Macular Pigment Shows Ringlike Structures

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Purpose. The spatial distribution of macular pigment is generally assumed to monotonously decrease to very low values in the periphery. However, there are indications that this picture may be too simple. The purpose of this study was to examine the spatial distribution of the macular pigment optical density.

Methods. Fundus reflectance and autofluorescence maps at 488 and 514 nm Argon laser wavelengths were acquired in 53 healthy subjects with a custom-built scanning laser ophthalmoscope. Because the lens and the macular pigment are the only absorbers in this wavelength region, digital subtraction of log reflectance and log autofluorescence at the two wavelengths provides density maps of the sum of both absorbers.

Results. In approximately half of the subjects, we observed a distinct ring pattern at a mean distance of 0.7° of the fovea. In a few subjects, the ring had an even larger optical density than did the central peak. A simple model with an exponentially decaying density as a function of eccentricity, in combination with a Gaussian-distributed ring pattern, yielded a good description of the data for both methods. The widths of the central peak and the Gaussian ring, and also the eccentricity at which the ring peaks, were similar for both methods. The prominence of the ring did not depend on age and gender.

Conclusions. Both reflectance and autofluorescence maps showed ring patterns in the distribution of the macular pigment, which probably follow the inner plexiform layer. (*Invest Ophthalmol Vis Sci.* 2006;47:709–714) DOI:10.1167/iovs.05-0663

A decade ago Seddon et al.[1] observed an inverse association between a diet with a high content of the carotenoids lutein and zeaxanthin, and the prevalence of age-related macular degeneration (AMD). Although further studies yielded inconclusive results,[2–7] this finding triggered a renewed interest in the macular pigment (MP), because it is solely composed of these two carotenoids.[8,9] MP is concentrated in the central area of the retina along the axons of the cone photoreceptors.[10,11] There are some plausible arguments to assume it exerts a protective effect in the retinal area: It acts as a blue light filter, absorbing between 390 and 540 nm,[12–15] thereby decreasing chances for photochemical light damage.[16] In addition, MP is capable of scavenging free radicals.[17] Several studies addressed the possible role of MP more explicitly by measuring macular pigment optic density (MPOD) in patients with, or at risk of AMD. Results were ambiguous, however.[18–20] MP is entirely of dietary origin. In healthy subjects it has been shown that MPOD can be changed by a dietary modification[21] or by supplements.[22–24] In subjects with a diseased macula carotenoid, absorption may differ. However, a recent study indicated that MPOD can also be augmented by lutein supplementation in subjects with early AMD.[25]

The spatial distribution of MP is generally assumed to decrease monotonously to very low values at an eccentricity of approximately 10°. However, there are indications that this may not be true (Delori FC, et al. *IOVS* 2004;45:ARVO E-Abstract 1288).[26–28] The purpose of this study was to examine the spatial distribution of MPOD.

Methods

Fundus reflectance[29] and autofluorescence maps[30,31] at 488 and 514 nm argon laser wavelengths were made with a custom-built scanning laser ophthalmoscope (SLO).[32] The lens and the macular pigment are the only absorbers in this wavelength region. Therefore, digital subtraction at two wavelengths of log reflectance and digital subtraction at two wavelengths of log autofluorescence provided density maps of the sum of both absorbers (see Fig. 1). For the reflectance, we assumed a double pass through the MP, for the autofluorescence a single pass. The measured densities were corrected with a factor for the slightly lower difference in the MP absorbance spectrum at 488 and 514 nm, compared with the peak at 460 nm and null at $\lambda > 540$ nm.[14] We assumed the MPOD distribution to be circularly symmetric and determined MPOD as a function of eccentricity by calculating for each pixel the distance to the peak (see Fig. 1). The subjects' pupils were dilated with tropicamide 0.5%. Chin rests and temple pads were used to maintain head position. The alignment periods, lasting 1 to 2 minutes, also served to bleach the visual pigments almost completely.[33] MPOD estimates from the reflectance maps were based on two images only. Because of the low signal-to-noise ratio in autofluorescence, we averaged 10 images, at both 488 and 514 nm, before calculating the MPOD maps.

Subjects

A total of 53 subjects were measured. The mean age of the 53 subjects was 50 ± 17 years (range, 19–76; men/women, 29/24). Mean age differed significantly between men (57 ± 12 years) and women (42 ± 18 years, $P = 0.01$). MPOD for one eye was determined in the right eye in 43 subjects and the left eye in 10. All measurements were included in the analysis, because there is a high correlation between MPOD in both eyes.[34–36] All subjects had good general health. Inclusion criteria were refractive error between −4 and +3, no known eye disease, and no reports of poor visual acuity. The research adhered to the tenets of the Declaration of Helsinki and was approved by the local medical ethics committee. Before testing, all subjects gave written informed consent after the nature and possible consequences of the study were explained.

Statistical Analysis

A computer was used for data analysis (SPSS, Release 10.0.7; SPSS, Chicago, IL) The *t*-test was used to evaluate possible gender differences and paired *t*-tests to compare the two methods. Pearson correlation tests were used to study possible age effects.

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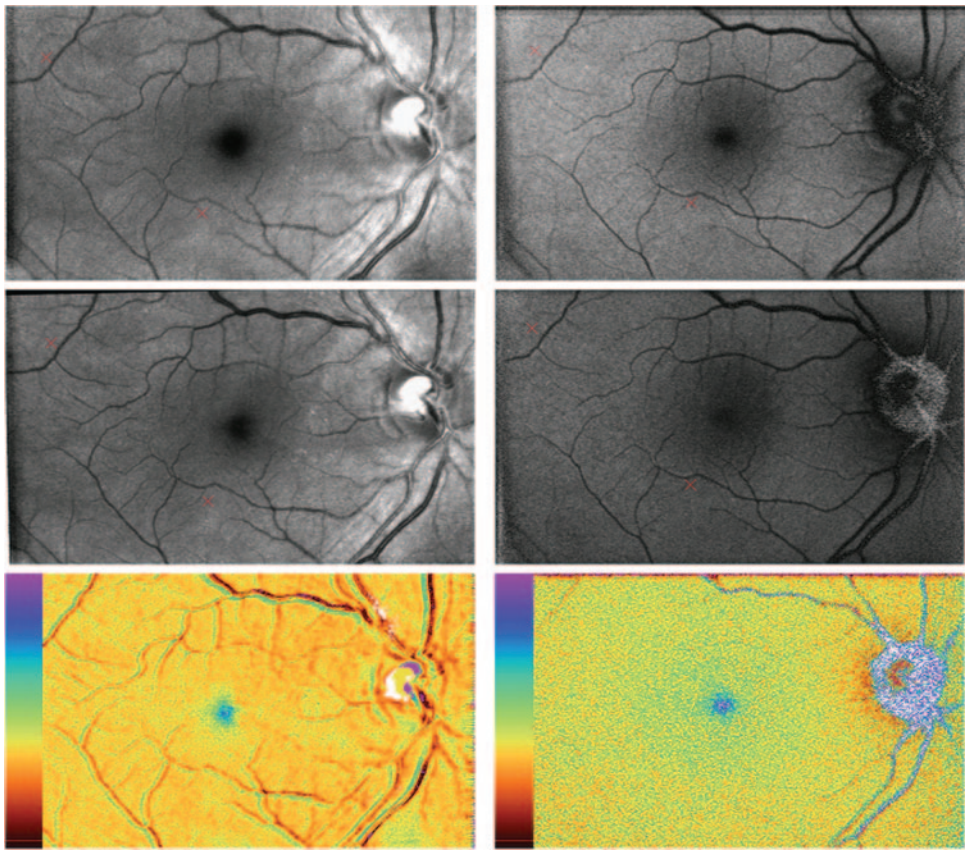

FIGURE 1. Reflectance (*left*) and autofluorescence maps (*right*) at a wavelength of 488 nm (*top*) and 514 nm (*middle*). The lower images show the corresponding color coded MPOD maps.

RESULTS

Mean peak MOPD (inner 0.5°) was 0.32 ± 0.10 for the reflectance method and 0.32 ± 0.11 for autofluorescence. We found no significant differences between the two techniques (−0.012; paired *t*-test, $P = 0.33$), and a significant Pearson correlation coefficient ($r = 0.637$, $P < 0.001$). Figure 2 shows five examples of MPOD as a function of eccentricity from reflectance and autofluorescence maps. From the top to the bottom images, an increasing ringlike structure was seen. The corresponding maps are shown as insets. In approximately half of the subjects we observed a distinct ring pattern at a mean distance of 0.7° of the fovea. In some subjects, the ring had an even larger OD than did the central peak (Fig. 2, bottom). An exponentially decaying density as a function of eccentricity in combination with a Gaussian-distributed ring pattern gave a good description of the data, as

$$\mathrm{MPOD}(x) = A_1 \times 10^{-\rho_1 x} + A_2 \times 10^{-\rho_2 [x - x_2]^2}$$

where x is the eccentricity, A_1 and A_2 are the amplitudes of the distributions, ρ_1 and ρ_2 the peakednesses, and x_2 the eccentricity at which the Gaussian distribution peaks. The solid lines in Figure 2 are results of fits with the latter model. The fits provided a similar value of x_2 for the reflectance and the autofluorescence methods (Fig 2). The same held for ρ_1 and ρ_2. Therefore, to obtain more reliable estimates, we assumed these three to be equal for reflectance and autofluorescence and fitted reflectance and autofluorescence data simultaneously. We found a mean A_1 of 0.28 ± 0.13 for the reflectance method and 0.31 ± 0.12 for the autofluorescence method ($P = 0.10$). Mean A_2 was 0.13 ± 0.07 and 0.11 ± 0.08, respectively ($P = 0.22$). Mean ρ_1 was 0.38 ± 0.24°. Some subjects did not or hardly showed a ring (see e.g., Fig. 2, top). In these subjects x_2 and ρ_2 were undetermined. To get more reliable estimates of x_2 and ρ_2, we therefore excluded all subjects with $A_2 < 0.03$. We then found a mean ρ_2 of 1.2 ± 1.1 deg^2 and mean x_2 of 0.70 ± 0.66°. To quantify the prominence of the ring, we defined a ring index as the quotient of the integrated amount of MP in the Gaussian-distributed ring and the amount of MP in the main exponential decaying function. We found a mean ring index of 0.43 ± 0.32 for the reflectance method and of 0.35 ± 0.38 for the autofluorescence method. For the reflectance data, the ring index exceeded 0.25 in 57% of the subjects. For the autofluorescence data it exceeded 0.25 in 41% of the subjects. We found no differences between men and women for all fit parameters and the ring indices. Neither did we find any age effect.

DISCUSSION

The MPOD has generally been assumed to decrease monotonically with eccentricity. However, Staurenghi et al. (*IOVS* 2003; 44:ARVO E-Abstract 5118) and Delori et al. (*IOVS* 2004;45: ARVO E-Abstract 1288), in studying autofluorescence images, found strong evidence of deviations from this behavior. This study confirms their results, and strengthens their observation, because we added the technique of reflectance spectroscopy.

An easy subjective measurement to observe MP is Maxwell's spot (after its 19th-century discoverer[37]). After a subject stares at a bright yellow surface for a while and then at a blue surface, the subject sees a dark spot, that shows the presence of MP. The darker it is, the more MP. Approximately 50 years ago, Miles[38] studied this phenomenon in detail. In line with the present findings, he found a ring in 14 of the 19 subjects that were able to see Maxwell's spot.

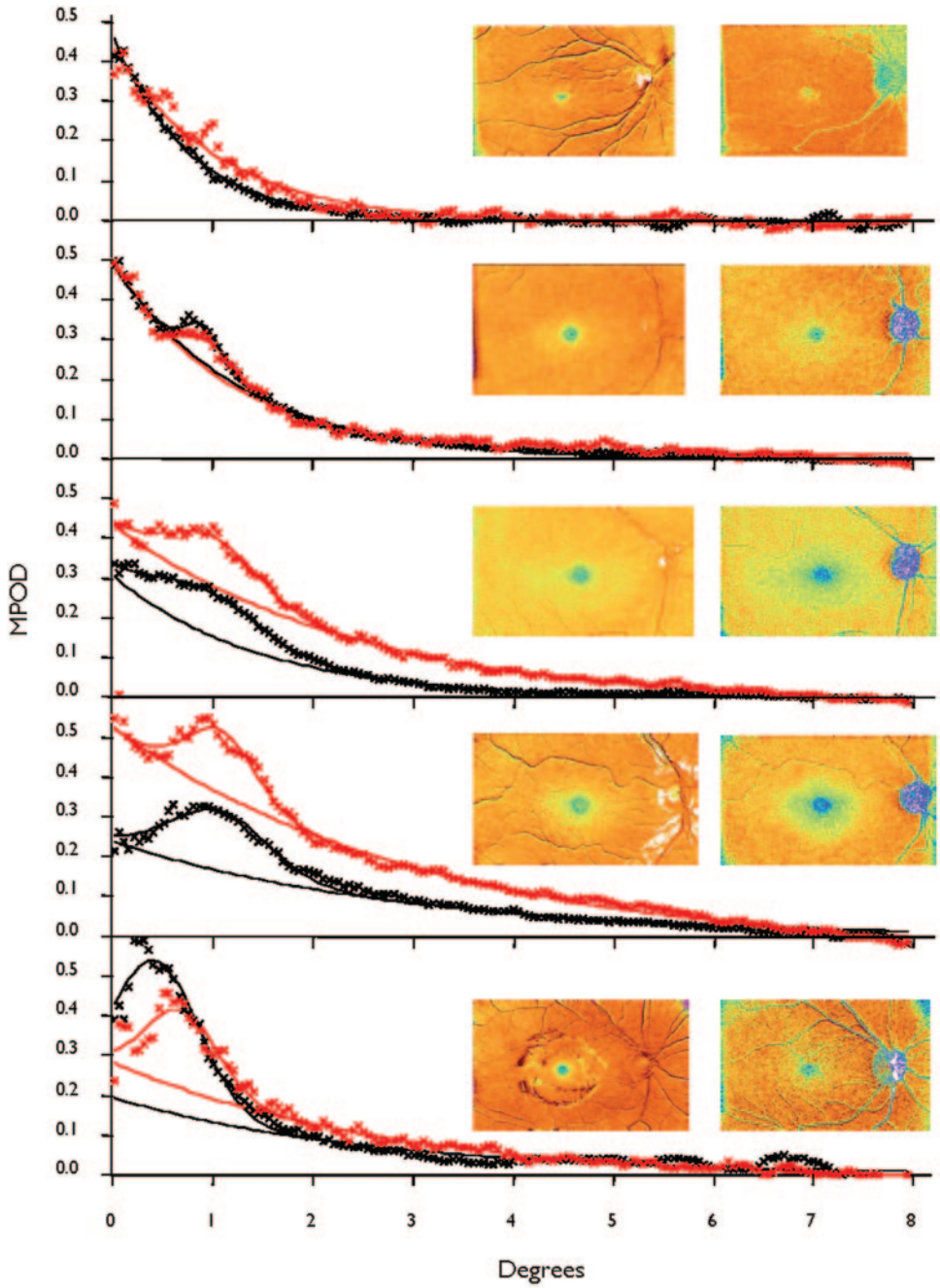

FIGURE 2. MPOD as a function of eccentricity from reflectance (*black*) and autofluorescence (*red*) maps. Macular pigment maps obtained from reflectance (*left*) and autofluorescence (*right*) are shown as *insets*. The MPOD distribution was assumed to be circularly symmetric and MPOD was determined as a function of eccentricity by calculating for each pixel the distance to the peak. *Solid lines*: results of a model fits.

Twenty years ago, Snodderly et al. [11] measured MP density profiles by two-wavelength microdensitometry in macaque and cebus monkeys. They found a central peak, with shoulders or flanking peaks around 0.8° eccentricity. The main peak was associated with MP along the receptor axons, whereas the shoulders followed the inner plexiform layer. In a second paper, Snodderly et al.[39] studied the spatial distribution of lutein and zeaxanthin in squirrel and macaque monkeys together with the spatial profiles if the MPOD. Both lutein and zeaxanthin reached their highest concentrations at the center of the fovea and declined monotonically with eccentricity. However, MPOD again showed shoulders or flanking peaks around 0.8° eccentricity. They proposed that variations in the orientation of the dichroic lutein and zeaxanthin as a function of eccentricity might cause the differences between the profiles of the optical density and the amount of the MP.[36,40]

Hammond et al.[26] studied the spatial distribution of the MPOD in 32 subjects with heterochromatic flicker photometry. An exponentially decreasing MPOD with eccentricity with a $\rho_1 = 0.39 \pm 0.20$ provided a good description of their MPOD profiles, in line with $\rho_1 = 0.38 \pm 0.24$ found in this study. They also noted small but significant deviations between 0.5° and 1.0° that took the form of valleys or flanking peaks. These occurred in 40% of their subjects. No explanation was provided. Another characteristic of their MP spatial profile was the significant correlation between the width of the distribution and the peak density: MP distributions tend to be wider in individuals with higher MP peak densities. We could not confirm this finding.

Using fundus reflectometry with an SLO, as in our study, Elsner et al.[41] compared MPOD maps with maps of the visual pigment. In some of their subjects they found large round

alterations in the MPOD or visual pigment distribution. All the women included in their study showed some alterations, and the alterations increased with age.

Recently, Delori et al.[28] studied the spatial distribution using autofluorescence. In more than half of the subjects, they found an annulus of higher density superimposed on a central exponential-like distribution. The annulus was located at a similar position as in this study of approximately 0.7° from the fovea. As in Elsner et al.,[41] women were more likely to exhibit the bimodal distribution. We could not confirm the latter finding.

In another recent study De Lint et al.[42] observed a yellow ring-shaped reflection at the center of the macula with indirect ophthalmoscopy. They attributed their observation to a difference in reflectance of the incoming light between cones at the foveal center and cones at eccentricities beyond 1°. Apart from this cause, a lower MPOD at the foveal center than in a ring around 1° may enhance the ringlike appearance.

Several other studies measured MPOD profiles, but did not find rings, or did not look in detail to their particular distribution. Berendschot et al.[22] used a custom-built SLO to measure reflectance maps at 488 and 514 nm in studying the influence of lutein supplementation on MPOD. Data were analyzed with an exponential decaying function with eccentricity that seemed to provide a reasonable fit to the data. No obvious rings were seen at that time, perhaps because of the small sample ($n = 8$). Chen et al.[43] used a modified fundus camera and a cooled CCD for in vivo imaging reflectometry. They found a mean $\rho_1 = 0.19 \pm 0.04$ in a young aged group ($n = 24$, mean, 24.8 ± 2.6 years), $\rho_1 = 0.16 \pm 0.03$ in a middle-aged group ($n = 13$, mean, 40.2 ± 8.3 years), and $\rho_1 = 0.12 \pm 0.02$ in an old-aged group ($n = 17$, mean, 67.5 ± 7.1 years). These ρ_1s are much lower (inferring a broader distribution) than those in the present study. In a fundus camera, stray light caused by vitreous backscatter and reflectance at the inner limiting membrane is hard to avoid. This factor may result in an underestimate of the MPOD. Indeed, absolute MPODs were low compared with those in other studies.[29] Also, it causes a broadening of the apparent spatial distribution, because the influence of the stray light is more pronounced for higher MPODs. As a result, the observed increase in ρ_1 with age may in part be an artifact caused by an increase in stray light with age. Chen et al.[43] noted shoulders on the MPOD profile; however, these were found at much larger eccentricities of approximately 4°. Bour et al.[44] used photographic images obtained with a fundus camera to study the MPOD distribution in 23 pediatric subjects. They found a mean $\rho_1 = 0.25 \pm 0.05$. This rather low ρ_1, accompanied with a rather low absolute value,[29] may again be caused by stray-light problems as in Chen et al.[43] Ringlike structures were not mentioned. Trieschmann et al.[27] extracted MPOD from autofluorescence images at 488 nm only. They reported no ring structures. However, they did not perform a second reference measurement at a wavelength that is not or is hardly absorbed by the MP. Therefore, the observed MPOD distribution shows the real MPOD distribution, diluted by various lipofuscin and melanin concentrations as a function of eccentricity, the exact shape of which is unknown. This makes their results difficult to interpret. Robson et al.[45] used minimum motion photometry to determine MPOD profiles in 18 subjects. Broad shoulders at approximately 4° were observed in two subjects. Their spatial resolution of 0.5° was probably too low to find ring structures as were found in this study. They also measured autofluorescence images. However, as in Trieschmann et al., they used only one wavelength. Wüstemeyer et al.[30] used reflectance and autofluorescence maps to determine MPOD distributions by comparing maps obtained at 488 and 514 nm. However, they looked only at the peak values and did not study the spatial profiles in detail.

In healthy subjects, it has been shown that MPOD can be changed by dietary modification[21] or by the ingestion of supplements.[22–24] In subjects with macular disease, carotenoid absorption may differ. However, a recent study indicated that MPOD can also be augmented by lutein supplementation in subjects with early AMD.[25] All modification studies mentioned measured the peak MPOD. A recent supplementation study in rhesus monkeys, however, stated that the increase in MPOD was substantially higher in the periphery than in the central fovea.[46] Also, various new studies have shown differences in spatial distribution with supplementation (Köpcke W, et al. *IOVS* 2005;46:ARVO E-Abstract 1768; Snodderly DM, et al. *IOVS* 2005;46:ARVO E-Abstract 1766; Sheehan JP, et al. *IOVS* 2005;46:ARVO E-Abstract 1763). It may well be that the prominence of the rings depends on differences in lutein and zeaxanthin intake.

Apart from the macular pigment, the visual pigments also absorb light at 488 and 512 nm.[14] For the middle-wavelength–sensitive cones, peaking at 534 nm, the absorption at 488 nm is 62% of its peak density and 91% at 514 nm. For the long-wavelength–sensitive cones, peaking at 556 nm, the absorption is 0.32% at 488 nm and 0.64% at 514 nm. Thus, if visual pigment is not fully bleached, there will be a difference in absorption between the two wavelengths of approximately 30% of the peak density. Similar to MPOD, visual pigment optical density has its maximum in the foveal center and decreases with eccentricity.[47–49] We attempted nearly fully bleaching the visual pigments during the alignment period. However, if visual pigment could not be bleached, it could have resulted in an apparent spatially dependent decrease in MPOD, giving rise to the observed rings. To study this effect in its most enhanced way, we measured a MPOD map from two single reflectance images after a 10-minute period of dark adaptation. Fast shutters in our SLO allow the capture of one frame only. Because of this short exposure to the measuring light, the retina stays in its dark-adapted state.[33,47] We compared these MPOD maps with maps obtained after a 2-minute bleach with a 514 nm, 6.3 log troland bleaching light. We did not find significant differences among eccentricity, width, and prominence of the ring between the two maps.

In Figure 1 the autofluorescence image at 488 nm is substantially brighter than the one at 514 nm. The autofluorescence method uses the intrinsic fluorescence of the lipofuscin that is normally present in the human retinal pigment epithelium. This fluorescence has its absorption peak at 490 nm, which makes the 488 nm more efficient than the 514-nm excitation. Further, the filters to capture the autofluorescence had a cutoff wavelength of 510 nm for the blue excitation and a cutoff wavelength of 530 nm for the green excitation. As a result, more fluorescence was captured from the 488 nm excitation than from the 514 excitation. Finally, in some subjects the voltage of the photomultiplier tube differed between the two wavelengths. All these phenomena result in an apparent difference in gain between the 488- and 514-nm maps. However, MPOD maps are obtained by digital subtraction at two wavelengths of log autofluorescence. This implies that a difference in gain shows up as an offset in the MPOD map. In our analysis, we assumed that the MPOD has vanished at 8°; thus, at this point we define the optical density to be zero. This seems a very reasonable assumption, as can be observed in Figure 2. Differences in illumination gradient between the two excitation wavelengths may also cause apparent density differences. Therefore, before calculating the MPOD, possible differences in illumination are corrected for, although in our setup they hardly differ.

There was no age dependency in the MPOD. This finding is discussed in another paper.[50]

In conclusion, both reflectance and autofluorescence maps showed similar ring patterns in the distribution of the macular pigment, which probably follow the inner plexiform layer. A better understanding of this distribution may be important in epidemiologic studies on the role of the MP in AMD.

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