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Quantitative reflection spectroscopy at the human ocular fundus

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Abstract

A new model of the reflection of the human ocular fundus on the basis of the adding–doubling method, an approximate solution of the radiative transport equation, is described. This model enables the calculation of the concentrations of xanthophyll in the retina, of melanin in the retinal pigment epithelium and the choroid, and of haemoglobin in the choroid from fundus reflection spectra. The concentration values found in 12 healthy subjects are in excellent agreement with published data. In individual cases of pathologic fundus alterations, possible benefits to the ophthalmologic diagnostics are demonstrated.

1. Introduction

The reflection spectra of the human ocular fundus are characterized by the absorption of melanin, haemoglobin and xanthophyll. The knowledge of the concentration of these pigments may be of clinical interest for diagnostic purposes as well as for the planning of laser treatment. Thus, it was the goal of this investigation to separately determine these concentrations in the retina, the retinal pigment epithelium (RPE), and in the choroid from *in vivo* measured ocular fundus reflection spectra.

A first attempt to model the reflection spectra on the basis of Lambert–Beer's law was published by van Norren and Tiemeijer (1986). This model yields stable results and is very useful for relative measurements. A comparison of the obtained concentrations with physiological data, however, is difficult. Firstly, concentrations are given as optical densities only, which had to be corrected for the thickness of the respective fundus layer. Secondly, there is a deviation from the values known from psychophysical or *in vitro* measurements. Besides the influence of the size of the stimulus in psychophysical measurements, the reason for this deviation clearly consists in the disregard for the scattering in the tissue. In order to overcome this disadvantage, Delori and Pflibsen (1989) as well as Schweitzer *et al* (1990) proposed fundus reflection models on the basis of the two-photon-flux theory by Kubelka and Munk (1931). Unfortunately, these models did not converge to the measurements taken by

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Hammer *et al* (1997) within a reasonable parameter range. This may be due to the fact that the experimental conditions do not fit the requirements of the theory: the scattering in the ocular fundus tissues is neither isotropic, nor is a diffuse illumination possible at the fundus *in situ*.

Due to the transparency of the cornea, the aqueous humor, the lens and the vitreous, the propagation of light in these media is described by geometrical optics. Inside the ocular fundus, however, the spread of the light is governed by the radiation transfer law. Furthermore, the fundus has to be regarded as a structure of thin layers showing very different optical properties (Hammer *et al* 1995). Unfortunately, an analytic solution of the radiation transport equation is not available for this geometry (Ishimaru 1978). Thus, the problem has to be solved numerically or by an approximation. First, a Monte Carlo simulation (Wang and Jacques 1992) of light transport in a stack of different layers was considered. This simulation certainly gives a correct description of the light backscattered from the fundus into the pupil of the eye. Due to its computational expenditure, however, it is not useful for the inverse problem of the calculation of optical properties of single layers from the reflection spectrum of the stack. Another solution widely used in biomedical optics, the diffusion approximation (Ishimaru 1978), requires almost isotropic radiation fields and scattering coefficients much bigger than the absorption coefficients. Both conditions are violated in our case: due to the high concentrations of melanin and haemoglobin in the retinal pigment epithelium and in the choroid respectively, the scattering and absorption coefficients of these layers are in the same order. Furthermore, isotropic fields cannot be established in very thin layers under nearly collimated illumination. Consequently, the use of the diffusion approximation for the interpretation of ocular fundus reflection spectra did not yield in reasonable results. The drawbacks of the two-photon-flux theory were already mentioned, the extension of this theory to four fluxes on appropriate boundary conditions is equivalent to the diffusion approximation (Star *et al* 1988).

An elegant solution of the problem, however, was found by applying the adding–doubling method developed by van de Hulst (1963, 1980) for the description of the reflection of planetary atmospheres and introduced to tissue optics by Prahl (1988, 1995).

2. Theory

The radiance reflected from (I_{ref}) or transmitted through (I_{tr}) a thin absorbing and scattering layer is given by $I_{\mathrm{ref}} = \mathbf{R} \cdot I_0$ and $I_{\mathrm{tr}} = \mathbf{T} \cdot I_0$, respectively, where I_0 is the incident radiance and $\mathbf{R}$ and $\mathbf{T}$ denote the reflection and transmission operators of the layer. Then, the reflection and transmission of a double layer is described by infinite series converging to the operators (van de Hulst 1980) (doubling)

$$\begin{aligned} \mathbf{R}_{\mathrm{double}} &= \mathbf{R} + \mathbf{TRT} + \mathbf{TRRRT} + \cdots = \mathbf{R} + \mathbf{T}(1 - \mathbf{RR})^{-1}\mathbf{RT} \\ \mathbf{T}_{\mathrm{double}} &= \mathbf{TT} + \mathbf{TRRT} + \mathbf{TRRRRT} + \cdots = \mathbf{T}(1 - \mathbf{RR})^{-1}\mathbf{T}. \end{aligned} \tag{1}$$

Once having the operators for an infinitesimal thin layer, the operators for a layer of an arbitrary thickness can be calculated by iterative doubling of the initial thin layer. In the same way, two layers of different optical properties can be combined (Prahl 1995) (adding)

$$\begin{aligned} \mathbf{R}_{\mathrm{adding}} &= \mathbf{T}_1(1 - \mathbf{R}_2\mathbf{R}_1)^{-1}\mathbf{R}_2\mathbf{T}_1 + \mathbf{R}_1 \\ \mathbf{T}_{\mathrm{adding}} &= \mathbf{T}_2(1 - \mathbf{R}_1\mathbf{R}_2)^{-1}\mathbf{T}_1 \end{aligned} \tag{2}$$

where indices 1 and 2 denote the top (illuminated) and the bottom layer, respectively. Now we consider the following geometry suitable for the treatment of ocular fundus reflection: let I_0 be constant within the illuminated spot at the surface of the retina with an angular

distribution symmetric around an axis vertical to the infinitely extended stack of tissue layers. This geometry eliminates the azimuth dependence, consequently the reflected radiance can be given in terms of the cosines η_0 and η of the elevation angles of illumination and observation:

$$I_{\mathrm{ref}}(\eta) = \int_0^1 R\,(\eta, \eta_0)\, I_0\,(\eta_0)\, 2\eta_0\, \mathrm{d}\eta_0. \tag{3}$$

The same holds for the transmission as well as for the products of reflection and transmission operators in equations (1) and (2). All the appearing integrals can be solved by numerical quadrature resulting in the following expression for the product of two reflection or transmission operators **A** and **B**:

$$\int_0^1 A(\eta, \eta')B(\eta', \eta'')2\eta'\,\mathrm{d}\eta' = \sum_{j=1}^{n} A_{ij} 2\eta_j w_j B_{jk} = \mathbf{A} * \mathbf{B}. \tag{4}$$

This is a special type of matrix multiplication with the identity matrix $\left[\frac{1}{2\eta_j w_j}\delta_{ij}\right]$, where w_j is the weight of the jth quadrature angle whose cosine is η_j. Thus, the reflection and transmission operators are transformed into matrices. The ij element of these matrices gives the radiance reflected or transmitted from the ith to the jth angular element, respectively. Though Gaussian quadrature is the most popular one, we followed Prahl (1995) and used Radau quadrature since this gives the opportunity to chose the cosine $\eta_0 = 1$ (normal incidence of the illumination) as a quadrature point. The η_i and w_i are for n quadrature angles given by (Prahl 1995)

$$\eta_i = \frac{1}{2} - \frac{1}{2}x_i \qquad \text{and} \qquad w_i = \frac{1}{2(1 - x_i)\left[P'_{n-1}(x_i)\right]^2} \tag{5}$$

where x_i with $1 < i < n - 1$ are the solutions of

$$P_{n-1}(x) + \frac{x - 1}{n} P'_{n-1}(x) = 0 \tag{6}$$

and $P_{n-1}(x)$ and $P'_{n-1}(x)$ are the $(n - 1)$th Legendre polynomial of zero order and its first derivative, respectively, whereas $x_n = 1$ and $w_n = w_n(-1)$.

The reflection and transmission matrices for an infinitesimal thin layer are obtained from the solution of the one-dimensional radiation transfer equation using discretization of the angle:

$$\pm\eta_i \frac{\mathrm{d}I(\tau, \pm\eta_i)}{\mathrm{d}\tau} + I(\tau, \pm\eta_i) = \frac{W_0}{2} \sum_{j=1}^{n} w_j [h(\eta_i, \eta_j) I(\tau, \pm\eta_j) + h(\eta_i, -\eta_j) I(\tau, \mp\eta_j)] \tag{7}$$

where τ is the optical depth. The redistribution function $h(\eta_i, \eta_j)$ is the phase function integrated over the azimuth angle ϕ:

$$h(\eta_i, \eta_j) = \frac{1}{2\pi} \int_0^{2\pi} p\left(\eta_i \eta_j + \sqrt{1 - \eta_i^2}\sqrt{1 - \eta_j^2}\cos\phi\right) \mathrm{d}\phi. \tag{8}$$

Approximating the phase function by the sum of a Dirac delta function and a series on $n - 1$ Legendre polynomials and substituting

$$\tau' = \left(1 - W_0 \cdot g^n\right)\tau \qquad \text{and} \qquad W'_0 = \frac{1 - g^n}{1 - W_0 \cdot g^n} W_0 \tag{9}$$

where W_0 is the albedo and g is the scattering anisotropy, yields

$$h(\eta_i, \eta_j) = \sum_{k=0}^{n-1} (2n + 1)\chi_k P_k(\eta_i) P_k(\eta_j). \tag{10}$$

Assuming the Henyey–Greenstein phase function (Henyey and Greenstein 1941), we find $\chi_k = \frac{g^k - g^n}{1 - g^n}$. Substituting equation (9) into equation (7) and integrating over an infinitesimal layer thickness

$$\Delta\tau' = \tau_1' - \tau_0' < \eta_1 \tag{11}$$

yields

$$\pm\eta_i[I(\tau_1', \pm\eta_i) - I(\tau_0', \pm\eta_i)] + \Delta\tau' I_{0/1}(\pm\eta_i) = \frac{W_0'}{2} \sum_{j=1}^{n} w_j \Delta\tau' [h(\eta_i, \eta_j) I_{0/1}(\pm\eta_i) + h(\eta_i, -\eta_j) I_{0/1}(\mp\eta_i)]$$

with

$$I_{0/1}(\eta) = \frac{1}{\Delta\tau'} \int_{\tau_1'}^{\tau_2'} I(\tau', \eta)\, \mathrm{d}\tau'. \tag{12}$$

Following Wiscombe (1976), we replace the integral in equation (12) by the average of the radiances at the top and at the bottom of the layer:

$$I_{0/1}(\eta) = \tfrac{1}{2}[I(\tau_0', \eta) + I(\tau_1', \eta)]. \tag{13}$$

Thus, equation (12) is an approximate solution of the one-dimensional discretized radiative transfer equation resulting in the following expressions for the reflection and transmission matrices of a thin layer:

$$\mathbf{R}_{\Delta\tau'} = 2\mathbf{G} \cdot \mathbf{B} \cdot ([\delta_{ij}] + \mathbf{A})^{-1} \qquad \text{and} \qquad \mathbf{T}_{\Delta\tau'} = 2\mathbf{G} - [\delta_{ij}]$$

with

$$\begin{aligned} \mathbf{A} &= \left[\frac{\delta_{ij}}{\eta_i}\right] \cdot \left([\delta_{ij}] - \frac{W_0'}{2}[h(\eta_i, \eta_j)] \cdot [w_i \delta_{ij}]\right) \frac{\Delta\tau'}{2} \\ \mathbf{B} &= \frac{W_0'}{2} \left[\frac{\delta_{ij}}{\eta_i}\right] \cdot [h(\eta_i, -\eta_j)] \cdot [w_i \delta_{ij}] \frac{\Delta\tau'}{2} \end{aligned} \tag{14}$$

and

$$\mathbf{G} = \left([\delta_{ij}] + \mathbf{A} - \mathbf{B} \cdot ([\delta_{ij}] + \mathbf{A})^{-1} \cdot \mathbf{B}\right)^{-1}.$$

The calculation of the reflection of a layer with finite thickness requires the following steps. First, choosing the number of quadrature points and calculating the cosines of quadrature angles and their weights from equation (5). Using the transformation equation (9), the optical thickness of the infinitesimal layer is estimated by $2^m \cdot \Delta\tau' = \tau'$, where τ' is the transformed optical thickness of the layer under investigation and m is chosen to fulfil the condition of equation (11). Now, the reflection and transmission matrices can be calculated from equation (14) and an m-fold doubling according to equation (1) results in the reflection and transmission of the finite layer under consideration. Multiplying that matrices with the incident radiance $\mathbf{I}_0(\eta_i)$, the reflected and transmitted radiance, respectively, were obtained. Summing up these radiances over all angular elements, the total reflection and transmission was found.

Applying this formalism to the single layers of the ocular fundus (optical properties of the layers from Hammer *et al* (1995), thicknesses from Rohen (1977)), we found an excellent agreement with the results of a Monte Carlo simulation using eight quadrature angles only. Assuming collimated illumination ($\mathbf{I}_0 = \{0, \ldots, 0, 1\}$), the largest difference between the results of the adding–doubling method and the Monte Carlo simulation was found to be 2.6% for the retinal pigment epithelium. Thus, the adding–doubling method is a suitable radiation transport model for the reflection of ocular fundus tissue. Furthermore, it is suitable for the determination of chromophore concentrations from measured spectra in an inverse algorithm

(see section 3). This is practically impossible by Monte Carlo simulation because of the computational expenditure (iterative repeated simulations at several discrete wavelengths would be necessary).

3. Inverse adding–doubling model for the calculation of chromophore concentrations from reflection spectra

As the reflection of the ocular fundus, represented by a stack of plane parallel layers, can be obtained from the optical parameters and the thicknesses of these layers by the adding–doubling method, a complete reflection spectrum can be calculated if the spectra of the absorption and scattering coefficients as well as the ones of the scattering anisotropy of all layers is known. Our problem, however, is the reverse: we have to determine the chromophore concentrations from a measured spectrum. We assume the ocular fundus to consist of four layers: the retina containing oxygenated and reduced haemoglobin in their capillaries and xanthophyll in the macular region, the RPE heavily pigmented with melanin, the choroid containing haemoglobin and melanin, and the sclera. Thus, the absorption coefficients μ_a are related to the concentrations of these pigments in the following way:

$$\begin{aligned}
\mu_{a_{\mathrm{Retina}}}(\lambda) &= c_{\mathrm{X}}\varepsilon_{\mathrm{X}}(\lambda) + c_{\mathrm{Hb}}\varepsilon_{\mathrm{Hb}}(\lambda) + c_{\mathrm{HbO_2}}\varepsilon_{\mathrm{HbO_2}}(\lambda) \\
\mu_{a_{\mathrm{RPE}}}(\lambda) &= c_{\mathrm{Me}}^{\mathrm{RPE}}\varepsilon_{\mathrm{Me}}(\lambda) \\
\mu_{a_{\mathrm{Chorioidea}}}(\lambda) &= c_{\mathrm{Me}}^{\mathrm{Ch}}\varepsilon_{\mathrm{Me}}(\lambda) + c_{\mathrm{HbO_2}}^{\mathrm{Ch}}\varepsilon_{\mathrm{HbO_2}}(\lambda)
\end{aligned} \tag{15}$$

where c are the chromophore concentrations and ε the extinction coefficients. The indices Hb and HbO_2 denote reduced and oxygenated haemoglobin, respectively, X and Me xanthophyll and melanin, and the superscript Ch the choroid. Reduced choroidal blood was neglected since the arteriovenous difference in oxygen saturation is only 3% for the choroid (Naumann 1997). The molar extinction coefficients for haemoglobin were obtained from van Assendelft (1970). Melanin and xanthophyll, however, are not distinct chemical compounds but composite substances. Therefore, we obtained the melanin extinction spectrum from microspectrophotometric measurements by Gabel *et al* (1978) and that of xanthophyll from a survey of psychophysical data given by Wyszecki and Stiles (1967). Thus, the concentrations of haemoglobin are given in mmol l^{-1} whereas the ones of melanin and xanthophyll can be given in relative units only, which are factors to the physiological values found in the literature. The scattering coefficient μ_s and the anisotropy of scattering g for each layer were obtained from *in vitro* measurements of Hammer *et al* (1995). Since post mortem changes as well as inter-individual differences of the tissue scattering are possible, the scattering coefficients were allowed to be multiplied with wavelength-independent constants c_s. The scattering anisotropy values were kept constant, i.e., the number of scatterers was assumed to be variable at constant scatterer size and geometry. Furthermore, the absorption and scattering coefficient of the sclera were kept constant at the literature data since a variation of these values showed negligible effect on the reflection of the whole fundus (<1% at variations of μ_a and μ_s by a factor of 8). This is because most of the light backscattered from layers anterior to the sclera. In order to take the transmission of the anterior ocular media into account, we multiplied the calculated reflection of the fundus with the double ocular media transmission given by van Norren and Vos (1974) corrected for the age according to the formula by Pokorny *et al* (1987).

Additionally, a variable factor was introduced to account for different eye lengths. Thus, we established a model of ocular fundus reflection f on the basis of the adding–doubling theory that had to be fitted to the measured spectra $R(\lambda)$ using a least squares procedure:

$$\sum_{\lambda} \Big(R(\lambda) - f\left(c_X\varepsilon_X(\lambda), c_{Hb}\varepsilon_{Hb}(\lambda), c_{HbO_2}\varepsilon_{HbO_2}(\lambda), c_{Me}^{RPE}\varepsilon_{Me}(\lambda), c_{Me}^{Ch}\varepsilon_{Me}(\lambda),\right.$$
$$\left. c_{HbO_2}^{Ch}\varepsilon_{HbO_2}(\lambda), c_s^R\mu_s^R(\lambda), c_s^R\mu_s^{RPE}(\lambda), c_s^{Ch}\mu_s^{Ch}(\lambda)\right)\Big)^2 \to \text{Min} \tag{16}$$

where c_s are factors to the scattering coefficients and the superscripts R and Ch denote the retina and the choroid, respectively. Equation (16), however, is a non-linear optimization problem with the disadvantage that the reflection model f cannot be given analytically but is the result of the iterative adding–doubling process. Thus, a least squares fit according to the Gauss–Newton method turns out to be very expensive since the Jacobi matrix has to be determined numerically performing small variations of each parameter at any iteration step. In this case, a derivative-free method is more comfortable. Consequently, we used a self-learning procedure on the basis of Powell's conjugate direction set method (Brent 1973).

In order to optimize the model, we fixed some of the parameters. First, we neglected the haemoglobin absorption in the retina since this turned out to be far below that of the choroid in preliminary fits. This is in agreement with the anatomy: the capillary density of the retina is much smaller than the one of the choroid. The thickness of the retina was measured by OCT. The thickness of RPE, choroid and sclera were fixed at 10 μm, 250 μm and 700 μm, respectively (Rohen 1977). Furthermore, we fixed factors c_s of the scattering coefficients in the following way: we ran the program according to equation (16) for three macular reflection spectra of normal volunteers using six different sets of initial parameter values. From this calculation, we found the mean values and standard deviations $c_s^R = 0.24 \pm 0.019$, $c_s^{RPE} = 0.59 \pm 0.14$ and $c_s^{Ch} = 1.64 \pm 1.32$ which were used in all further fits. An interesting alternative would be the *in vivo* measurement of the scattering parameters by optical coherence tomography (OCT) (Hammer *et al* 2000).

Thus, the remaining parameters to be determined from ocular fundus reflection spectra are the retinal xanthophyll concentration c_X, the concentration of melanin in the RPE and in the choroid c_{Me}^{RPE} and c_{Me}^{Ch}, as well as the choroidal haemoglobin concentration $c_{HbO_2}^{Ch}$.

4. Validation of the reflection model in clinical examples

4.1. Methods

The model for the determination of ocular fundus chromophore concentrations introduced in section 3 was tested in first experiments. The fundus reflection spectra of 12 normal caucasians as well as patients suffering from macular holes, age-related macular degeneration (AMD), juvenile macular degeneration and high myopia were measured by imaging fundus reflectometry. The technique is described in detail elsewhere (Hammer 1997). In principle, the light of the xenon flash of a Zeiss fundus camera (Carl Zeiss Jena, Germany) reflected from the ocular fundus was dispersed by an imaging spectrograph (Jobin Yvon, France) and detected by an intensified slow scan CCD camera (Princeton Instruments, New Jersey). The reflected intensity was measured spectrally and locally resolved in relative units dividing the signal detected from the fundus by the one detected from an artificial eye with a diffuse white reflector (Spectralon, Labsphere Inc, New Hampshire) in its focal plane.

The thickness of the retina was measured by an optical coherence tomography scanner (Humphrey Instruments Inc, California). Unfortunately, the depth resolution and the sensitivity of this device were not sufficient to measure the thickness of the other layers. Therefore, we used literature values (Rohen 1977) instead: RPE: 10 μm, choroid: 250 μm, sclera: 700 μm.

The investigation in human subjects were performed with their informed consent, according to the guidelines of the Declaration of Helsinki, and under the approval of a

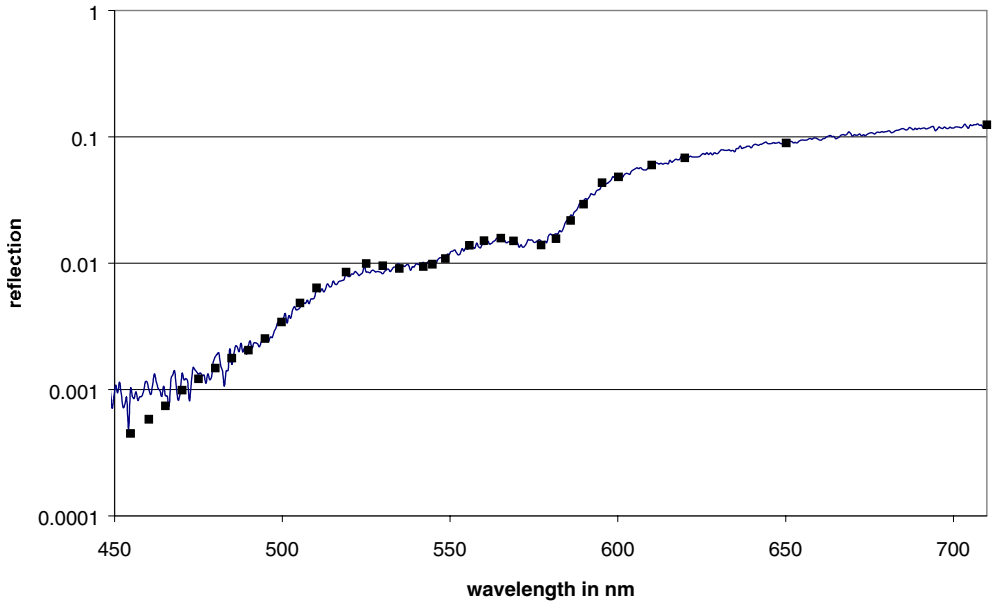

Figure 1. Macular fundus reflection spectrum (solid line) and its representation by the fitted adding–doubling model (squares).

Table 1. Mean values and standard deviations of chromophore concentrations (haemoglobin: mmol l^{-1}, all other pigments: relative units, see section 3 for definition) at and besides the macula in healthy subjects.

	Xanthophyll	Melanin (RPE)	Melanin (chorioid)	Haemoglobin
Macula	1.01 ± 0.23	0.99 ± 0.71	0.020 ± 0.036	1.71 ± 1.49
Paramacular	0.03 ± 0.14	0.88 ± 0.74	0.054 ± 0.070	1.73 ± 1.80

local ethics committee. The subject's pupil was dilated to a diameter of at least 6 mm by Tropicamide before the investigation.

4.2. Results

A typical macular reflectance spectrum and the reflections calculated by the model described in section 3 to fit to the spectrum at 23 wavelengths are shown in figure 1. Generally, we found a good agreement between measurement and model. In order to obtain reliable values for the concentration of the macular pigment, we repeated the calculation for 14 wavelengths between 455 nm and 520 nm fixing all parameters except the xanthophyll concentration c_X.

Table 1 gives the mean values and standard deviations of the concentrations of xanthophyll, melanin and haemoglobin in the macula and about two degrees temporal to the fovea in 12 normal subjects. Though the inter-individual variations, represented by the standard deviations, are high for some pigments, no negative values were found. However, the distribution of the values was non-symmetric. The mean macular concentrations of xanthophyll and melanin in the RPE were found to be in complete agreement with the data published by Wyszecki and Stiles (1967) and Gabel *et al* (1978) whereas the standard deviations show large inter-individual variations. Towards the paramacular region, the xanthophyll

Table 2. Correlation coefficients R^2 describing correlation between the concentrations of different chromophores. Here n.s. means no significant correlation.

	Melanin (RPE)	Melanin (chorioid)	Haemoglobin
Macula			
Xanthophyll	n.s.	n.s.	n.s.
Melanin (RPE)		0.53	0.43
Melanin (chorioid)			0.46
Paramacular			
Xanthophyll	n.s.	n.s.	n.s.
Melanin (RPE)		n.s.	n.s.
Melanin (chorioid)			0.71

concentration reduces drastically. The melanin concentration in the pigment epithelium is also reduced as already found *in vitro* by Gabel *et al* (1978). This decrease, however, is statistically (Student's *t*-test, $p = 0.05$) not significant. In contrast, we found a non-significant increase of the choroidal melanin concentration two degrees temporal to the fovea compared to the macula. This is in agreement with the observations by Gabel *et al* (1976), too. Assuming a choroidal thickness of 250 μm, the total choroidal melanin content in the fovea is as half as high as in the RPE whereas it is 1.5 times higher parafoveal. There was no difference between the choroidal haemoglobin concentration at the investigated fundus sites. We found it 1.7 mmol l^{-1} which is equivalent to a blood content of the choroid at 20%. However, the inter-individual differences are extremely high and a correlation with the melanin concentration was found, indicating that the haemoglobin concentration has to be interpreted with care. The correlation coefficients R^2 describing correlation between the concentrations of different chromophores are given in table 2. Besides the correlation between melanin and haemoglobin concentrations mentioned above, only a weak correlation was found between macular melanin concentration in the RPE and in the choroid.

After the good agreement of the chromophore concentrations derived from *in vivo* measurements at healthy subjects with published data has been shown, the capability of the technique and its limitations was investigated in a few pathologic cases. Examining a group of four patients suffering from full thickness macular holes, we found no xanthophyll in the fovea (obviously due to the absence of the retina in this location) but a para-macular xathophyll concentration of 0.13 ± 0.06, which is significantly higher than the one in the normal control group (see table 1). This finding is explained by the parafoveal deposit of primarily macular retinal tissue as it can also be observed by OCT showing a thickening of the retina besides the hole. The macular measurement in a 72-year-old female suffering from non-exudative age-related macula degeneration (AMD) showed a xanthophyll concentration of 0.38 and a melanin concentration in the RPE of 0.23 (normal values are unity according to the definition of relative concentrations in section 3). All other values were normal. The reduction of the xanthophyll, which is under discussion as a protective agent against oxidative stress in the photoreceptors (Bone *et al* 1988, Gerster 1991, Schalch and Werner 1994, Sommerburg *et al* 1998, Young 1988), seems to be typical for AMD (Schweitzer *et al* 1996). The method described here enables the objective measurement of xanthophyll concentration and can, therefore, be beneficial for the early detection of subjects with the risk to develop AMD. The depigmentation we found is one of the clinical features of non-exudative AMD and can be quantified now. Noteworthy are, furthermore, the findings in a 33-year-old male patient suffering from juvenile macula degeneration on both eyes:

reduced xanthophyll concentrations (left eye: 0.41, right eye: 0.63) were found in this case, too, whereas the RPE melanin concentration remained normal in the eye with the higher xanthophyll concentration (right eye: 0.94) but was reduced in the other eye (left eye: 0.59). All other values were normal. This may also be a hint about the neuroprotective effect of xanthophyll. That the model introduced in section 3 is able to distinguish between melanin in the RPE and in the choroid despite their absorption spectra being the same was demonstrated in the case of a 60-year-old female showing a hyperpigmentation of the posterior pole. It is known that such hyperpigmentation is located in the choroid (Sachsenweger 1994). From our macular measurement, we calculated a normal melanin concentration of 0.93 in the RPE but a concentration which was 11 times higher than in the normal control group (0.23) in the choroid. The paramacular choroidal melanin concentration was found to be five times higher than the normal one.

However, we also found examples, in which our model did not give correct results: in an eye with a myopia of -13 diopters, we found choroidal melanin concentrations of -0.033 (macula) and -0.028 (paramacular region) as well as haemoglobin concentration of $0.29\,\mathrm{mmol\,l^{-1}}$ and $0.24\,\mathrm{mmol\,l^{-1}}$, respectively. The reason of these obviously incorrect values is, however, rather an effect of the reduced thickness of the choroid of the myopic eye than a reduction of the concentrations. Thus, the model needs the input of the correct thickness for all layers. The thickness of the choroid could be measured *in vivo* by high resolution ultrasound sonography which, unfortunately, was not available. From the reflectance spectra of the drusen and hard exudates in the patient with AMD, we found negative values for the pigmentation of the RPE (druse: -0.3, exudate: -0.23). All other values were normal. These values are clearly due to enhanced scattering of deposits of hyalin (druse) and lipids (exudate) and not to a lack in pigmentation. This shows that the scattering coefficients are critical constants of the model, which had to be determined separately.

5. Discussion

The model given in section 3 yields reasonable values for the concentration of melanin, haemoglobin and xanthophyll at the ocular fundus as shown by the results presented in section 4.2. From a theoretical point of view, however, some items need further discussion.

Since the adding–doubling method uses a discretization of the elevation angle, it is generally able to represent the illumination and observation geometry of the used fundus camera. In order to average over at least three radiance values within the cone determined by the observation aperture of the camera, 200 quadrature angles were necessary. Inversion of reflection and transmission matrices (equations (1) and (2)), however, needs a computing time proportional to the cube of the number of quadrature angles. This makes the handling of matrices of 200×200 values virtually impossible. Therefore, eight quadrature angles were used and the collimated incidence of the light was assumed to be a reasonable model for fundus camera illumination. In contradiction to the geometry of the fundus camera allowing an observation within 2.15°, we integrated the reflection over all quadrature angles. Since the measured reflection was normalized to that of a white reflection target with Lambertian characteristics, this integral value represents the fundus reflection into the observation aperture in the case of a Lambertian reflection of the ocular fundus only. This fact has already been pointed out by Delori and Pflibsen (1989). Thus, we performed an angle-resolved Monte Carlo simulation (Wang and Jacques 1992) of the reflection of the fundus with the optical properties given by Hammer *et al* (1995) at 560 nm. As shown in figure 2, the Lambertian cosine law of reflection is a good representation for the reflection of the ocular fundus. The mean relative deviation between the calculated data and the theoretical Lambertian radiation

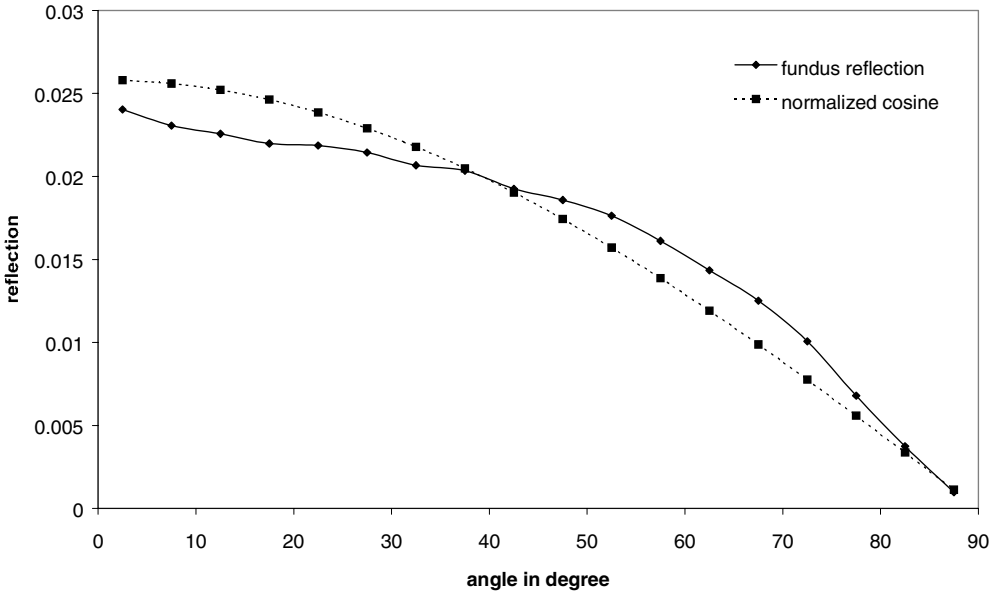

Figure 2. Comparison of the ocular fundus reflection modelled by angle-resolved Monte Carlo simulation with the suggestions of the Lambertian reflection law (cosine).

distribution was found to be 0.12. The deviation within the camera observation pupil, i.e. the error made by the assumption of a Lambrertian reflecting fundus, was 0.068.

This error was determined for homogeneous layers. The anatomical structure of the retina, however, shows inhomogenities affecting the reflection of light. Specifically, the waveguide nature of the photoreceptors (Snyder 1975) oriented towards the centre of the pupil leads to the so-called Stiles–Crawford effect of brighter appearance of light entering the eye through the pupil centre. Van de Kraats *et al* (1996) modelled the receptors as optical antennas not only receiving but also sending out light. This has an anatomic equivalence in the photoreceptor discs which are lipoprotein membranes (Naumann 1997) oriented perpendicular to the direction of the incident light, not only absorbing but also reflecting light. This effect is called the optical Stiles–Crawford effect (DeLint *et al* 1998): the reflection of incident light through the centre of the pupil on the axis of the receptors is higher than that of light with oblique incidence. This is used in first clinical attempts for the detection of the misalignment of photoreceptors by De Lint *et al* (1998). However, van Blokland and van Norren (1986) found no optical Stiles–Crawford effect at an eccentricity of the illumination of three millimetre in the pupil plane. Since the fundus camera used in our experiment has an annular illumination aperture with a mean diameter of 2.7 mm, we assumed virtually no light coupled into the receptors and hence the Lambertian reflection characteristics are not superimposed by a directed reflex according to the Stiles–Crawford effect. Furthermore, van Blokland and van Norren (1986) showed that the reflection is almost independent from the concentration of the photoreceptor pigments in the case of eccentric illumination. Therefore, these pigments were neglected in the model introduced in section 3.

Since we found the chromopore concentrations determined by our model to be dependent on the thicknesses of the fundus layers and their scattering behaviour (see section 4.2), we investigated this point in more detail: starting from the optical properties of the tissues measured *in vitro* (Hammer *et al* 1995) and from the thickness values given by Rohen (1977),

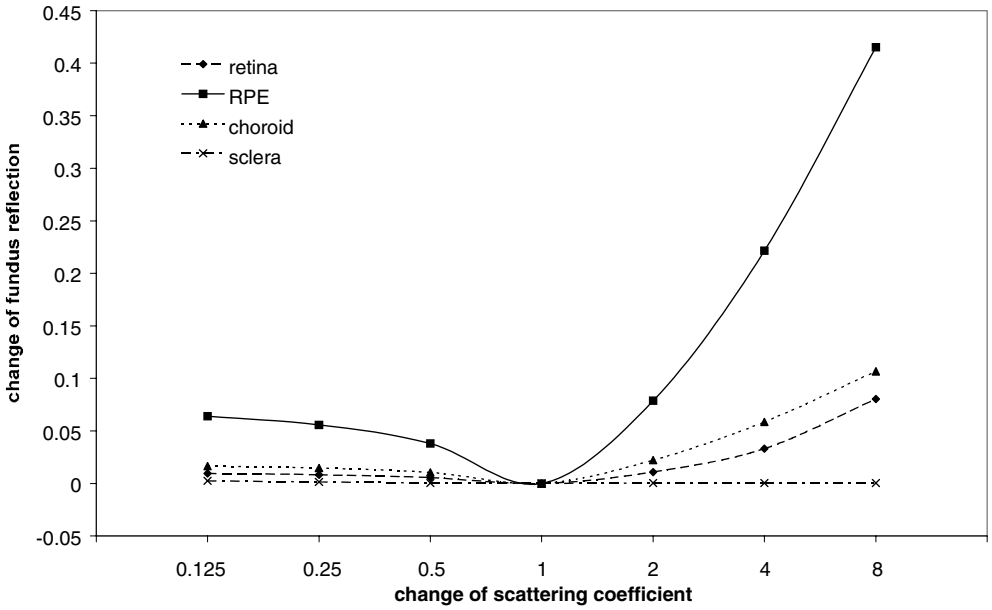

Figure 3. Ocular fundus reflection versus variation of the scattering coefficients.

we calculated the reflection at the wavelengths 413 nm, 430 nm, 450 nm, 488 nm, 514 nm, 540 nm, 560 nm, 586 nm, 633 nm and 700 nm. Then, we changed the scattering coefficient of one layer by a factor between 0.125 and 8 or the thickness of one layer by a factor between 0.5 and 2, keeping all other parameters constant, repeated the calculation and determined the square root of the sum of the quadratic deviations of the reflection at all wavelengths. The results are shown in figures 3 and 4. The strongest effects are due to changes in the RPE. Despite its small thickness and its high absorption coefficient, the reflection of this layer is not negligible. This fact, already pointed out in the fundus reflection models by van Norren and Tiemeijer (1986) as well as by Delori and Pflibsen (1989), is due to the very high scattering coefficient and the relatively small scattering anisotropy (Hammer *et al* 1995) of $g = 0.84$. Virtually no effect is due to changes in the outermost layer of the eye, the sclera. The parameters of all other layers should be known from separate measurements at best. The scattering properties of the single layers can, in principle, derived from OCT scans though the signal-to-noise ratio of these measurements is too poor to determine scattering coefficient and anisotropy with sufficient accuracy at present (Hammer *et al* 2000). As a matter of routine, OCT is used for the measurement of the thickness of the retina as it was done in this investigation (section 4.1). The depth resolution of the instrument used here was not sufficient to measure the thickness of the RPE. Since the RPE, however, is a monocellular layer, its thickness should be relatively constant within 9 to 12 μm given by Rohen (1977). Attention should be paid to the measurement of the thickness of the choroid by high resolution ultrasound sonography (Cristini *et al* 1991, Nickla *et al* 1998) since this value may vary with age (Ramrattan *et al* 1994) and with the circadian cycle (Nickla *et al* 1998) as well. Furthermore, pathologic alterations are reported in the case of AMD (Ramrattan *et al* 1994) and glaucoma (Kubota *et al* 1993, Yin *et al* 1997).

Despite the limitations discussed above, the model of the ocular fundus reflection developed on the basis of the adding–doubling model of radiative transfer has proven to

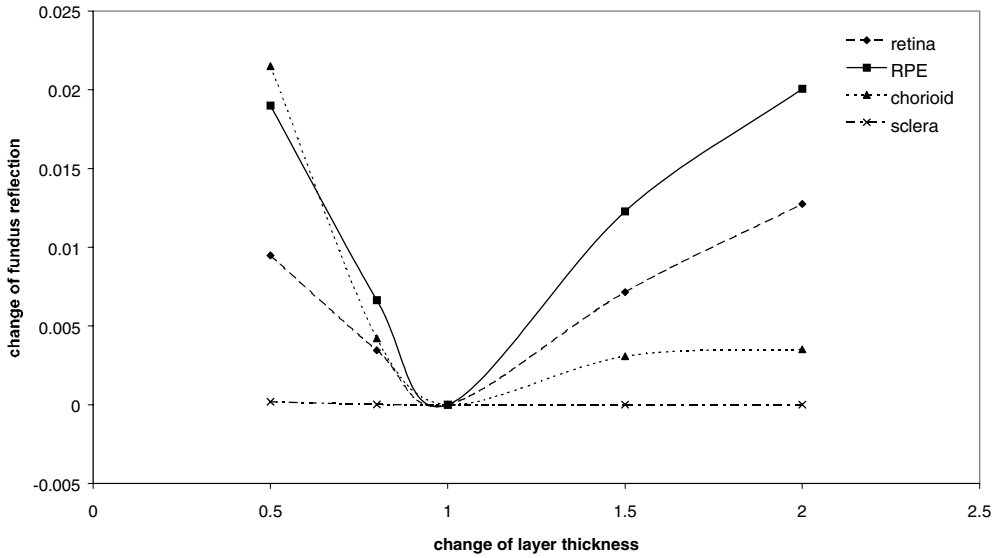

Figure 4. Ocular fundus reflection versus variation of the layer thickness.

be useful in the interpretation of spectrally resolved fundus reflectometry measurements. The reasonable concentration values for haemoglobin, melanin and xanthophyll determined in single patients as well as in a healthy control group give grounds for believing that the developed method may be of interest for clinical diagnostics.

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