investigate the impact, if any, of cataract on MP measurements obtained using the Spectralis, by measuring MP before and after cataract surgery in each eye of patients scheduled for cataract surgery in one eye.

Methods

Study Design and Population

Thirty-six patients scheduled for cataract surgery at the Institute of Eye Surgery (IOES; www.ioes.ie), Whitfield Clinic, Waterford, Ireland, were enrolled in this study. Figure 1 shows the flow diagram summarizing the study design, patient enrollment, and follow-up. Ethical approval was granted by the Research Ethics Committee, University Hospital Waterford (UHW), Ireland. Written informed consent was obtained from each patient, and the experimental procedures adhered to the tenets of the Declaration of Helsinki. Patients were eligible for this study if they were scheduled for cataract surgery and if they had no evidence of coexisting ocular disease (e.g., AMD, glaucoma) other than mild ocular surface disease (i.e., dry eye and/or blepharitis).

Study clinical assessments (Table 1) were conducted before (baseline; V1) and after cataract surgery (final visit; V2) at the IOES. Study visits began in February 2015 (i.e., first patient visit) and were completed in November 2015 (last patient visit). Each study visit lasted approximately 30 minutes; the interval between visits ranged from 19 to 107 days, with a mean interval per patient of 37.6 days.

Demographic and Lifestyle Questionnaire

The following details were obtained from each patient before cataract surgery: contact information, age, sex, body mass index (BMI in kg/m^2), and spectacle lens prescription.

Visual Acuity

Visual acuity (VA) was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) logarithm of the minimum angle of resolution (logMAR) chart (Test Chart 2000 Pro; Thomson Software Solutions, Hatfield, UK), viewed at 4 meters.

Dietary Carotenoid Assessment

Dietary intake of L and Z was estimated using a crude carotenoid screener known as the "L/Z screener," which was developed by Elizabeth Johnson (Tufts University, Boston, Massachusetts). This screener gives a dietary score (from 0 to 75), which can be categorized as follows: low intake, category 1, 0 to 15: $\leq$2 mg/day; medium intake, category 2, 16 to 30: 3 to 13 mg/day; high intake, category 3, 31 to 75: >13 mg/day. This tool has been described in detail elsewhere.[24,25]

Blood Sample Collection and Serum Carotenoid Assessment

Nonfasting blood samples were collected in 9-mL VACUETTE tubes containing the Z Serum Sep Clot Activator (BD Vacutainer SST Serum Separation Tubes; Becton, Dickinson and Company, Plymouth, UK), adhering to standard venipuncture protocols. The blood samples were allowed to clot at room temperature for approximately 30 minutes and then centrifuged at 725*g* for 10 minutes in a Gruppe GC 12 centrifuge (Desaga Sarstedt, Hampshire, UK) to separate the serum from the whole blood. The resulting serum samples were stored in light-resistant microtubes at −80°C until the time of batch analysis using high-performance liquid chromatography (HPLC). Serum carotenoid analysis was conducted using a method previously described.[26]

Pupillary Dilation

Pupils were dilated using a drop each of 0.5% proxymetacaine hydrochloride and 1% tropicamide prior to performing MP measurement using the Spectralis, optical coherence tomography (OCT), and cataract grading.

Cataract Grading

Cataract grading was performed by a trained and certified grader (CK), using the Haag-Streit 900 Slit Lamp biomicroscope (Haag-Streit AG, Koeniz, Switzerland), adhering to the Lens Opacities Classification System III (LOCS III).[27] The degree of nuclear opalescence (NO) and color (NC) was graded on a scale ranging from 0.1 to 6.9 while cortical (C) and posterior subcapscular (P) opacities were graded on a scale ranging from 0.1 to 5.9.

Optical Coherence Tomography

Optical coherence tomography was performed using the Spectralis HRA+OCT MultiColor (Heidelberg Engineering

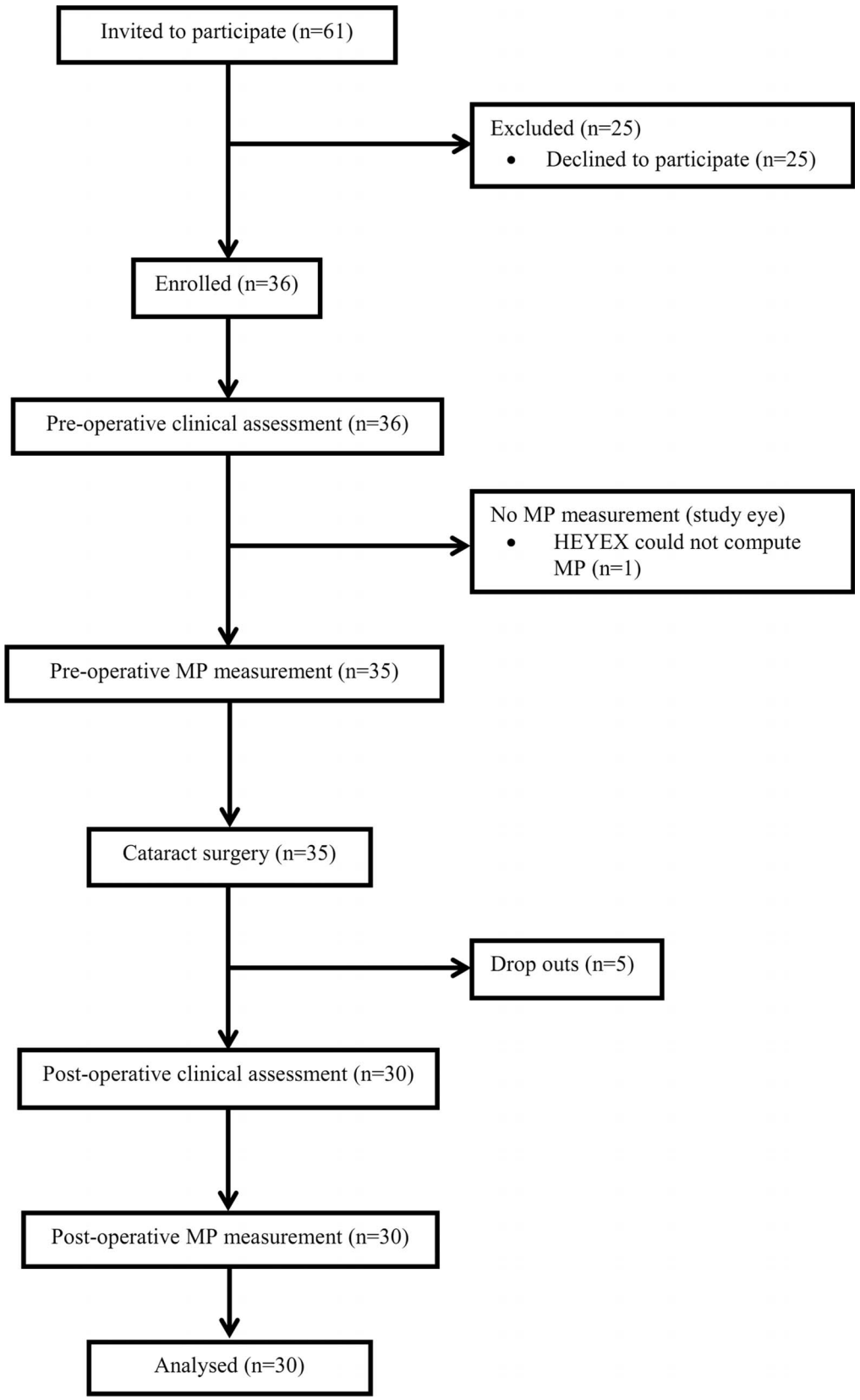

FIGURE 1. Flow chart showing study design, enrollment, clinical procedures, follow-up, and participants included in study analyses. MP, macular pigment; HEYEX: Heidelberg Eye Explorer software. The study eye was the patient's eye that was designated for cataract surgery and that fulfilled the inclusion criteria.

Investigative Ophthalmology & Visual Science

GmbH, Heidelberg, Germany).[28] The following acquisition protocol was used: 20×15 volume scan, 19 scans each 239 μm apart at high speed, automatic real-time mean (ART) of 8 frames per B-scan. Foveal thicknesses (minimum and mean) were recorded following analysis using Heidelberg Eye Explorer software (HEYEX, version 1.9.10.0).

Cataract Surgery

Cataract surgery was performed at the IOES by a single surgeon (SB) using standard phacoemulsification technique, with the implantation of either a Tecnis ZCB00 (Advanced Medical Optics, Inc., Santa Ana, CA, USA) or the toric version of the same intraocular lens (Tecnis ZCT, Advanced Medical Optics, Inc.), as described elsewhere.[29] The two intraocular lenses have the same lens design and absorbance properties and do not block blue light, but may differ only in refractive power. Only one study eye was implanted with a toric Tecnis ZCT, with all other eyes being implanted with a monofocal Tecnis ZCB00. Pre-, intra-, and postoperative procedures adhered to standard protocols at the IOES, which have recently been published.[30] No intra- or postoperative complications were encountered.

Measurement of Macular Pigment Using Dual-Wavelength Fundus Autofluorescence

Macular pigment was measured using the Spectralis HRA+OCT MultiColor. The Spectralis has a confocal scanning laser ophthalmoscope (cSLO) with diode lasers and uses dual-wavelength AF technique (two excitation wavelengths, one that is well absorbed by MP [486 nm, blue] and one that is not well absorbed by MP [518 nm, green]) for measuring MP.

During the measurement, the patient's head was positioned with the help of the canthus alignment mark, and forehead and chin rest. The patient was then instructed to fixate on an internal fixation target. Initial camera alignment, illumination, and focus were done in infrared (IR) mode. Once the image was evenly illuminated, the camera mode was switched to simultaneous blue AF and green AF imaging (BAF+GAF) mode for MP measurement acquisition. After additional adjustments to illumination and focus in order to ensure optimal image quality, a 30-second video was recorded.

The AF images in the video were aligned and digitally subtracted using the Heidelberg Eye Explorer software (HEYEX, version 1.9.10.0), generating the MP spatial distribution profile. Macular pigment at 0.23°, 0.47°, 0.98°, and 1.72° and MP volume were recorded, with the parafoveal reference set at 7°.

When tear film was so poor as to interfere with MP measurement, a drop of Hyloforte (an intensive ocular lubricant; Scope Ophthalmics, London, UK/Dublin, Ireland) was applied. Approximately 1 minute following instillation of the lubricant, a further attempt was made to measure MP. In these cases, the application of the lubricant facilitated acquisition of MP measurements.

Statistical Analysis

One eye (the study eye) of each patient composed the unit of our primary analyses. The study eye was the patient's eye that was designated for cataract surgery and fulfilled the inclusion criteria. We also analyzed MP data from the nonstudy eye as control (secondary analyses). In our analyses of study eyes, we excluded one patient because the MP measurement had a high level of "noise," indicating questionable reliability. In the nonstudy eyes, we excluded two eyes because of macular hole; some nonstudy eyes (14, 38.9%) were pseudophakic, precluding the need to grade cataract, and such eyes were necessarily excluded from some analyses. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY, USA); the statistical programming language R[31] was used to generate agreement statistics such as the concordance correlation coefficient.[32] Disagreement in pre- and postoperative measures of MP was expressed in terms of the ratio of postsurgery (visit 2, V2) measurements to presurgery (visit 1, V1) measurements. The 1-sample *t*-test was used to test if this ratio was significantly different from 1, a ratio of 1 being ideal.

The relationship between the MP V2/V1 ratio and grade of cataract (and other study variables, such as age, sex, BMI) was investigated in the study eye using correlation analyses and general linear models.

Results

Of the 61 patients invited to participate, 36 (56.3%) were assessed before cataract surgery with 30 (46.9% of those invited to participate, but 83.3% of those deemed eligible to participate at the preoperative assessment) completing final study visits after cataract surgery (Fig. 1). Table 2 presents the demographic, lifestyle, cataract, dietary, and serum carotenoid characteristics of all patients who completed clinical assessment before cataract surgery.

Before cataract surgery in the study eye, MP measurements were not obtained in one patient because the HEYEX software failed computation of MP spatial density profile. In the nonstudy eye, MP measurements were not obtained in two patients before cataract surgery and in three patients after cataract surgery, mainly because HEYEX software failed computation of MP spatial density profile.

Serum concentrations of L and Z did not change significantly ($P > 0.05$ for all), on average, in the course of the study.

Comparing Study and Nonstudy Eyes for Disagreement in MP Measurements

Figure 2 shows the scatter plots of the pre- and postsurgery MP values (in study eyes) at 0.23°, 0.47°, 0.98°, and 1.72° and MP volume, with the line $y = x$ superimposed. Figure 3 shows the corresponding scatter plots for nonstudy eyes.

In Figure 2 (study eyes), nearly all points lie to the left of the line $y = x$, indicating that postsurgery MP measurements are consistently higher than presurgery measurements. In Figure 3, there is no such pattern evident for nonstudy eyes. The concordance correlation coefficients[32] for MP0.23° are 0.98 in the nonstudy eyes but only 0.50 in the study eyes; the corresponding indices for MP volume are 0.93 in the nonstudy eyes and 0.84 in the study eyes.

Table 3 provides summary statistics underpinning this graphical representation. For study eyes, the average V2/V1 ratios are significantly different from 1 in all cases ($P < 0.0005$ at 0.23°, 0.47°, and 0.98°; $P = 0.007$ at 1.72°; and $P = 0.014$ for MP volume; 1-sample *t*-test). Thus, statistically significant percentage increases in measured MP following cataract surgery are seen at each eccentricity and for MP volume. Of note, the greatest average apparent increase in MP readings was seen centrally (i.e., 35% at 0.23°), which is approximately double the increase observed at outer eccentricities and for overall MP volume (16%–18%).

In contrast, in nonstudy eyes, none of the average V2/V1 ratios (lower part of Table 3) is significantly different from 1 ($P > 0.05$ for all; 1-sample *t*-test). It is even more revealing to compare, side by side, the V2/V1 ratios in the study versus nonstudy eyes for individual patients' measures of MP at 0.23°

Table 2. Demographic, Lifestyle, Cataract, Dietary, and Serum Carotenoid Characteristics of Patients Before Cataract Surgery

Variable	*n* (%)	Mean ± SD	Range
Age, y	36 (100)	72.92 ± 7.47	54 to 87
Sex			
Male	11 (30.56)		
Female	25 (69.44)		
Body mass index, kg/m^2	36 (100)	26.26 ± 3.68	20.40 to 34.20
Spectacle prescription SE, diopters	34 (94.44)	0.86 ± 2.04	−5.25 to 3.88
Visual acuity SE, VAR	36 (100)	87.92 ± 8.11	75 to 101
Diet score	36 (100)	15.44 ± 11.01	0 to 42
Serum carotenoids, μM			
Lutein	35 (97.22)	0.79 ± 0.41	0.26 to 2.18
Zeaxanthin	35 (97.22)	0.15 ± 0.08	0.02 to 0.33
Foveal thickness SE, μm			
Minimum	36 (100)	225.08 ± 26.31	146 to 277
Mean	36 (100)	292.75 ± 80.25	219 to 742
Axial length SE, mm	30 (83.33)	22.19 ± 0.87	22.19 to 25.89
Cataract SE			
Nuclear opalescence	36 (100)	3.42 ± 1.22	1.2 to 5.9
Nuclear color	36 (100)	4.14 ± 1.14	1.8 to 6.4
Cortical	36 (100)	1.91 ± 1.54	0.1 to 5.1
Posterior subcapsular	36 (100)	0.70 ± 0.70	0.1 to 3.2
Cataract NSE			
Nuclear opalescence	22 (61.11)	3.43 ± 1.07	1.8 to 5.5
Nuclear color	22 (61.11)	4.11 ± 1.07	2.2 to 6.0
Cortical	22 (61.11)	1.45 ± 1.43	0.1 to 4.3
Posterior subcapsular	22 (61.11)	0.69 ± 0.99	0.1 to 4.2
MPOD SE			
0.23°	35 (97.22)	0.48 ± 0.17	0.15 to 0.83
0.47°	35 (97.22)	0.43 ± 0.14	0.12 to 0.73
0.98°	35 (97.22)	0.35 ± 0.11	0.10 to 0.55
1.72°	35 (97.22)	0.16 ± 0.07	0.06 to 0.35
MP volume	35 (97.22)	5,880.57 ± 2,332.97	1,807 to 12,809
MPOD NSE			
0.23°	33 (91.67)	0.56 ± 0.24	0.17 to 1.44
0.47°	33 (91.67)	0.48 ± 0.20	0.14 to 1.14
0.98°	33 (91.67)	0.36 ± 0.15	0.17 to 0.95
1.72°	33 (91.67)	0.17 ± 0.09	0.08 to 0.55
MP volume	33 (91.67)	6,215.42 ± 3,062.16	1,502 to 18,964

Data displayed are mean ± standard deviation (SD) for interval data and percentages for categorical data; SE, study eye; NSE, nonstudy eye; diet score, estimated dietary intake of lutein (L) and zeaxanthin (Z) using the L/Z screener; visual acuity measured with Thompson Test Chart 2000 Pro and recorded in visual acuity rating (VAR); spectacle prescription reported in spherical equivalent refraction (SER); serum carotenoids analyzed using high-performance liquid chromatography (HPLC); axial length measured using the IOLMaster, Version 5 (Carl Zeiss Meditec, AG, Jena, Germany); foveal thickness and macular pigment optical density at 0.23°, 0.47°, 0.98°, and 1.72° eccentricity obtained using the Heidelberg Spectralis HRA+OCT MultiColor; cataracts graded using the Lens Opacities Classification System (LOCS) III; $n \neq 36$ for all tests/measures because certain tests/measures could not be obtained.

eccentricity (Table 4). The V2/V1 ratios in Table 4, with few exceptions ($n = 3$; 11.5%), are closer to 1 in the nonstudy eye than in the study eye. Indeed, the interocular disparity in V2/V1 ratios is dramatic in many cases.

Other Results for Study Eyes

Uniformity of Disagreement in Measured MP at Different Eccentricities, Before and After Surgery (Study Eyes). Disparities in measures of MP before and after surgery hold true across the different retinal eccentricities in a given eye. For example, the pairwise correlations of the V2/V1 ratios at the different eccentricities were statistically significant ($P < 0.001$ for all) and very close to 1 for eccentricities 0.23°, 0.47°, and 0.98° (between 0.94 and 0.97). The pairwise correlations of these ratios at 1.72° were statistically significant ($P < 0.001$ for all) and still high (between 0.68 and 0.79). Thus, there is strong evidence that observed agreement/disagreement within a given eye is maintained across eccentricities for that eye.

Is Disagreement in Measured MP (Study Eyes) Related to V1 Cataract Scores? Preliminary analysis, based on Pearson correlations, showed that NO and NC were positively and significantly associated with V2/V1 ratios at all retinal eccentricities, and with the MP volume ratio ($P < 0.05$ for all). Posterior subcapsular cataract was positively and significantly associated with ratios at eccentricities from 0.23° up to 0.98° ($P < 0.05$ for all). However, C (cortical cataract) was not significantly associated with any ratio ($P > 0.05$ for all).

Nuclear opalescence and NC cataract scores are themselves highly correlated ($r = 0.889$, $P < 0.0005$). When we proceeded

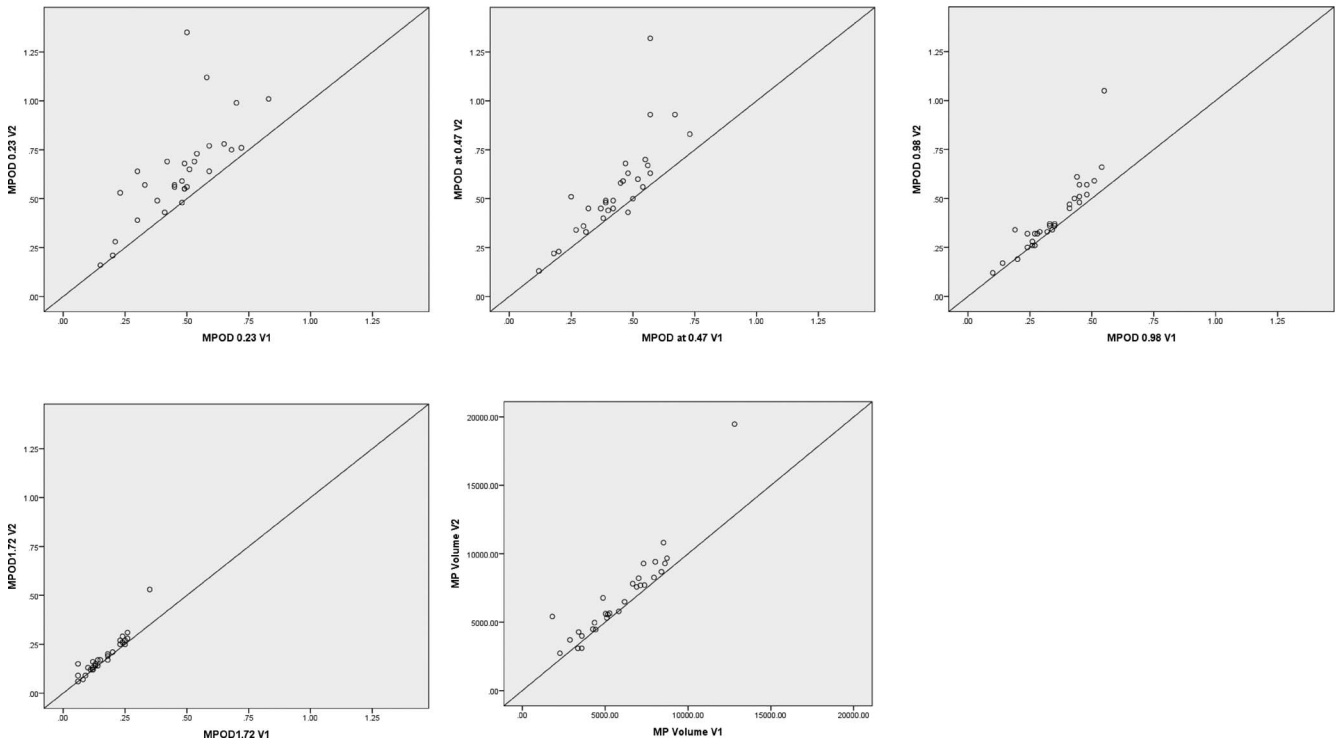

Figure 2. Scatter plots with the line $y = x$ superimposed comparing macular pigment optical density (MPOD) measurements in the study eye using the Heidelberg Spectralis HRA+OCT MultiColor before cataract surgery (V1) and after cataract surgery (V2).

to fit general linear models for the V2/V1 ratio (such as ratio ~ NO+NC+P, where " ~ " means "is modeled as depending on"), the effect of this high correlation, in all cases, was that either NO or NC became redundant in any model already containing the other variable. Selecting from just these three cataract variables, the following models emerged as best: ratio ~ NC+P at 0.23° and 0.47°, ratio ~ NO+P at 0.98°, ratio ~ NC at 1.72° (or ratio ~ NO, i.e., NC and NO are equally strongly related to the V2/V1

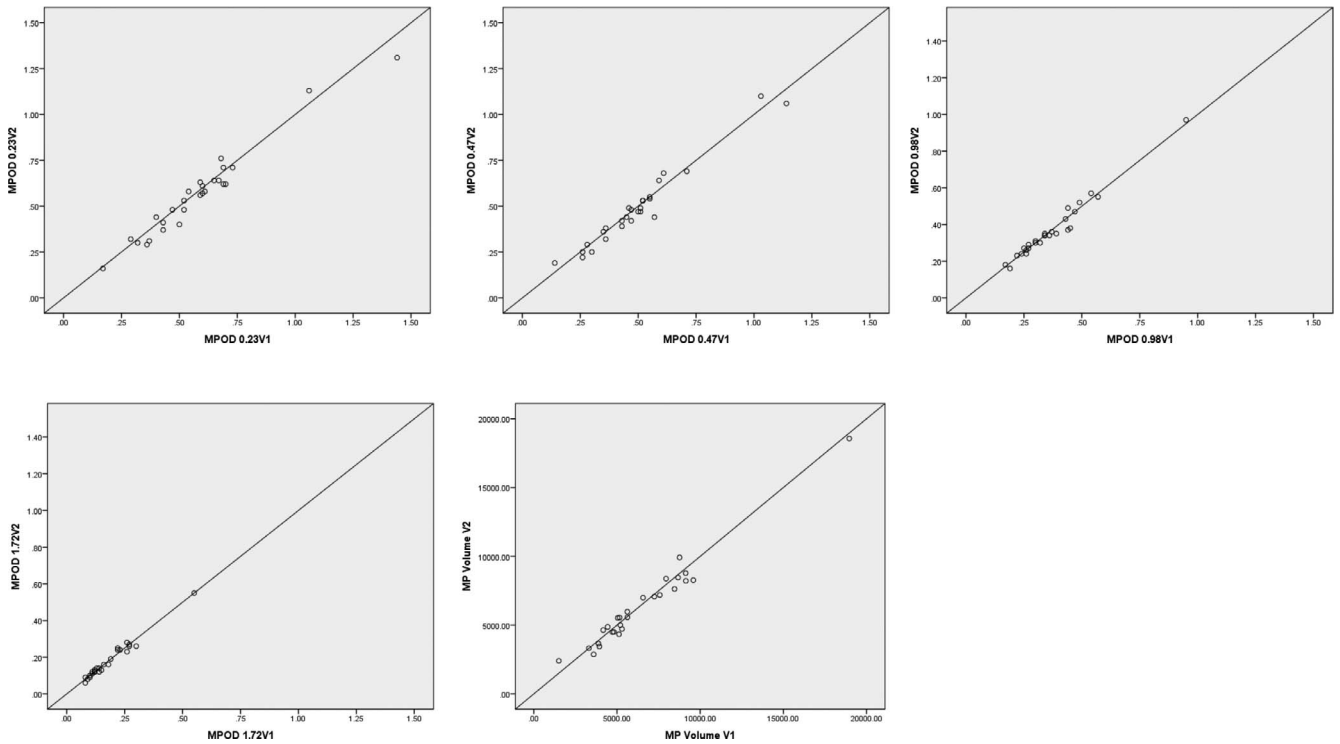

Figure 3. Scatter plots with the line $y = x$ superimposed comparing macular pigment optical density (MPOD) measurements in the nonstudy eye using the Heidelberg Spectralis HRA+OCT MultiColor before cataract surgery (V1) and after cataract surgery (V2).

Investigative Ophthalmology & Visual Science

Table 3. Change in Measured Macular Pigment, Before and After Surgery, Based on the Ratio of Measured Macular Pigment After Cataract Surgery (V2) to Before Cataract Surgery (V1)

MP Ratio (V2/V1)	*n*	Mean ± SD	Range
SE 0.23°	29	1.35 ± 0.37	1.00–2.70
SE 0.47°	29	1.27 ± 0.30	0.90–2.32
SE 0.98°	29	1.17 ± 0.21	0.95–1.91
SE 1.72°	29	1.16 ± 0.29	0.88–2.50
SE MP volume	29	1.18 ± 0.37	0.86–2.99
NSE 0.23°	26	0.97 ± 0.08	0.80–1.12
NSE 0.47°	26	0.99 ± 0.10	0.83–1.36
NSE 0.98°	26	0.99 ± 0.07	0.84–1.11
NSE 1.72°	26	0.98 ± 0.10	0.75–1.14
NSE MP volume	26	1.00 ± 0.15	0.80–1.60

SD, standard deviation; SE, study eye; NSE, nonstudy eye.

ratio at this eccentricity), and ratio ~ NO for MP volume. In all models, coefficients of explanatory variables are positive, indicating that, in all cases, more severe cataract in the study eye (higher NC, NO, or P scores) is associated with higher ratios, that is, with greater disagreement. R^2 values for these fitted models (the proportion of variance in the V2/V1 ratio explained by the cataract scores) ranged from 0.18 up to 0.38, the higher R^2 values being found at central eccentricities.

Is Observed Disagreement in Measured MP (Study Eyes) Related to Other V1 Variables? We examined a wide range of other study variables (including all V1 variables listed in Table 2) in relation to observed disagreement in measures of MP before and after surgery**.** Statistically significant relationships with disagreement were found for six of these variables: age, VA, axial length, serum L, serum Z, and V1 MP at 1.72° eccentricity. We also found a significant negative correlation between change in serum Z (V2 − V1) and disagreement in measurement of MP volume ($r = –0.40$, $P = 0.031$).

Just two of these variables, however, remained significant when included alongside the cataract variables in the earlier fitted models. When V1 MP1.72° is included in some of these models, it has the effect of substantially increasing R^2 values for these models, and also makes the cataract variable P redundant. This is also true of V1 serum L, which could replace P (and increase R^2 to 0.43, from 0.33) in model iii below. However, as measurement of serum L requires specialized laboratory facilities and personnel, model iii below (which requires only cataracts to be measured) is presented as being more practical for the purpose of estimating postsurgery MP from measured presurgery MP.

The final fitted models, therefore, are as follows:

(i) Ratio at 0.23° = 0.222 + 0.184 × NC + 2.193 × MP1.72° V1, $R^2 = 0.49$
(ii) Ratio at 0.47° = 0.41+ 0.14 × NC + 1.677 × MP1.72° V1, $R^2 = 0.45$
(iii) Ratio at 0.98° = 0.866 + 0.074 × NO + 0.085 × P, $R^2 = 0.33$
(iv) Ratio at 1.72° = –0.690 + 0.113 × NC, $R^2 = 0.19$
(v) Ratio for MP volume = 0.764 + 0.123 × NO, $R^2 = 0.18$.

All coefficients of explanatory variables in these models were positive, indicating that greater disagreement is associat-

Table 4. Comparison of Disagreement, Before and After Surgery, in Measured Macular Pigment Between Study and Nonstudy Eye for Individual Patients, Based on the Ratio of Measured Macular Pigment After Cataract Surgery (V2) to Before Cataract Surgery (V1) at 0.23° Eccentricity

	Nonstudy Eye				Study Eye								
ID	0.23V1	0.23V2	Diff0.23	0.23V2/V1	0.23V1	0.23V2	Est.0.23V2*	Diff0.23	0.23V2/V1	NO	NC	C	P
SS002	0.36	0.29	−0.07	0.81	0.83	1.01	1.07	0.18	1.22	1.3	2.8	0.1	0.3
SS003	0.69	0.71	0.02	1.03	0.59	0.77	0.78	0.18	1.31	1.7	3.2	0.7	0.1
SS004	0.52	0.53	0.01	1.02	0.49	0.55	0.55	0.06	1.12	2.8	3.3	2.4	0.1
SS005	0.68	0.76	0.08	1.12	0.49	0.68	0.67	0.19	1.39	2.7	3.3	2.3	1.4
SS006	0.29	0.32	0.03	1.10	0.21	0.28	0.22	0.07	1.33	2.8	3.8	1.3	1.2
SS008	1.06	1.13	0.07	1.07	0.50	1.35	0.95	0.85	2.70	4.8	4.9	0.4	1.8
SS010	0.73	0.71	−0.02	0.97	0.70	0.99	1.14	0.29	1.41	4.3	4.8	2.4	1.2
SS011	0.61	0.58	−0.03	0.95	0.59	0.64	0.78	0.05	1.08	3.7	4.4	0.1	1.2
SS013	0.54	0.58	0.04	1.07	0.45	0.57	0.60	0.12	1.27	2.8	4.3	1.3	1.2
SS015	0.43	0.37	−0.06	0.86	0.45	0.56	0.62	0.11	1.24	3.1	3.2	2.2	1.1
SS018	0.69	0.62	−0.07	0.90	0.48	0.59	0.62	0.11	1.23	3.6	4.4	0.8	0.2
SS019	0.40	0.44	0.04	1.10	0.49	0.55	0.81	0.06	1.12	5.1	5.4	0.6	0.6
SS020	0.70	0.62	−0.08	0.89	0.72	0.76	0.89	0.04	1.06	2.9	3.4	0.1	0.1
SS021	0.52	0.48	−0.04	0.92	0.50	0.56	0.47	0.06	1.12	1.4	1.8	3.4	0.1
SS024	0.32	0.30	−0.02	0.94	0.30	0.39	0.41	0.09	1.30	4.8	4.7	5.1	1.4
SS026	0.60	0.61	0.01	1.02	0.51	0.65	0.77	0.14	1.27	3.8	4.3	0.8	0.8
SS029	0.43	0.41	−0.02	0.95	0.48	0.48	0.52	0.00	1.00	2.4	3.3	0.1	0.1
SS030	0.50	0.40	−0.10	0.80	0.42	0.69	0.75	0.27	1.64	4.7	5.4	2.8	1.2
SS031	0.59	0.56	−0.03	0.95	0.53	0.69	0.64	0.16	1.30	2.8	3.7	2.4	0.1
SS032	0.59	0.63	0.04	1.07	0.65	0.78	0.80	0.13	1.20	2.3	3.8	2.2	0.1
SS033	0.60	0.57	−0.03	0.95	0.54	0.73	0.61	0.19	1.35	1.2	2.8	4.7	0.5
SS034	0.37	0.31	−0.06	0.84	0.20	0.21	0.26	0.01	1.05	2.9	4.8	3.7	0.1
SS035	0.67	0.64	−0.03	0.96	0.33	0.57	0.53	0.24	1.73	5.9	6.4	4.3	1.3
SS036	0.47	0.48	0.01	1.02	0.41	0.43	0.43	0.02	1.05	3.8	3.4	4.3	0.1
SS037	1.44	1.31	−0.13	0.91	0.58	1.12	1.10	0.54	1.93	4.9	6.2	4.3	0.9
SS038	0.65	0.64	−0.01	0.98	0.68	0.75	0.73	0.07	1.10	2.7	3.3	0.1	0.1

Diff0.23, difference in macular pigment optical density (V2 − V1); NO, NC, C, P refer to cataract grades in the study eye before cataract surgery based on the Lens Opacities Classification System III (LOCS III).

* Est0.23V2 is the product of the estimated macular pigment 0.23° V2/V1 ratio (using the model: ratio = 0.222 + 0.184 × NC + 2.193 × MP1.72° V1) and macular pigment optical density at 0.23° V1 in the study eye.

Table 5. Comparison of Measured and Estimated Macular Pigment Measurement Ratios Before and After Cataract Surgery at 0.23° Eccentricity

Variable	*n*	Mean ± SD	Range
Measured MP0.23° V2/V1 ratio	29	1.35 ± 0.37	1.0–2.7
Estimated MP0.23° V2/V1 ratio*	29	1.00 ± 0.18	0.68–1.46

* Estimated macular pigment 0.23° V2/V1 ratio based on the model: ratio = 0.222 + 0.184 × NC + 2.193 × MP1.72 V1; *n* = number included in analysis.

ed with more severe cataracts and with higher measures of presurgery MP1.72°.

Using the Fitted Models (Study Eyes) to Adjust MP Measures at V1. The fitted models, in addition to describing the relationship between measured MP and cataracts, may be used to adjust the presurgery measure of MP (at any eccentricity or MP volume) for cataract in a given eye. To illustrate: Suppose we measure MP0.23° as 0.6 at V1 for a patient whose cataract score at V1 is NC = 4.0 and whose MP1.72° is measured as 0.2 at V1. Then, the V2/V1 ratio for this patient (at 0.23°) is estimated from the model as follows: $0.222 + 0.184 \times 4.0 + 2.193 \times 0.2 = 1.4$ approximately. Thus, for this patient, we would estimate postsurgery MP0.23° as $1.4 \times 0.6 = 0.84$.

Table 5 compares the measured V2/V1 ratio for measured MP0.23° with the ratio V2 MP0.23°/predicted MP0.23°, where predicted MP0.23° uses the above model to predict MP0.23° post surgery from each patient's NC and MP1.72° scores presurgery. It is apparent from Table 5 that the predicted V2 MP0.23° is, generally, much closer to the measured V2 MP0.23° than is the V1 MP0.23°. The mean ratio is now 1, as it should be, and the variation around this ratio (measured by the standard deviation or the range) is much less. Nevertheless, there is still considerable discrepancy between the measured and predicted MP0.23° for some patients. For one patient (minimum ratio = 0.68), predicted MP0.23° is 32% below measured MP0.23°, and for another (maximum ratio = 1.46), the predicted MP0.23° is 46% above measured MP0.23°.

Analysis of Outliers (Study Eyes). Six patients had a V2/V1 ratio in excess of 1.4 at 0.23° eccentricity; that is, their MP0.23° measurement post surgery was at least 40% higher than the presurgery measurement. The lowest of the six NO scores, before surgery, for these patients was 4.3 (which is the 74th percentile for presurgery NO in this study), and the lowest NC score was 4.8 (77th percentile). Thus, all six patients with very poor agreement in measured MP0.23° also exhibited NO and NC grades very much at the upper end of the range. Given that there was high correlation between agreement/disagreement in MP readings at different eccentricities, it is reasonable to hypothesize that these findings extend to other eccentricities. Thus, in general, we report that the greatest discrepancy between pre- and postoperative measures of MP (whether centrally or peripherally) was seen in eyes with more severe lens opacification.

Does the Parafoveal Reference Location Influence the Effect of Cataract on MP Measures Before (V1) and After Cataract Surgery (V2) in the Study Eye? Table 6 presents

Table 6. Location of Parafoveal Reference and Its Impact on Macular Pigment Measurement at 0.23° Eccentricity Before (V1) and After Cataract Surgery (V2) in the Study Eye

	Parafoveal Reference at 5°				Parafoveal Reference at 7°				Parafoveal Reference at 10°			
ID	MP0.23V1	MP0.23V2	0.23V2/V1	Diff0.23	MP0.23V1	MP0.23V2	0.23V2/V1	Diff0.23	MP0.23V1	MP0.23V2	0.23V2/V1	Diff0.23
SS002	0.79	0.97	1.23	0.18	0.83	1.01	1.22	0.18	0.86	1.04	1.21	0.18
SS003	0.55	0.73	1.33	0.18	0.59	0.77	1.31	0.18	0.62	0.81	1.31	0.19
SS004	0.46	0.51	1.11	0.05	0.49	0.55	1.12	0.06	0.51	0.57	1.12	0.06
SS005	0.45	0.63	1.40	0.18	0.49	0.68	1.39	0.19	0.52	0.72	1.38	0.20
SS006	0.20	0.26	1.30	0.06	0.21	0.28	1.33	0.07	0.22	0.30	1.36	0.08
SS008	0.42	1.24	2.95	0.82	0.50	1.35	2.70	0.85	0.57	1.44	2.53	0.87
SS009	0.14	0.15	1.07	0.01	0.15	0.16	1.07	0.01	0.17	0.18	1.06	0.01
SS010	0.68	0.96	1.41	0.28	0.70	0.99	1.41	0.29	0.72	1.01	1.40	0.29
SS011	0.57	0.62	1.09	0.05	0.59	0.64	1.08	0.05	0.61	0.66	1.08	0.05
SS013	0.43	0.54	1.26	0.11	0.45	0.57	1.27	0.12	0.47	0.59	1.26	0.12
SS015	0.40	0.51	1.28	0.11	0.45	0.56	1.24	0.11	0.48	0.58	1.21	0.10
SS018	0.45	0.57	1.27	0.12	0.48	0.59	1.23	0.11	0.50	0.61	1.22	0.11
SS019	0.45	0.51	1.13	0.06	0.49	0.55	1.12	0.06	0.52	0.58	1.12	0.06
SS020	0.67	0.71	1.06	0.04	0.72	0.76	1.06	0.04	0.77	0.80	1.04	0.03
SS021	0.48	0.52	1.08	0.04	0.50	0.56	1.12	0.06	0.53	0.60	1.13	0.07
SS023	0.36	0.47	1.31	0.11	0.38	0.49	1.29	0.11	0.39	0.50	1.28	0.11
SS024	0.28	0.37	1.32	0.09	0.30	0.39	1.30	0.09	0.33	0.41	1.24	0.08
SS025	0.29	0.61	2.10	0.32	0.30	0.64	2.13	0.34	0.31	0.67	2.16	0.36
SS026	0.47	0.62	1.32	0.15	0.51	0.65	1.27	0.14	0.54	0.68	1.26	0.14
SS029	0.44	0.44	1.00	0.00	0.48	0.48	1.00	0.00	0.51	0.51	1.00	0.00
SS030	0.37	0.63	1.70	0.26	0.42	0.69	1.64	0.27	0.46	0.74	1.61	0.28
SS031	0.49	0.65	1.33	0.16	0.53	0.69	1.30	0.16	0.56	0.72	1.29	0.16
SS032	0.61	0.75	1.23	0.14	0.65	0.78	1.20	0.13	0.68	0.80	1.18	0.12
SS033	0.50	0.69	1.38	0.19	0.54	0.73	1.35	0.19	0.57	0.76	1.33	0.19
SS034	0.17	0.19	1.12	0.02	0.20	0.21	1.05	0.01	0.22	0.23	1.05	0.01
SS035	0.32	0.56	1.75	0.24	0.33	0.57	1.73	0.24	0.35	0.58	1.66	0.23
SS036	0.39	0.42	1.08	0.03	0.41	0.43	1.05	0.02	0.42	0.44	1.05	0.02
SS037	0.55	1.09	1.98	0.54	0.58	1.12	1.93	0.54	0.61	1.15	1.89	0.54
SS038	0.65	0.73	1.12	0.08	0.68	0.75	1.10	0.07	0.70	0.77	1.10	0.07

Diff0.23, difference in macular pigment optical density (V2 − V1); macular pigment optical density at 0.23° eccentricity obtained using the Heidelberg Spectralis HRA+OCT MultiColor.

data on MP0.23° (study eye) obtained for three parafoveal reference locations (5°, 7°, and 10°). It is evident from this table that the absolute values of MP at 0.23° eccentricity differ slightly for the three reference locations, with the farther-out reference eccentricities producing slightly higher estimates of MP. However, there is very strong consistency between the MP0.23° V2/V1 ratios for the three parafoveal reference locations. In fact, the intraclass correlation coefficient for the three ratio columns in Table 6 is 0.98 (95% confidence interval 0.97, 0.99); that is, 98% of the variation in ratios in these three columns is between subjects, and only 2% is between columns.

Macular Pigment Profile Assessment

Visual assessment of the MP spatial profile was performed for each patient in the study eye before and after cataract surgery. Of note, 58% of patients had typical profile types (exponential decline from center), and 42% exhibited atypical profile types (e.g., ring-like structures). Profile type did not change following cataract surgery in these patients.

Discussion

We investigated the impact of cataract on measures of MP using the Spectralis, and found statistically significant disagreement between MP readings before and after cataract surgery in our primary analyses (study eyes), with postsurgery measures being higher than those acquired prior to surgical intervention (Fig. 2). Disagreement was statistically significant at all eccentricities, the greatest disagreement being observed centrally; and disagreement was related to severity of lens opacification. In nonstudy eyes (secondary analyses), and in contrast, we found no statistically significant disagreement between measures of MP taken before and after cataract surgery (Fig. 3); in fact, given that test–retest measurements were taken an average of 38 days apart, we report concordance correlation coefficients that are remarkably high in these nonstudy eyes.

In study eyes, we investigated the use of general linear models to adjust for the impact of cataract on MP measurement. While patient cataract scores (LOCS III), as expected, featured prominently as explanatory variables in these models, it was a surprise that presurgery measure of MP, at 1.72° eccentricity, was also a significant predictor of disagreement between pre- and postsurgery measures of MP centrally. A possible explanation, supported by our statistical observations for the different eccentricities, is that cataract affects presurgery MP less at this outer eccentricity; in other words, high presurgery measures of MP at 1.72° reflect high presurgery MP at central eccentricities, the latter being disproportionately affected by cataract. Of note, three of four cataract scores (NO, NC, and P, but not C) were significantly and positively associated with disagreement in measured MP before and after surgery. Nuclear opalescence, NC, and P reflect opacification in the nucleus and posterior subcapsular region of the lens, and are dominant centrally (along the visual axis), whereas C reflects opacification of the cortical region of the lens (and is distributed radially, in a manner that is not dominant along the visual axis).[27] Our findings are therefore not counterintuitive, given that disagreement was greater at central eccentricities (and that disagreement was related to those measures of opacification that are dominant centrally).

Predicting postsurgery central MP with a general linear model, including the MP1.72° variable alongside the cataract variable NC, did go some way toward addressing the observed downward bias in presurgery measures of MP centrally. Our final models (e.g., estimated MP0.23° V2/V1 ratio = 0.222 + $0.184 \times$ NC + $2.193 \times$ MP1.72° V1) may therefore be useful for addressing the impact of lens opacification on MP using the Spectralis.

Furthermore, we found that MP volume is less affected by cataract, in line with the observation that MP values at outer eccentricities are less affected than those yielded for central MP. Thus, in an older population with varying severity of cataract, MP volume would appear to be a more appropriate surrogate of overall MP than, say, MP at central retinal eccentricities (e.g., 0.23°). Moreover, central MP does not always predict total amount of MP because of variability in MP spatial profile (e.g., narrow peak versus broad peak, with same central value).

Our results are consistent with a study by Sasamoto et al.,[23] which examined the effect of cataract on MP measurement using the dual-wavelength AF technique. In that study, MP was measured before and after cataract surgery in 45 eyes of 41 subjects using the Heidelberg Retina Angiograph (HRA; Heidelberg Engineering, Dossenheim, Germany), but at only one eccentricity (0.5°) and utilizing the wavelengths 488 and 514 nm; the authors concluded that MP measurements are affected by cataracts (especially by nuclear cataracts). Of note, the fellow eye was not used as control in the study by Sasamoto et al.,[23] which represents a limitation of that study.

Our secondary analyses (nonstudy eyes) demonstrate that, in the absence of cataract surgery or cataract progression, MP measurement using the Spectralis is robust to test–retest variability over short periods of time, and this observation is consistent with a study by You et al.[33] and will have important implications for clinical practice in the future, as well as for those research studies measuring MP over time.

We could not obtain MP measurements in some patients because the HEYEX software could not compute the MP spatial density profile from the acquired video. Possible explanations include too much eye movement during MP measurement acquisition and poor image quality, which may be related to cataract severity. For example, the cataract severity grade (LOCS III) in one of these patients was NO: 5.5; NC: 5.5; C: 1.4; P: 0.2.

In the current study, we examined the effect of the parafoveal reference point on the discrepancy between pre- and postoperative measures of MP (Table 6). In addition to the 7° reference point (standard device reference point), we examined the effect of the parafoveal reference location at 5° and 10° on V2/V1 ratios (study eye) for MP0.23° eccentricity. We found that the choice of parafoveal reference location had very little influence on the ratio data for our analysis, and so we would have arrived at the same conclusions (about the effect of cataract on MP measurement with the Spectralis) whichever reference point had been chosen.

It is known that central retinal thickness is positively correlated with MP optical density.[34] We investigated whether central foveal thickness could also help explain the discrepancy between measures of MP before and after cataract surgery. We report that discrepancy between MP measurements, before and after cataract surgery, was not associated with baseline central retinal thickness.

Another important point is that detector sensitivity remained unchanged throughout the current study and therefore the effect of different detector settings on pre- and postoperative measures of MP was not examined. Future studies should examine the effect of different detector settings (high versus low) on measures of MP using the Spectralis.

The Spectralis uses the dual-wavelength AF technique, which rests on the assumption of a relatively clear ocular media. The optical density of the crystalline lens is particularly variable among persons at any age.[35] This should be borne in mind in interpreting the results of the current study.

Assumptions and possible mechanisms that could contribute to the observed discrepancy in macular pigment optical density (MPOD) measurements before and after cataract surgery are discussed below. First, the basic idea to overcome the effect of the lens scattering is the following: The ratio of green AF to blue AF (GAF/BAF) for the center is referenced to the ratio GAF/BAF in the periphery (5°, 7°, and 10°). Here the following assumptions were made:

a) The scattering effect (for the ratio green excitation light to blue excitation light) is similar for light entering the pupil at an angle of 0° central to the fovea as for light entering the pupil at 5°, 7°, or 10°.
b) The MP density is negligible in the periphery (5°, 7°, 10°); therefore the periphery can be used as a reference point.
c) Bleaching of the photopigment leads to a stable ratio (green excitation light to blue excitation light) in the periphery as well as in the center.
d) Fluorescence signal from the lens does not contribute significantly since the signal is suppressed by the confocal detection (pinhole).

All assumptions are reasonable, although it is difficult to say to what extent they are really valid especially in severe cataracts. Assumption a) is most likely better fulfilled for smaller angles 5° vs. 10°, whereas assumption b) is most likely better fulfilled for the 10° reference compared to 5° reference. Assumptions a), c), and d) could depend on the extent of the cataract, whereas assumption b) does not. Especially assumption d) could contribute, since severe cataract will reduce the signal from the retina and at the same time fluorescence from the lens can be increased. The detection unit in the Spectralis is designed to detect light simultaneously from several layers (e.g., choroidal and retinal blood system); therefore the pinhole is larger than the diffraction limit and confocal suppression is not optimum. This could be possibly the major effect for erroneous MP measurements in patients with cataract.

Bleaching of the retina (photopigment) could be different for fovea compared with the periphery, since for the center, due to the presence of MP, a higher luminance is required to bleach the photopigments. It is possible that for eyes with cataracts, where less light reaches the retina, the bleaching effect is still sufficient in the periphery but not complete within the fovea. This could explain larger gray value changes in the fovea compared with changes in the periphery after cataract surgery.

Second, regarding sensor sensitivity, the higher the sensitivity, the broader the noise distribution for a given AF value, and clipping could occur during the averaging procedure. However, this effect has been carefully considered in the design of the Spectralis MP software. The Spectralis has a sensitivity wheel (next to the touch screen), which can be adjusted between 31 and 107 in arbitrary sensitivity units. These sensitivity units are adjusted according to the intensity of light; that is, for IR reflection, very low sensitivity settings (e.g., 50) are used, whereas for AF images the sensitivity increases to >90 (maximum 107). However, when measuring MP, the Spectralis limits the sensitivity to 90 (i.e., the very high sensitivity settings are blocked for MP measurements), and by shifting of the digitization range, it is guaranteed that the complete zero light distribution is measured and no clipping occurs. The offset level is very carefully analyzed from laser offset measurements acquired during the resetting period of the Y-scanners. Therefore, the high-sensitivity setting should have no or only minor effects on MP measurements with the Spectralis; this could differ to some extent in the older HRA devices.

Other mathematical assumptions and potential explanations:

1. Wavelength-dependent absorption of lens: Assume that Beer's law is valid for the lens. In this case the intensity of blue and green light after passing the lens becomes:

$$B(x) = B_0 \times \exp(-b \times x)$$
$$G(x) = G_0 \times \exp(-g \times x)$$

with

B_0 = intensity of incoming blue laser light
G_0 = intensity of incoming green laser light
b = absorption coefficient of the lens for blue laser light
g = absorption coefficient of the lens for green laser light
x = thickness of the lens
B = intensity of the blue laser light after passing the lens
G = intensity of the green laser light after passing the lens

Assume $B_0 = G_0 = 1$, density of fluorophores is constant, and there is no MP. In this case the measured optical density would be related to

$$\log\Big(B(x)/G(x)\Big) = (g - b) \times x \neq 0.$$

If b, g, and x vary with the scanning angle, then this variation in addition to the optical density of the retina is measured.

If this is the case, then there should be a similar effect in the cHFP. The optical density of MP after cataract operation differs from the optical density before cataract operation. However, the changes may be different due to different wavelengths of the devices.

2. Fluorescence of the lens: If this is the case, the detected optical density would be:

$$\mathrm{OD} = \log\Big((B + BAF)/(G + GAF)\Big) \neq \log(B/G)$$

with

B = intensity of fluorescence light of the retina excited with the blue laser light
G = intensity of fluorescence light of the retina excited with the green laser light
BAF = intensity of fluorescence light of the lens excited with the blue laser light
GAF = intensity of fluorescence light of the lens excited with the green laser light

Please note that this effect would occur in cHFP.

3. Little fluorescence of the retina: If there is only little fluorescence of the retina, then the signal-to-noise ratio becomes poor. Small errors in the measurement of the underground or the signal may have a large effect. There could be a problem with averaging optical densities if the signal-to-noise ratio is poor. For example, if the blue and green signals have a value of 1 and noise is 0.01, the quotient of blue and green will be somewhere between 0.99/1.01 and 1.01/0.99. The average is close to 1.00. However, if the blue and green signals have a value of 1 and noise is 0.25, the quotient of blue and green will be somewhere between 0.75/1.25 and 1.25/0.75. The average is 1.13.

The strengths of this study include the following: All cataract surgery procedures were performed by a single surgeon using a single model of non–blue-blocking intraocular lens, thereby eliminating potential bias in these respects; cataract grading was conducted by a trained and certified LOCS

III grader; one trained examiner performed MP measurements before and after cataract surgery, thereby eliminating interexaminer bias and variability; dietary and serum carotenoid assessment was performed to control for any variability in MP measurement attributable to these parameters; and the fellow eye was used as a control (i.e., in the absence of cataract surgery). Limitations of this study include its small sample size and a large number of potential study patients who were ultimately unable to participate due to the need for an accompanying person for transport purposes (because of the need to pharmacologically dilate the pupils at the study visits, a measure that would not be part of routine clinical evaluation of an eye before or after cataract surgery at the IOES[36]).

In conclusion, we recommend that cataract be graded as a matter of routine during measurement of MP in older adults using currently available AF techniques, and suggest that such grading may be useful to correct for the impact of cataract on MP readings using such devices. However, over short periods of time, the Spectralis device does yield reliable and reproducible MP values in patients with cataracts that have not been surgically removed.

Acknowledgments

We thank Elizabeth Johnson, Tufts University, Boston, Massachusetts, United States, for permission to use the L/Z screener for estimating dietary intake of lutein and zeaxanthin.

Supported by Heidelberg Engineering GmbH, Heidelberg, Germany, a high-tech medical device company that designs, manufactures, and distributes diagnostic instruments for eye care professionals. KOA, RP, LC, and JMN are funded by the European Research Council, grant agreement number 281096. JMN is also funded by the Howard Foundation, Cambridge, United Kingdom.

Disclosure: **K.O. Akuffo**, None; **J.M. Nolan**, Nutrasight Consultancy Limited (C, E); **J. Stack**, None; **R. Power**, None; **C. Kirwan**, None; **R. Moran**, None; **L. Corcoran**, None; **N. Owens**, None; **S. Beatty**, Nutrasight Consultancy Limited (C, E)

References

1. Hirsch J, Curcio CA. The spatial resolution capacity of human foveal retina. *Vis Res*. 1989;29:1095–1101.
2. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. 1. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:660–673.
3. Bone RA, Landrum JT, Cains A. Optical-density spectra of the macular pigment in vivo and in vitro. *Vis Res*. 1992;32:105–110.
4. Sujak A, Gabrielska J, Grudzinski W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch Biochem Biophys*. 1999;371:301–307.
5. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch Biochem Biophys*. 2010;504:56–60.
6. Wrona M, Rozanowska M, Sarna T. Zeaxanthin in combination with ascorbic acid or alpha-tocopherol protects ARPE-19 cells against photosensitized peroxidation of lipids. *Free Radic Biol Med*. 2004;36:1094–1101.
7. Tian Y, Kijlstra A, van der Veen RL, Makridaki M, Murray IJ, Berendschot TT. Lutein supplementation leads to decreased soluble complement membrane attack complex sC5b-9 plasma levels. *Acta Ophthalmol*. 2015;93:141–145.
8. Tian Y, Kijlstra A, van der Veen RL, Makridaki M, Murray IJ, Berendschot TT. The effect of lutein supplementation on blood plasma levels of complement factor D, C5a and C3d. *PLoS One*. 2013;8:e73387.
9. Li SY, Fung FK, Fu ZJ, Wong D, Chan HH, Lo AC. Anti-inflammatory effects of lutein in retinal ischemic/hypoxic injury: in vivo and in vitro studies. *Invest Ophthalmol Vis Sci*. 2012;53:5976–5984.
10. Firdous AP, Kuttan G, Kuttan R. Anti-inflammatory potential of carotenoid meso-zeaxanthin and its mode of action. *Pharm Biol*. 2015;53:961–967.
11. Liu R, Wang T, Zhang B, et al. Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:252–258.
12. Akuffo KO, Nolan JM, Howard AN, et al. Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration. *Eye (Lond)*. 2015;29:902–912.
13. Ma L, Yan SF, Huang YM, et al. Effect of lutein and zeaxanthin on macular pigment and visual function in patients with early age-related macular degeneration. *Ophthalmology*. 2012;119:2290–2297.
14. Loughman J, Nolan JM, Howard AN, Connolly E, Meagher K, Beatty S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci*. 2012;53:7871–7880.
15. Hammond BR, Fletcher LM, Roos F, Wittwer J, Schalch W. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on photostress recovery, glare disability, and chromatic contrast. *Invest Ophthalmol Vis Sci*. 2014;55:8583–8589.
16. Age-Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA*. 2013;309:2005–2015.
17. Bone RA, Landrum JT. Heterochromatic flicker photometry. *Arch Biochem Biophys*. 2004;430:137–142.
18. Delori FC. Autofluorescence method to measure macular pigment optical densities fluorometry and autofluorescence imaging. *Arch Biochem Biophys*. 2004;430:156–162.
19. Wooten BR, Hammond BR. Spectral absorbance and spatial distribution of macular pigment using heterochromatic flicker photometry. *Optom Vis Sci*. 2005;82:378–386.
20. Stringham JM, Hammond BR, Nolan JM, et al. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res*. 2008;87:445–453.
21. Dennison JL, Stack J, Beatty S, Nolan JM. Concordance of macular pigment measurements obtained using customized heterochromatic flicker photometry, dual-wavelength autofluorescence, and single-wavelength reflectance. *Exp Eye Res*. 2013;116:190–198.
22. Akuffo KO, Beatty S, Stack J, et al. Concordance of macular pigment measurement using customized heterochromatic flicker photometry and fundus autofluorescence in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:8207–8214.
23. Sasamoto Y, Gomi F, Sawa M, Sakaguchi H, Tsujikawa M, Nishida K. Effect of cataract in evaluation of macular pigment optical density by autofluorescence spectrometry. *Invest Ophthalmol Vis Sci*. 2011;52:927–932.
24. Akuffo KO, Beatty S, Stack J, et al. Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials. *Ophthalmic Epidemiol*. 2014;21:111–123.
25. Nolan JM, Loskutova E, Howard A, et al. The impact of supplemental macular carotenoids in Alzheimer's disease: a randomized clinical trial. *J Alzheimers Dis*. 2015;44:1157–1169.
26. Nolan JM, Loskutova E, Howard AN, et al. Macular pigment, visual function, and macular disease among subjects with

Alzheimer's disease: an exploratory study. *J Alzheimers Dis*. 2014;42:1191–1202.

27. Chylack LT Jr, Wolfe JK, Singer DM, et al. The Lens Opacities Classification System III. The Longitudinal Study of Cataract Study Group. *Arch Ophthalmol*. 1993;111:831–836.
28. Wolf-Schnurrbusch UE, Ceklic L, Brinkmann CK, et al. Macular thickness measurements in healthy eyes using six different optical coherence tomography instruments. *Invest Ophthalmol Vis Sci*. 2009;50:3432–3437.
29. Charalampidou S, Loughman J, Nolan J, et al. Prognostic indicators and outcome measures for surgical removal of symptomatic nonadvanced cataract. *Arch Ophthalmol*. 2011; 129:1155–1161.
30. Kirwan C, Nolan JM, Stack J, Moore TC, Beatty S. Determinants of patient satisfaction and function related to vision following cataract surgery in eyes with no visually consequential ocular co-morbidity. *Graefes Arch Clin Exp Ophthalmol*. 2015;253: 1735–1744.
31. R Development Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2008.
32. Lin L, Hedayat AS, Wu W. *Statistical Tools for Measuring Agreement*. New York: Springer; 2012.
33. You QS, Bartsch DG, Espina M, et al. Reproducibility of macular pigment optical density measurement by two-wavelength autofluorescence in a clinical setting [published online ahead of print December 11, 2015]. *Retina*. doi:10.1097/IAE.0000000000000893.
34. Liew SH, Gilbert CE, Spector TD, et al. Central retinal thickness is positively correlated with macular pigment optical density. *Exp Eye Res*. 2006;82:915–920.
35. Norren DV, Vos JJ. Spectral transmission of the human ocular media. *Vis Res*. 1974;14:1237–1244.
36. Saeed A, Guerin M, Khan I, et al. Deferral of first review after uneventful phacoemulsification cataract surgery until 2 weeks: randomized controlled study. *J Cataract Refract Surg*. 2007;33:1591–1596.

Investigative Ophthalmology & Visual Science