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

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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.

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

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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
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 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.

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.

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 BaSO4 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. 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 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. 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 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...
increasingly absorbs nearly all incoming light. Consequently,
in the blue region of the spectrum reflection is about a factor
twenty times lower than in the red region.

Density of macular pigment (MPOD) was derived from the
spectral reflectance curves with the van de Kraats model, as
mentioned in Sec. 2.4. MP 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
between the data obtained with reflectometry (condition natu-
ral pupil, dimly lit room) and HFP is given. Again a signifi-
cant 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 fix-
ate the stimulus for a few seconds. Such a task is far easier
than the performance required with the widespread HFP tech-
nique. HFP asks for a substantial number of settings, which
are not particularly easy to make with peripheral stimuli, par-
ticularly for elderly subjects. Depending on the instrument
used, this probably accounts for the much lower optical den-
sities. 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, in-
cluding 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 den-
sity. 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 absorp-
tion curves that match the extinction spectrum of xanthophylls.21 With the new device, a model uses the extinc-
tion 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 tech-
nique.4 In addition, in a comparison of 5 different tech-
niques, Berendschot et al. found highly significant correla-
tions 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 opthalmosco-
scope (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
covary with other parameters in the model analysis, except
with the ILM reflex. However, we found no correlation be-
tween MP density and the ILM reflex in this data set, indicat-
ing that the MPOD estimates were not biased by incorrect
ILM values.
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 MPOD. This is a limitation in view of the increasing interest in the retinal distribution of MPOD.23,24 HFP devices generally provide fixation targets at a number of locations and hence data on the distribution of MPOD. 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 $R_{chord}$. Light in the choroidal space is absorbed by blood and melanin. The density of a layer of blood with a thickness $T_{blood}$ in microns is

$$D_{blood}(\lambda) = T_{blood} \alpha_{blood}(\lambda)$$

with $\alpha_{blood}(\lambda)$ as the density of 1 micron 95% oxygenated blood. 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 $T_{blood}$. The transmission can be calculated as

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

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

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

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

\[ T_{\text{melanin}}(\lambda) = D_{\text{melanin}} \alpha_{\text{melanin}}(\lambda). \]

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

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

with \( R_{\text{rpe}} \) 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 reflection 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

\[ T_{\text{media}}(\lambda) = 10^{-\left[D_{\text{lenyoung}} \alpha_{\text{lenyoung}}(\lambda) + D_{\text{lensold}} \alpha_{\text{lensold}}(\lambda) + 2 \alpha_{\text{water}}(\lambda) \right]} \]

Parameter \( D_{\text{lenyoung}} \) is the density of the lens at 420 nm for the young age component having the spectral shape \( \alpha_{\text{lenyoung}}(\lambda) \) of the Pokorny aging template normalized at 420 nm.\(^{20}\) Similarly, \( D_{\text{lensold}} \) is the density at 420 nm for the old age component, with \( \alpha_{\text{lensold}}(\lambda) \) as the corresponding Pokorny nonaging template normalized at 420 nm.\(^{20}\) \( \alpha_{\text{water}}(\lambda) \) is the density of 1-mm water.\(^{15}\) The transmission of the MP is described by

\[ T_{\text{macpig}}(\lambda) = 10^{-\left[D_{\text{macpig}} \alpha_{\text{macpig}}(\lambda) \right]} \]

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

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

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

\[ R_{\text{eye}}(\lambda) = \left[ T_{\text{media}}(\lambda) \right]^2 R_{\text{lim}} + \left[ T_{\text{macpig}}(\lambda) \right]^2 R_{\text{recep}}. \]

\( R_{\text{lim}} \) represents a parameter for a neutral reflection at the back of the vitreous at the ILM. Thus the model contains seven free parameters: \( R_{\text{choroid}}, T_{\text{blood}}, D_{\text{melanin}}, R_{\text{rpe}}, D_{\text{lenyoung}}, D_{\text{lensold}} \) and \( D_{\text{macpig}} \).

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