Fast assessment of the central macular pigment density with natural pupil using the macular pigment reflectometer

Jan van de Kraats
University Medical Center Utrecht
Department of Ophthalmology
AZU E03.136
P.O. Box 85500
NL-3508 GA Utrecht
The Netherlands

Tos T. J. M. Berendschot
University Medical Center Utrecht
Department of Ophthalmology
AZU E03.136
P.O. Box 85500
NL-3508 GA Utrecht
The Netherlands
and
University Eye Clinic Maastricht
P.O. Box 5800
NL-6202 AZ Maastricht
The Netherlands

Suze Valen
Dirk van Norren
University Medical Center Utrecht
Department of Ophthalmology
AZU E03.136
P.O. Box 85500
NL-3508 GA Utrecht
The Netherlands

Abstract. We built a new macular pigment reflectometer (MPR) for fast and objective measuring of the optical density of macular pigment in the human eye, using the undilated eye. The design is based on the spectral reflectance from a spot of white light at the fovea. To evaluate its performance, we measured the macular pigment of 20 healthy subjects, ages 18 to 79 years, under four conditions: (1) natural pupil in the dark, (2) natural pupil with dim room light, (3) dilated pupil in the dark, and for comparison with a different technique, (4) heterochromatic flicker photometry (HFP) in dim room light with natural pupil. Condition 1 was repeated in a subset of 10 subjects after an interval of at least 3 days. Data analysis with a model of reflectors and absorbers in the eye provided the density of the macular pigment in conditions 1 to 3. Dim room light and pupil dilatation had no influence on measured density. Mean within subjects variation was typically 7%. Mean difference between test and retest after at least 3 days was 1%. Correlation between MPR and HFP was $r=0.56$ ($p=0.012$). Mean within subjects variation with HFP was 19%. The new instrument holds promise for specific applications such as epidemiological research. [DOI: 10.1117/1.2398925]

Keywords: ophthalmology; reflectometers; optical systems; spectroscopy; reflectance; vision.

Paper 06116R received May 8, 2006; revised manuscript received Aug. 2, 2006; accepted for publication Aug. 16, 2006; published online Nov. 22, 2006.

1 Introduction

Macular pigments invoke increasing interest because of their putative role in preventing chronic adverse effects of light on the retina. In particular, there is an ongoing debate on the positive effects of the macular pigments lutein and zeaxanthin in preventing age-related macular degeneration (AMD).[1–3] Several studies have shown that it is relatively easy to manipulate the amount of macular pigment either by eating food rich in lutein and zeaxanthin or by taking supplements.[4–8] A pivotal element is assessing the amount of the macular pigments in the human retina. This is not a trivial task. Their overlapping spectral characteristics make individual assessments of lutein and zeaxanthin difficult, if not impossible in the living eye. Generally, overall macular pigment optical density (MPOD) is measured. There are two main approaches. The most widespread method makes use of psychophysical techniques. The subject adjusts color or luminosity, generally through a minimum flicker or a minimum motion task.[9,10] The psychophysical approach has the advantage of requiring no special measures like pupil dilatation or head fixation, but a complete measurement is rather time-consuming, and the task, in particular when making a match in the peripheral retina, is not trivial. The second approach is through analysis of light returning from the retina. It relies either on spectral analysis,[11,12] autofluorescence,[13] or on Raman spectroscopy.[14] It has the advantage of being objective and relatively fast, but it has the drawback of requiring sophisticated equipment that is only available in a few specialized laboratories.

In this study, we aimed to build a specialized, but simple device for fast, reliable MPOD measurements, without the need for pupil dilatation. We compared the results of the new instrument with one based on heterochromatic flicker photometry (HFP).

2 Methods

2.1 *Macular Pigment Reflectometer*

The optical arrangement is shown in Fig. 1. Light from a 30-W halogen lamp is delivered at the eye through three optical relay systems. The first relay (L1, L2) images the filament of the lamp on a mask. Between L1 and L2, filtering (GG395, Schott, Mainz, Germany) occurs to cut off uv. This to reduce the level of dangerous light in subjects with their

Address all correspondence to: Jan van de Kraats, University Medical Center Utrecht, Department of Ophthalmology, AZU E03.136, P.O. Box 85500, NL-3508 GA Utrecht, The Netherlands; Tel.: +31 30 2507872; Fax: +31 30 2505417; E-mail: jkraats@umcutrecht.nl

1083-3668/2006/11(6)/064031/7/$22.00 © 2006 SPIE

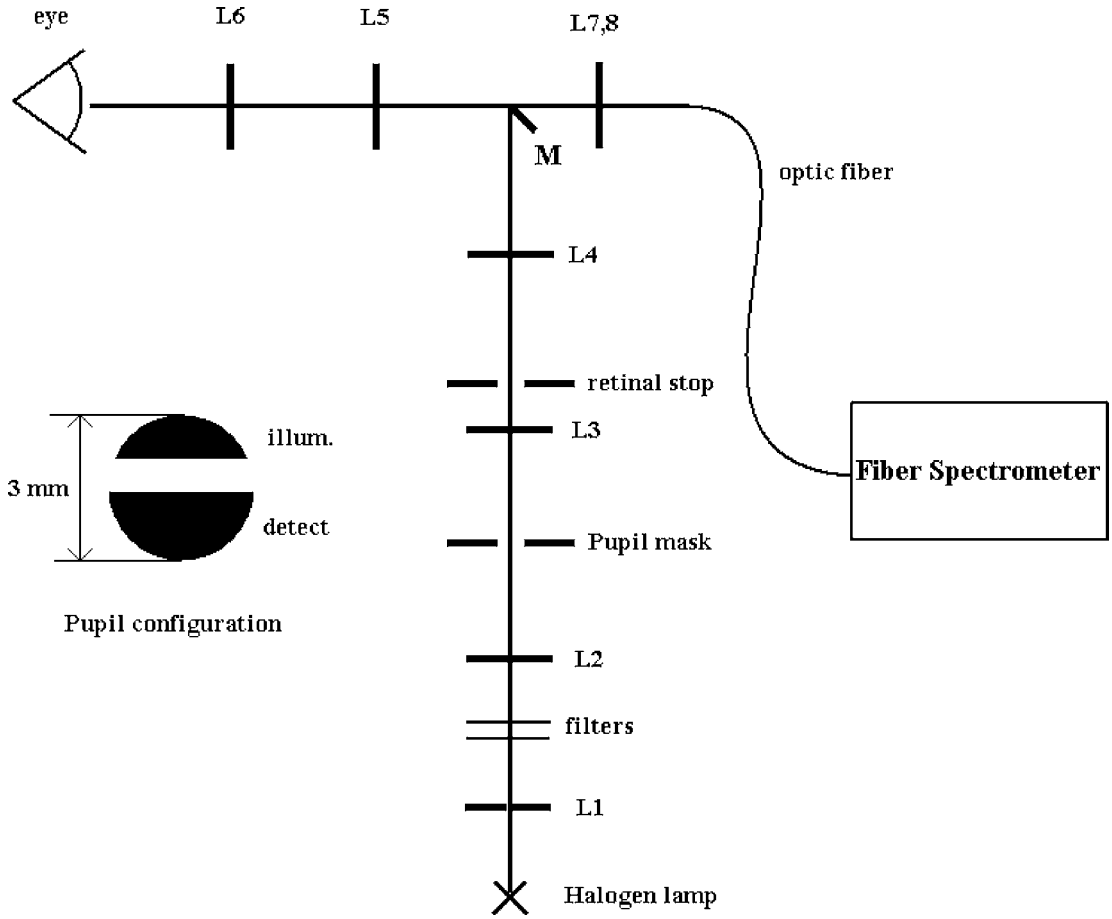

Fig. 1 Optical scheme of the MPR. An image of the filament of the halogen lamp is relayed by lenses L1 to L4 to a mask and then to a mirror *M*. Filters (GG395, Schott) are inserted to reduce uv light. The Badal system L5 to L6 has L6 as a movable lens to focus a retinal stop (between L3 and L5) on the retina. The detection channel has the Badal system in common with the illumination system, without overlap of the light paths in lenses. Lenses L7 to L8 form an image of the retinal field on the tip of a fiber that connects to a commercial spectrometer. The insert shows the dimensions of the illumination and detection beams at the level of the pupil.

eye lenses removed. The second relay (L3, L4) images the filament within the mask on a small mirror *M* at 45 deg. The last relay (L5, L6) forms an image of the eye pupil at the plane of *M*, where the illumination beam coming from *M*, and detection beam, passing over *M* are separated ("pupil separator"). The relay (L5, L6) forms a Badal system, as L6 is movable to focus a stop (between L3 and L4) at the retina. Focusing range is from −15D to +15D. The resulting retinal spot has a diameter of 1-deg visual field, a retinal illuminance of 1.04×10^7 Td, and a retinal irradiance of 4.6 mW/cm^2. The calculated maximum exposure time, based on the recommendations of the Health Council of the Netherlands (1993)[15] is 26 min; for people with aphakia, it is 20 min. The detection channel has the Badal system (L5, L6) in common with the illumination system, with no overlap in the lenses itself to keep instrumental stray light to a minimum. The pupil separator contains masks to limit the illumination and detection configuration (separation 0.8 mm) in the pupil to a 3-mm circle (Fig. 1). For subjects with a pupil diameter smaller than 3 mm, absolute calibration of percentage reflection no longer holds. However, this does not immediately make accurate measurements of macular pigment impossible, as these are determined from the shape of the spectral reflection. A diameter of 2.3 mm is about the practical lower limit. Lenses L7 and L8 form an image of the retinal spot on the tip of an optical fiber. A mask at the tip of the fiber defines a diameter of 1 deg at the retinal plane. The fiber is the receiving part of a commercial fiber spectrometer (Ocean Optics SD2000, Ocean Optics Inc. Dunedin, Florida). Optical resolution is 5.8 nm (FWHM); the range 400 to 800 nm was used for analysis. The output of the spectrometer is sent to a personal computer. Integration time was set at 1 s. The displayed reflection spectrum is the mean and standard deviation of two succeeding 1-s periods.

Reflectance was calibrated with a surface painted with Eastman 6080 white at a distance of 230 mm from the pupil plane position ("white calibration"). The surface was mounted at the end of a black anodized tube to eliminate room light. The Eastman 6080 white surface was compared with a $BaSO_4$ surface. Differences were accounted for in the software. During the calibration, the spectrometer averaged 30 measurements of 1 s each. The first lens was set to a position such that the white surface was conjugate with the retinal planes of the instrument. Because the distance of 230 mm from the pupil plane is 10 times the focal distance of the eye, the measured spectrum equaled a 1% reflection from the eye. This reflection

calibration accounts both for the spectral sensitivity of the detection system, and for the spectral content of the illumination. The white calibration was corrected for spurious reflections in the front lenses by averaging 30 measurements with a light trap at the position of the eye ("dark calibration"). The light trap consisted of two polished black plates at a tapering angle, positioned at the pupil plane of the instrument. The dark calibration was taken with the lens position optimized for each subject (see Sec. 2.3). Wavelength was calibrated with a lamp containing mercury and argon lines (Avalight-CAL, Avantes Eerbeek, The Netherlands).

2.2 *HFP*

We used a portable instrument developed by Mellerio et al.[16] In summary, a test field flickers between a blue light that is highly absorbed by the macular pigment (MP) and a green light that is not absorbed by the MP. A minimum flicker match is made by adjusting the intensity of the blue light when the retinal image of the 1-deg test field lies on the fovea. Another match is made away from the fovea, where the MPOD is assumed to be negligible. The test field was an annulus of 5-deg inner radius and of 1-deg width, centered on the foveal fixation spot. The logarithm of the ratio of the blue luminosity for the foveal match to that for the extramacular match gives the MPOD.

2.3 *Protocol*

The research followed the tenets of the Declaration of Helsinki and was approved by the local medical ethics committee. All subjects ($n=20$, ages 18 to 79 years) were Caucasian, not known to have an eye disease, and with a visual acuity of at least 0.8. The purpose was explained at the beginning of the experiment, and written informed consent was obtained. Chin rest and temple pads were used to help maintain head position. The instrument was aligned using a joystick with the subject's eye. The (clearly visible) illumination beam was set in the subject's pupil a little above its center, allowing room for the invisible detection beam. The subject was asked to fixate the center of the 1-deg spot. Initial focusing was obtained with a diopter scale on the instrument using the spectacle prescription. Next the subject was asked whether the spot edges were sharply focused. If necessary, an adjustment was made. Correcting refraction resulted in concentric illumination and detection fields at the retina. Without focusing, they are concentric posterior or anterior to the retina, and reflected light is lower in amplitude. Therefore, a final adjustment was made by optimizing the amplitude of the measured reflection spectrum. The experimenter viewed a reflectance curve, updated once per second, together with the highest curve (with maximum reflectance between 500 to 600 nm) obtained since the start of the measurement. This feature also facilitated optimizing the position of illumination and detection beam with regard to the subject's pupil. When the front lens was optimized, the subject leaned back, allowing the experimenter to perform the dark calibration. Thereafter, final alignment took place. Dark calibration changed very little over the course of hours, thus in fact it could have been taken less frequently.

Reflections from the corneal surface and/or lens were minimized by adjusting the distance between eye and the instrument, and minor adjustments in the position of the illumination beam. Corneal reflections show up as a relatively high signal at the short wavelengths compared to the middle wavelengths. We estimated that alignment time was sufficient to bleach more than 95% of the visual pigments. Subjects were asked to occasionally blink but keep their eyes wide open during measurements. Five spectra were measured and stored on a disk for further analysis in three conditions: (1) natural pupil, dark room, for getting statistics on measurements with natural pupil in an optimal environment; (2) natural pupil dim room light, for getting statistics on measurements with natural pupil in a less optimal environment with a disturbing light, in this case from a dimmed incandescent lamp set at 3 lux at the eye; (3) dilated pupil, dark room, for having an optimal reference with no disturbing room light and no possible influence of interception of measurement light by the pupil edges. Ten subjects were remeasured in condition 1 after an interval of at least 3 days to check reproducibility.

With the HFP instrument, according to the prescribed protocol, also five measurements were taken.

2.4 *Data Analysis*

A slightly modified model of pathways of light in the eye was used to analyze the spectral data.[17] Briefly, the model contains three reflectors, the inner limiting membrane (ILM), the cone receptor disks, and the choroidal space. Absorption anterior to the receptor layer takes place in the lens and in the macular pigment. Absorption posterior to the receptor layer is in melanin and blood. Absorption in visual pigment is neglected because of bleaching by the high light levels. The retinal area of 1 deg to be analyzed was considered homogeneous in macular pigment and melanin content, although we recognize that this is a simplification. A more detailed description of the model is in the appendix.

The Levenberg-Marquardt routine[18] was used to fit the data with the model by minimizing chi squares. The wavelength range used was from 400 to 800 nm. All parameters were allowed to vary simultaneously. For a single measurement, the noise in the data points was assumed to originate mainly from photon noise, with a small addition from head or eye movements of the subject. The standard deviation in the spectral data points were therefore calculated from two succeeding 1-s periods, and were used to weight the data points in the fitting process. For a typical example of this noise, see the error bars in Fig. 2 in Sec. 3. A single measurement containing relatively large eye movements was easily recognized by a large standard deviation at the long wavelength points and was generally rejected and replaced immediately during the measurements.

3 Results

Measurements were successfully completed in all subjects. A typical reflectance spectrum is shown in Fig. 2. In the long wavelength region, reflection is high because the lens, MP, blood, and melanin have minimal absorption. At wavelengths below 600 nm, the reflection declines because of increased absorption in blood and melanin. Around 560 nm, a plateau is visible. Light is reflected by the spectrally neutral interfaces at the pigment epithelium/cone outer segment level. Below 510 nm, MP absorption sets in, and below 450 nm, the lens

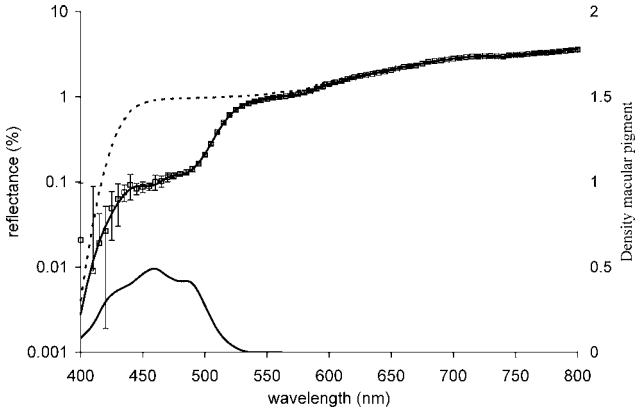

Fig. 2 Typical reflectance spectrum exhibiting high reflectance at long wavelengths, with a gradual decrease caused by absorption in melanin and blood. At about 550 nm, the MP causes a sharp decline, followed by a plateau between 500 and 450 nm. Thereafter, another sharp decrease is caused by absorption in the crystalline lens. A best fitting model curve is drawn through the data points. The error bars show the standard deviation in the data points. The same model curve, but with the absorption of MP set to zero, is shown as a dashed line. The absorption curve of MP from literature (Ref. 31), as used in the model, is shown at the bottom (see the scale on the right).

increasingly absorbs nearly all incoming light. Consequently, in the blue region of the spectrum reflection is about a factor of 100 below that of the red region.

Density of macular pigment (MPOD) was derived from the spectral reflection curves with the van de Kraats model, as mentioned in Sec. 2.4.[17] MPOD with the natural pupil in a dark room was 0.55 ± 0.21 (condition 1), with dim room light 0.56 ± 0.23 (condition 2), and with dilated pupil 0.57 ± 0.22 (condition 3). No significant differences existed between conditions 1 to 3. With the HFP (condition 3), a significantly lower MPOD of 0.35 ± 0.19 was found than in condition 1 (paired t-test; $p<0.001$). Figure 3 shows MPOD as a function of age. No change with age was found (condition 1, $r=0.15$, $p=0.54$). Test-retest results after at least 3 days [Fig. 4(a)] showed a very high reproducibility ($r=0.94$, $p<0.001$). Correlation between the conditions natural pupil in dim light and dark room were high [$r=0.97$; $p<0.001$; Fig. 4(b)], as was the correlation between dark room natural and dilated pupil [$r=0.90$; $p<0.001$; Fig. 4(c)]. In Fig. 4(d), the relation

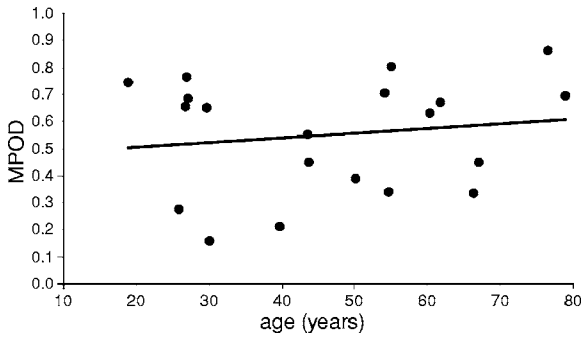

Fig. 3 MPOD showing no change with age. The solid line, provided as a guide to the eye, shows a positive, but not significant linear regression.

between the data obtained with reflectometry (condition natural pupil, dimly lit room) and HFP is given. Again a significant correlation was found ($r=0.56$; $p=0.012$). Five spectra were measured in each condition, which provided an estimate of the within subject variation. Mean within subjects variation for the MPR was typically 7%. Mean within subjects variation with HFP was 19%.

4 Discussion

With the new macular pigment reflectometer (MPR) fast and reproducible estimates were obtained of the MPOD. An advantage inherent to all the techniques with physical detectors is that they require only minimal cooperation of the subject. After the preliminaries, the subject is asked five times to fixate the stimulus for a few seconds. Such a task is far easier than the performance required with the widespread HFP technique. HFP asks for a substantial number of settings, which are not particularly easy to make with peripheral stimuli, particularly for elderly subjects. Depending on the instrument used, this probably accounts for the much lower optical densities. The HFP apparatus used in this study, for instance, lacked an individual setting of flicker rates.[16] Related to this is the assumed similarity in the receptor sensitivities between the foveal and peripheral sites, with perhaps different criteria attached. A second reason for lower densities with HFP may be the assumed zero MP density at the peripheral site for the HFP technique, and other effects resulting from the spatial distribution of macular pigment. These differences between HFP and other techniques are not uncommon; see the more detailed discussion in papers by Delori and Berendschot et al.[19,20] With the new device, the whole procedure takes, including calibration, less than 5 min, comparing favorably with the HFP that takes 10 to 15 min. As a benefit, it provides other parameters from the reflectance model, such as lens density. Discussing these parameters is beyond the scope of this paper.

Hammond et al. stated that the validity of noninvasive MP methods depends on that they must provide spectral absorption curves that match the extinction spectrum of xanthophylls.[21] With the new device, a model uses the extinction spectrum of MP and finds, with the density as a free parameter, the best fit to the spectral reflection.

Reflections at layers anterior to the MP are accounted for in the model, thus an estimate of MP is obtained, undiluted by stray light. An indication for the validity of the technique comes from showing an increase in density of MP with oral intake of lutein, similar to what was found with the HFP technique.[4] In addition, in a comparison of 5 different techniques, Berendschot et al. found highly significant correlations of MPOD in a group of 53 subjects with methods based on the present technique (model analysis of spectral fundus reflectance) on the one hand, and scanning laser ophthalmoscope (SLO) maps, SLO autofluorescence, and HFP on the other hand.[19] As mentioned before, just like with the present data, HFP showed the lowest correlation. The MPOD did not co-vary with other parameters in the model analysis, except with the ILM reflex. However, we found no correlation between MP density and the ILM reflex in this data set, indicating that the MPOD estimates were not biased by incorrect ILM values.

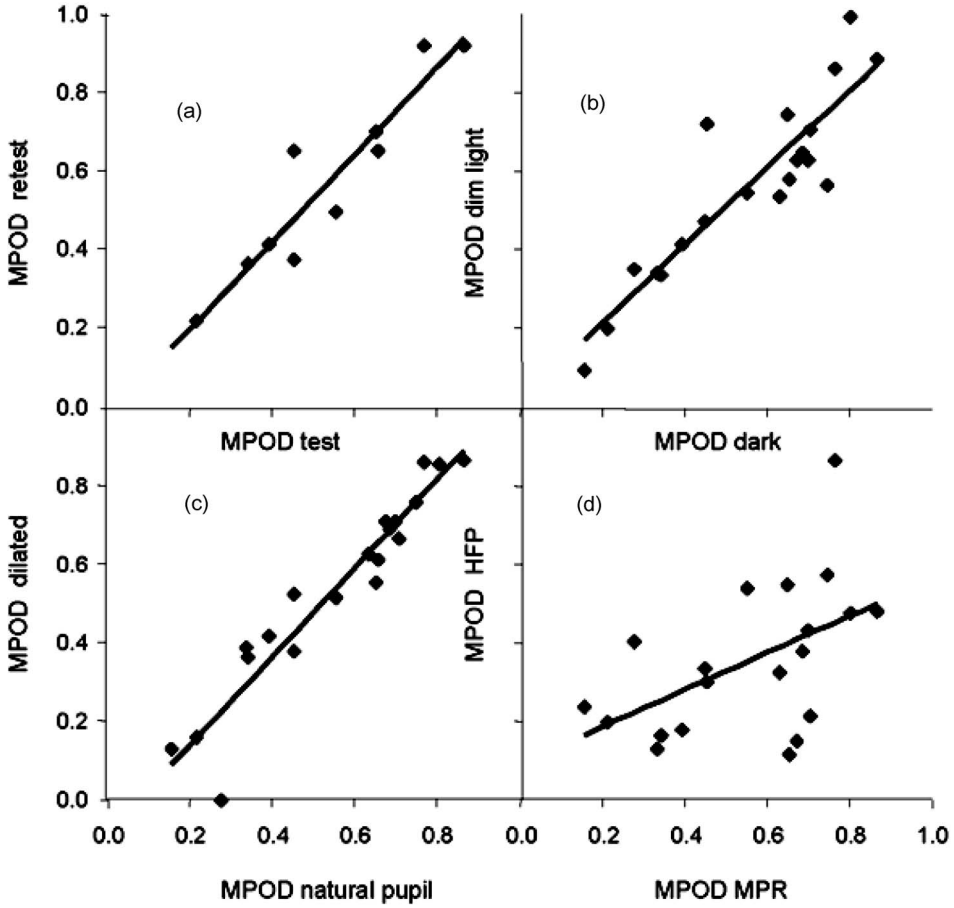

Fig. 4 (a) Test-retest results for 10 subjects with a minimum interval of 3 days. Correlation was 0.94 ($p<0.01$). The solid line shows equality. (b) MPOD in a condition with dim room light and no room light. Correlation was 0.97 ($p<0.001$). The solid line is the regression line. (c) MPOD with natural pupil versus dilated pupil. Correlation was 0.98 ($p<0.001$). The solid line is the regression line. (d) MPOD as measured with the MPR versus that with HFP. Correlation was 0.56 ($p=0.012$). The solid line is the regression line.

Despite the fact that the present technique has no separate fixation target at the center of the 1-deg test field, test-retest reliability was very high ($r=0.98$), even higher than in a HFP study with a very careful protocol (range 0.68 to 0.90 for different conditions).[22]

With no movable fixation target available, the new device only allows central measurement of MP. This is a limitation in view of the increasing interest in the retinal distribution of MP.[23,24] HFP devices generally provide fixation targets at a number of locations and hence data on the distribution of MP. The penalty is a series of time-consuming measurements (up to 45 min). In fact, two wavelength autofluorescence, measured with a SLO, seems ideal for providing detailed maps of MPOD. However such devices are costly, and often require pupil dilatation, rendering the method less suitable for larger scale investigations on MP.

MPOD showed no changes with age, a finding that is in line with most other studies,[19] and even, despite strong indications to the contrary,[25] with a recent study using Raman spectroscopy.[26]

In conclusion, we succeeded in building an instrument for fast, reliable measurement of MPOD. The instruments might be suitable for specific purposes in assessing MPOD. In particular, it holds promise for epidemiological research and quick assessments for patients on supplements.

Appendix: Reflectance Model

In the model, first the light reflected in the choroidal space is described. The back scattering of light in the choroidal tissues is taken as a neutral reflection $R_{choroid}$. Light in the choroidal space is absorbed by blood and melanin. The density of a layer of blood with a thickness Th_{blood} in microns is

$$D_{blood}(\lambda) = Th_{blood}\alpha_{blood}(\lambda) \quad (1)$$

with $\alpha_{blood}(\lambda)$ as the density of 1 micron 95% oxygenated blood.[27] To account for the variety of path lengths through the center and periphery of small and large blood vessels, we assumed a wedge shaped blood layer, with path lengths from zero to Th_{blood}. The transmission can be calculated as

$$T_{blood}(\lambda) = \{1.0 - 10.0^{[-D_{blood}(\lambda)]}\}/[D_{blood}(\lambda)\ln(10)]. \quad (2)$$

For the transmission through a uniform layer of melanin, we took the spectral data of Gabel,[28] approximated by

$$\alpha_{\text{melanin}}(\lambda) = 2.45 \times [0.007 + 0.165 \exp(-\lambda \times 0.0055) + 113 \exp(-\lambda \times 0.011) - 4300 \exp(-\lambda \times 0.022)]. \quad (3)$$

The term 2.45 is to normalize the function to 1 at 500 nm, so that $D_{melanin}$ is the parameter for the density of melanin at 500 nm. The transmission of melanin becomes

$$T_{\text{melanin}}(\lambda) = D_{\text{melanin}} \alpha_{\text{melanin}}(\lambda). \quad (4)$$

Reflection at the level of the receptor layer is than described by

$$R_{\text{recep}}(\lambda) = R_{\text{rpe}} + [T_{\text{blood}}(\lambda) T_{\text{melanin}}(\lambda)]^2 R_{\text{choroid}} \quad (5)$$

with R_{rpe} as the parameter for a neutral reflection at the retinal pigment epithelium (RPE), augmented with the reflection of the retinal cone receptors. We simplified the original model[17] by assuming the absence of visual pigments because of the high bleaching intensity level. Also, a single mean cone reflectance was taken, as the shape of the directional sensitivity cannot be discriminated with this simple instrument. Both the cone disk reflection and the RPE reflection are taken to be spectrally neutral. Therefore, they cannot be distinguished from each other, and both are represented by the single RPE reflection value.

Transmission of the media is described by

$$T_{\text{media}}(\lambda) = 10^{-[D_{\text{lensyoung}} \alpha_{\text{Lensyoung}}(\lambda) + D_{\text{lensold}} \alpha_{\text{Lensold}}(\lambda) + 24 \alpha_{\text{water}}(\lambda)]}. \quad (6)$$

Parameter $D_{lensyoung}$ is the density of the lens at 420 nm for the young age component having the spectral shape $\alpha_{Lensyoung}(\lambda)$ of the Pokorny aging template normalized at 420 nm.[29] Similarly, $D_{lensold}$ is the density at 420 nm for the old age component, with $\alpha_{Lensold}(\lambda)$ as the corresponding Pokorny nonaging template normalized at 420 nm.[29] $\alpha_{\text{water}}(\lambda)$ is the density of 1-mm water.[30] The transmission of the MP is described by

$$T_{\text{macpig}}(\lambda) = 10^{-[D_{\text{macpig}} \alpha_{\text{macpig}}(\lambda)]} \quad (7)$$

with $\alpha_{macpig}(\lambda)$ as Walraven's description of the MP data.[31] We normalized it to 1 at 460 nm, then parameter D_{macpig} is the density of macular pigment at 460 nm, and

$$\alpha_{\text{macpig}}(\lambda) = (1/0.35)\{0.32 \exp[-0.0012(436 - \lambda)^2] + 0.32 \exp[-0.0012(480 - \lambda)^2] - 0.123 \exp[-0.0012(458 - \lambda)^2] + 0.12042 \exp[-0.006(457 - \lambda)^2]\}. \quad (8)$$

At the level of the cornea, the reflection from the eye can now be described by

$$R_{\text{eye}}(\lambda) = [T_{\text{media}}(\lambda)]^2 \{R_{\text{ilm}} + [T_{\text{macpig}}(\lambda)]^2 R_{\text{recep}}(\lambda)\}. \quad (9)$$

R_{ilm} represents a parameter for a neutral reflection at the back of the vitreous at the ILM. Thus the model contains seven free parameters: $R_{choroid}$, T_{blood}, $D_{melanin}$, R_{rpe}, $D_{lensyoung}$, $D_{lensold}$, and D_{macpig}.

Acknowledgments

The authors thank John Mellerio for the use of his portable device for HFP.

References

1. S. Beatty, I. J. Murray, D. B. Henson, D. Carden, H. Koh, and M. E. Boulton, "Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population," *Invest. Ophthalmol. Visual Sci.* **42**, 439–446 (2001).
2. T. T. J. M. Berendschot, J. J. M. Willemse-Assink, M. Bastiaanse, P. T. V. M. de Jong, and D. van Norren, "Macular pigment and melanin in age-related maculopathy in a general population," *Invest. Ophthalmol. Visual Sci.* **43**, 1928–1932 (2002).
3. R. A. Bone, J. T. Landrum, S. T. Mayne, C. M. Gomez, S. E. Tibor, and E. E. Twaroska, "Macular pigment in donor eyes with and without AMD: A case-control study," *Invest. Ophthalmol. Visual Sci.* **42**, 235–240 (2001).
4. T. T. J. M. Berendschot, R. A. Goldbohm, W. A. Klöpping, J. van de Kraats, J. van Norel, and D. van Norren, "Influence of lutein supplementation on macular pigment, assessed with two objective techniques," *Invest. Ophthalmol. Visual Sci.* **41**, 3322–3326 (2000).
5. J. T. Landrum, R. A. Bone, H. Joa, M. D. Kilburn, L. L. Moore, and K. E. Sprague, "A one year study of the macular pigment: The effect of 140 days of a lutein supplement," *Exp. Eye Res.* **65**, 57–62 (1997).
6. R. A. Bone, J. T. Landrum, L. H. Guerra, and C. A. Ruiz, "Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans," *J. Nutr.* **133**, 992–998 (2003).
7. B. R. Hammond, Jr., E. J. Johnson, R. M. Russell, N. I. Krinsky, K. J. Yeum, R. B. Edwards, and D. M. Snodderly, "Dietary modification of human macular pigment density," *Invest. Ophthalmol. Visual Sci.* **38**, 1795–1801 (1997).
8. S. Beatty, J. Nolan, H. Kavanagh, and O. O'Donovan, "Macular pigment optical density and its relationship with serum and dietary levels of lutein and zeaxanthin," *Arch. Biochem. Biophys.* **430**, 70–76 (2004).
9. J. D. Moreland, "Macular pigment assessment by motion photometry," *Arch. Biochem. Biophys.* **430**, 143–148 (2004).
10. R. A. Bone and J. T. Landrum, "Heterochromatic flicker photometry," *Arch. Biochem. Biophys.* **430**, 137–142 (2004).
11. T. T. J. M. Berendschot, P. J. DeLint, and D. van Norren, "Fundus reflectance-historical and present ideas," *Prog. Retin Eye Res.* **22**, 171–200 (2003).
12. T. T. J. M. Berendschot and D. van Norren, "Objective determination of the macular pigment optical density using fundus reflectance spectroscopy," *Arch. Biochem. Biophys.* **430**, 149–155 (2004).
13. F. C. Delori, "Autofluorescence method to measure macular pigment optical densities fluorometry and autofluorescence imaging," *Arch. Biochem. Biophys.* **430**, 156–162 (2004).
14. P. S. Bernstein, D. Y. Zhao, M. Sharifzadeh, I. V. Ermakov, and W. Gellermann, "Resonance Raman measurement of macular carotenoids in the living human eye," *Arch. Biochem. Biophys.* **430**, 163–169 (2004).
15. Health Council of the Netherlands, Committee on optical radiation, "Optical Radiation. Health based exposure limits for electromagnetic radiation in the wavelength region from 100 nanometer to 1 millimeter," Health Council of the Netherlands, The Hague (1993).
16. J. Mellerio, S. Ahmadi-Lari, F. van Kuijk, D. Pauleikhoff, A. Bird, and J. Marshall, "A portable instrument for measuring macular pigment with central fixation," *Curr. Eye Res.* **25**, 37–47 (2002).
17. J. van de Kraats, T. T. J. M. Berendschot, and D. van Norren, "The pathways of light measured in fundus reflectometry," *Vision Res.* **36**, 2229–2247 (1996).
18. W. H. Press, B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling, *Numerical recipes in Pascal. The Art of Scientific Computing*. Cambridge University Press, Cambridge (1989).
19. T. T. J. M. Berendschot and D. van Norren, "On the age dependency of the macular pigment optical density," *Exp. Eye Res.* **81**, 602–609 (2005).
20. F. C. Delori, D. G. Goger, B. R. Hammond, D. M. Snodderly, and S. A. Burns, "Macular pigment density measured by autofluorescence spectrometry: Comparison with reflectometry and heterochromatic flicker photometry," *J. Opt. Soc. Am. A* **18**, 1212–1230 (2001).

21. B. R. Hammond, B. R. Wooten, and B. Smollon, "Assessment of the validity of in vivo methods of measuring human macular pigment optical density," *Optom. Vision Sci.* **82**, 387–404 (2005).
22. D. M. Snodderly, J. A. Mares, B. R. Wooten, L. Oxton, M. Gruber, and T. Ficek, "Macular pigment measurement by heterochromatic flicker photometry in older subjects: The carotenoids and age-related eye disease study," *Invest. Ophthalmol. Visual Sci.* **45**, 531–538 (2004).
23. T. T. J. M. Berendschot and D. van Norren, "Macular pigment shows ringlike structures," *Invest. Ophthalmol. Visual Sci.* **47**, 709–714 (2006).
24. F. C. Delori, D. G. Goger, C. Keilhauer, P. Salvetti, and G. Staurenghi, "Bimodal spatial distribution of macular pigment: Evidence of a gender relationship," *J. Opt. Soc. Am. A* **23**, 521–538 (2006).
25. P. S. Bernstein, "New insights into the role of the macular carotenoids in age-related macular degeneration. Resonance Raman studies," *Pure Appl. Chem.* **74**, 1419–1425 (2002).
26. K. Neelam, N. O'Gorman, J. Nolan, O. O'Donovan, H. B. Wong, K. G. A. Eong, and S. Beatty, "Measurement of macular pigment: Raman spectroscopy versus heterochromatic flicker photometry," *Invest. Ophthalmol. Visual Sci.* **46**, 1023–1032 (2005).
27. O. W. van Assendelft, *Spectroscopy of Hemoglobin Derivatives*, C.C. Thomas, Springfield, IL (1970).
28. V. P. Gabel, R. Birngruber, and F. Hillenkamp, "Visible and near infrared light absorption in pigment epithelium and choroid," in *Excerpta Medica*, International Congress Series, Elsevier, Amsterdam (1978). K. Shimizu and J. A. Oosterhuis, Eds., pp. 658–662.
29. J. Pokorny, V. C. Smith, and M. Lutze, "Aging of the human lens," *Appl. Opt.* **26**, 1437–1440 (1987).
30. R. C. Smith and K. S. Baker, "Optical properties of the clearest natural waters," *Appl. Opt.* **20**, 177–184 (1981).
31. P. L. Walraven, "CIE report TC 1-36 Draft 7, Personal communication," Soesterberg (2003).