Foveal cone mosaic and visual pigment density in dichromats

Tos T. J. M. Berendschot*, Jan van de Kraats* and Dirk van Norren*†

*Helmholtz Institute, Department of Ophthalmology, Utrecht University, PO Box 85500, NL-3508 GA Utrecht, and †Netherlands Aerospace Medical Centre, Kampweg 3, NL-3769 DE Soesterberg, The Netherlands

1. Optical reflectance spectra of the fovea were measured in ten subjects with normal colour vision, ten protanopes and seven deuteranopes. Four conditions were used: perpendicular and oblique angle of incident and reflected light on the retina, both in a dark-adapted and a fully bleached state.

2. The spectra were analysed to assess the effects of dichromacy on the cone mosaic. A replacement model, i.e. one where the total number of cones remains unchanged and all cones are filled with a single type of pigment, was found to fit our data best.

3. The analysis of the spectral fundus reflectance also provided estimates for densities of photo-labile and photo-stable retinal pigments and fraction of long wavelength-sensitive (LWS) cones. Visual pigment density was 0·39 for protanopes and 0·42 for deuteranopes, significantly lower than the 0·57 found for colour normals. Macular pigment density was 0·54 for colour normals, 0·46 for protanopes and 0·42 for deuteranopes.

4. For colour normals the LWS cone fraction was 0·56, in agreement with psychophysical literature. The LWS cone fraction for protanopes was −0·04, and for deuteranopes 0·96, consistent with their Rayleigh matches.

Colour normals have three classes of photoreceptor cells, each containing a specific visual pigment. Dichromats are presumed to have only two of these classes. Rushton (1963*a*) and Alpern & Wake (1977) measured visual pigment absorption by retinal densitometry and found that dichromats indeed lack one of the cone photo-pigments. Microspectrophotometric analysis (Dartnall, Bowmaker & Mollon, 1983) on colour-normal and dichromatic retinas confirmed this result. Dichromacy is caused by alterations in the genes that encode the different pigments (Nathans, Piantanida, Eddy, Shows & Hogness, 1986). This gene encoding also supports the theory of absence of one of the visual pigments. Three models can be put forward to explain the effect of the lack of one of the visual pigments on the cone mosaic. The first model specifies that a complete class of cones with their associated pigment is lost, leaving empty spaces in the cone mosaic – the empty spaces model (1). In the second and third model it is assumed that the total number of cones in dichromats is not different from the total number in normals. These cones are either completely filled with one of the remaining classes of pigments – the replacement model (2), or a fraction of the cones contain no visual pigment – the empty cones model (3). Careful examination of visual acuity to discriminate between the models yielded conflicting results (Hecht, 1949; François & Verriest, 1958; Brown, Phares & Fletcher, 1960; Wildner, 1970). Both Geller & Sieving (1993) and Seiple, Holopigian, Szlyk & Greenstein (1995) recently showed that a factor 10 decrease in foveal cone packing density, is still compatible with useful visual acuity. Discrimination between the different models seems thus not possible with this simple test. Support for the first or third model can be gleaned from a paper of Vos & Walraven (1970). They concluded, on deriving foveal receptor primaries, and analysing the Bezold–Brücke effect, that the total number of cones in dichromats is about 40% lower than in normals. The results of Cicerone & Nerger (1989*b*), and Wesner, Pokorny, Shevell & Smith (1991) support the second model. They presented estimates of the numerosity of cones in the dichromatic fovea, based on frequency of seeing curves, and found the packing of foveal cones of the dichromat to be comparable with that of the colour normal.

Recently, Van de Kraats, Berendschot & Van Norren (1996) forwarded a model for foveal fundus reflectance with three reflectors: the inner limiting membrane, the outer segments of the photoreceptors and the sclera. Empty spaces in the cone mosaic of dichromats would result in a decrease of the spectral fundus reflectance in a condition with all visual pigments bleached, since fewer cones are available as possible reflectors for the incoming light. Empty cones would result in a higher reflectance in the dark-adapted condition.

We measured the reflectance of the fundus in dichromats and colour normals and analysed the data with the Van de

Kraats model, to allow a choice between the different models for dichromacy. The analysis favours the replacement model, but to our surprise the visual pigment density of the remaining cones was abnormally low in dichromats, as was the density of the macular pigment.

METHODS

Reflection measurements were performed with a slightly modified version (Van de Kraats *et al.* 1996) of the Utrecht Retinal Densitometer (Van Norren & Van de Kraats, 1989*a*). We used an illumination field of 1·9 deg and a detection field of 1·6 deg. Entry and exit pupils were aligned to the peak of the Stiles–Crawford function (Van Blokland & Van Norren, 1986), called the perpendicular condition, and to a pupil position 2·5 mm from the peak, called the oblique condition. In both positions, reflection spectra were measured in a bleached and a dark-adapted condition, to monitor the influence of the visual pigment. Thus, four reflection spectra were obtained in each subject. A total of twenty-seven subjects was investigated. The local ethics committee approved the use of the experimental procedures and subjects gave informed, written consent. According to their Rayleigh matches (Nagel anomaloscope, type 2), ten subjects were classified as having normal colour vision, ten were protanopes and seven were deuteranopes.

RESULTS

Figure 1*A* shows mean reflectance spectra for the perpendicular measurement angle of the bleached foveae of ten colour normals, ten protanopes and seven deuteranopes. Symbols reflect the results of the measurements and lines are results of fitting the data with the Van de Kraats model. Error bars show the mean measurement error at each wavelength. For wavelengths greater than 500 nm the reflectance is slightly higher for colour normals than for both protanopes and deuteranopes; for wavelengths shorter than 500 nm it is lower. To emphasize the difference between colour normals and dichromats, Fig. 1*B* shows the difference between the logarithmically scaled spectra of colour normals and dichromats. The shape of the difference spectrum is highly suggestive for a difference in the density of macular pigment between colour normals and dichromats. The continuous line, discussed in the next section, represents a fit with the spectral extinction of macular pigment (DeMarco, Pokorny & Smith, 1992).

Figure 2*A* shows mean reflectance spectra of ten colour normals for perpendicular measurement angle in the bleached and dark-adapted condition. In Fig. 2*B* the

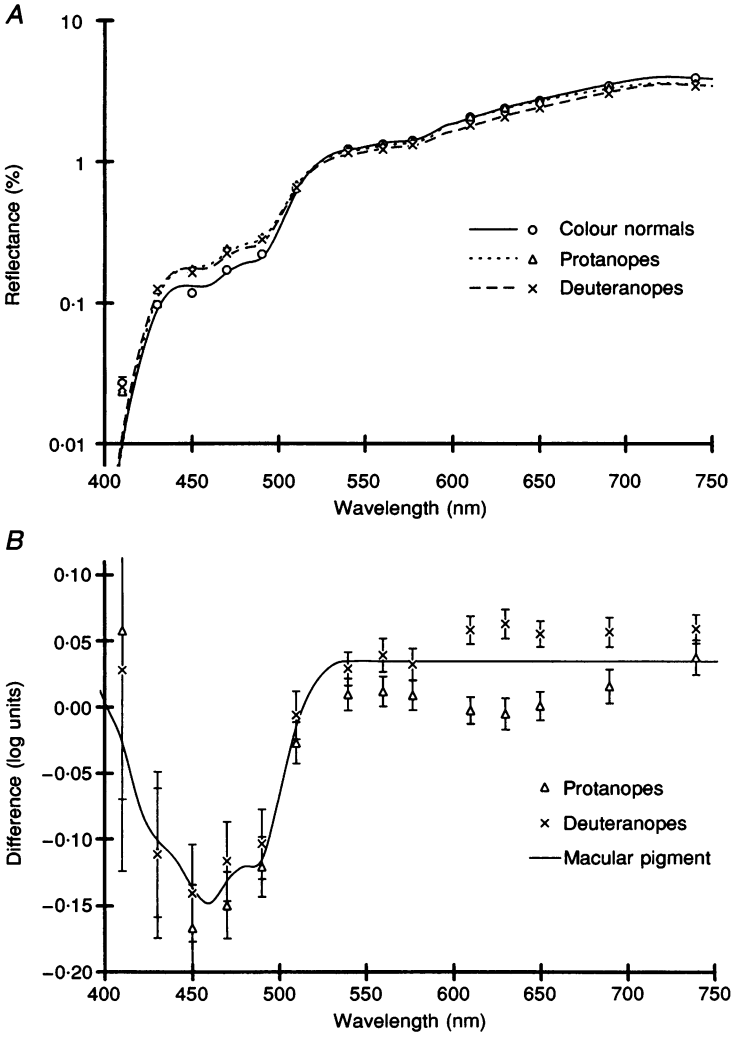

Figure 1. Mean reflectance spectra

A, mean reflectance spectra for a perpendicular measurement angle of the bleached retina in colour normals and dichromats. Symbols are measured data and lines are results of fits with the Van de Kraats model. Error bars indicate the mean measurement error at each wavelength. *B*, difference between the spectra for colour normals and protanopes and deuteranopes. The line represents the spectral density of macular pigment.

difference between the spectra shown in Fig. 2*A* is presented, together with similar differences for protanopes and deuteranopes. As expected, the wavelengths at which the differences are maximal differ between colour normals, protanopes and deuteranopes. These bell-shaped curves show for all wavelengths the so-called double density, the common result of retinal densitometry. Double density traditionally refers to a double traverse of the measuring light through the visual pigments. If no diluting stray light exists, it is a measure for twice the density of visual pigment. Table 1 shows double densities D_d obtained from the mean reflectance spectra of all subjects in a group and double densities obtained by averaging the individual data. The double density for colour normals (0·436) is significantly higher than for both protanopes (0·310) and deuteranopes (0·349).

Analysis

The reflectance of the bleached fundus differs between colour normals and dichromats. For the perpendicular, bleached condition the difference of the reflectance spectra between colour normals and dichromats can be grasped by the assumption that colour defectives have substantially less macular pigment than colour normals, in double traverse 0·18 log units. To account for the small differences in reflectance for wavelengths greater than 550 nm, a constant difference between colour normals and dichromats of 0·034 log units was added for all wavelengths. These values result in the continuous line in Fig. 1*B*.

The spectra were also analysed with a model for fundus reflectance presented by Van de Kraats *et al.* (1996). This model describes absorption and reflection by different retinal layers and uses these, and a limited number of known spectral extinctions to decompose the measured spectra. By fitting the four measured spectra simultaneously, data were obtained for the density of both the photo-stable absorbers (lens, macular pigment, melanin, blood) and the photo-labile visual pigments. In addition, the model provided data for reflections from three interfaces (inner limiting membrane, outer segment discs and sclera). To obtain the maximum likelihood estimate of the model parameters, we used the Levenberg–Marquardt fitting algorithm (Press, Flannery, Teukolsky & Vettering, 1989)

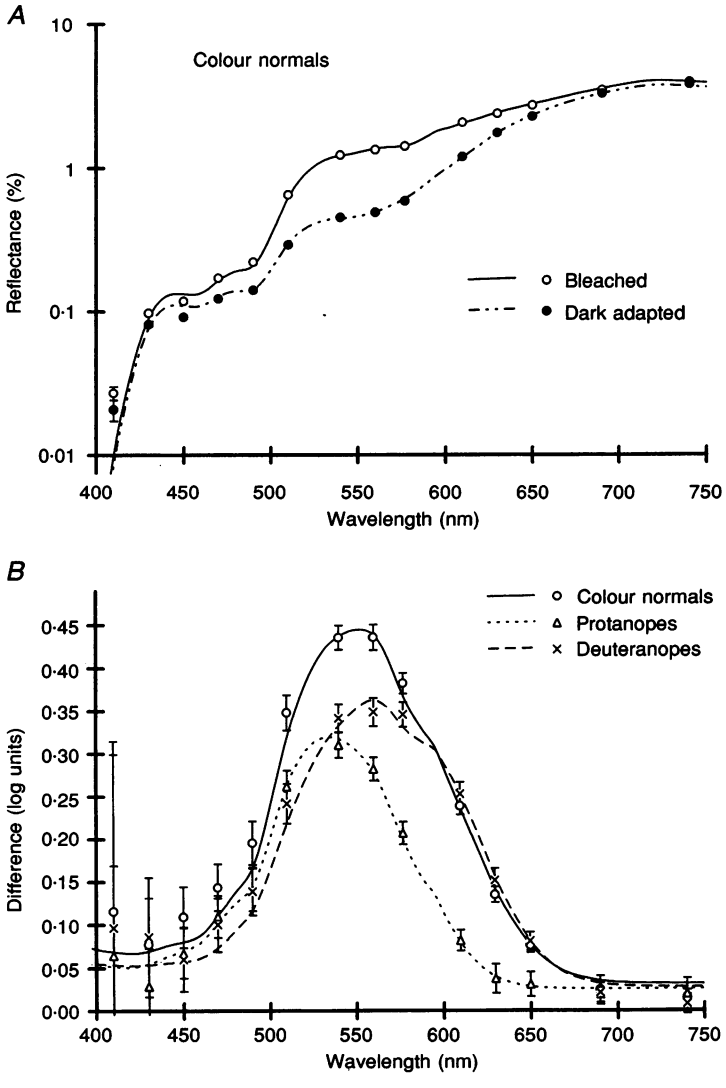

Figure 2. Double density of visual pigment

A, mean reflectance spectrum of colour normals for perpendicular measurement angle of the bleached retina (copy of curve in Fig. 1*A*), together with the spectrum measured in the dark-adapted retina. *B*, difference between the spectra shown in *A*, accompanied by similar differences for protanopes and deuteranopes. This bell-shaped curve called double density, the usual result of retinal densitometry, is traditionally ascribed to a double traverse of the measuring light through the visual pigments, and, as such, a measure for twice the density of visual pigment. In both *A* and *B* symbols are measured data and lines are results of fits with the Van de Kraats model (Van de Kraats *et al.* 1996). Error bars indicate the mean measurement error at each wavelength.

Table 1. Double densities of visual pigment for colour normals and dichromats

	Colour normals			Protanopes			Deuteranopes		
	D_d	Error	S.D.	D_d	Error	S.D.	D_d	Error	S.D.
D_d of mean	0·436	0·030	—	0·310	0·030	—	0·349	0·033	—
Mean D_d	0·433	—	0·072	0·303	—	0·086	0·351	—	0·048

Double density (D_d) obtained from the mean reflectance spectra, and obtained by averaging the individual double densities. Error represents the mean measurement error. Double densities of dichromats differ significantly ($P < 0·05$) from those of the colour normals.

Table 2. Parameters calculated with the Van de Kraats model from the mean reflectance spectra

	Colour normals	Protanopes	Deuteranopes
Lens density (420 nm)	0·534 ± 0·029	0·595 ± 0·017	0·515 ± 0·024
Macular pigment density (460 nm)	0·525 ± 0·025	0·411 ± 0·016	0·413 ± 0·019
Melanin density (500 nm)	1·274 ± 0·028	1·156 ± 0·019	1·336 ± 0·028
Effective blood layer thickness	17·0 ± 4·5	45·2 ± 14·6	13·3 ± 3·6
Choroid scatter loss	0·225 ± 0·006	0·281 ± 0·005	0·244 ± 0·005
LWS cone fraction	0·525 ± 0·048	−0·0080 ± 0·044	0·958 ± 0·050
Visual pigment density (peak)	0·587 ± 0·026	0·405 ± 0·016	0·421 ± 0·018
ILM reflectance (%)	0·260 ± 0·024	0·435 ± 0·027	0·270 ± 0·025
Outer segment discs reflectance	2·773 ± 0·095	2·831 ± 0·062	2·465 ± 0·080
ρ (mm^{-2})	0·110 ± 0·018	0·065 ± 0·006	0·076 ± 0·009

LWS, long wavelength sensitive; ILM, inner limiting membrane; ρ, optical quality of photoreceptor layer.

Table 3. Mean values of the individual parameters calculated with the Van de Kraats model

	Colour normals	Protanopes	Deuteranopes
Lens density (420 nm)	0·54 ± 0·11	0·55 ± 0·17	0·536 ± 0·085
Macular pigment density (460 nm)	0·54 ± 0·12	0·46 ± 0·15	0·42 ± 0·14
Melanin density (500 nm)	1·32 ± 0·23	1·16 ± 0·29	1·37 ± 0·19
Effective blood layer thickness	22·7 ± 16·9	52·8 ± 66·8	42·6 ± 41·4
Choroid scatter loss	0·226 ± 0·058	0·274 ± 0·053	0·238 ± 0·032
LWS cone fraction	0·56 ± 0·11	−0·036 ± 0·089*	0·983 ± 0·058*
Visual pigment density (peak)	0·60 ± 0·11	0·39 ± 0·12*	0·434 ± 0·091*
ILM reflectivity (%)	0·261 ± 0·084	0·37 ± 0·16*	0·285 ± 0·070
Outer segment discs reflectance	2·95 ± 0·94	2·79 ± 1·07	2·57 ± 0·88
Wavelength at peak D_d (nm)	552·1 ± 3·8	535·7 ± 3·2*	558·7 ± 3·0*
ρ (mm^{-2})	0·120 ± 0·047	0·098 ± 0·062	0·082 ± 0·036
Age	33·5 ± 9·6	29·9 ± 12·0	35·1 ± 13·9

For abbreviations see Table 2. * Significantly different ($P < 0·05$) from those of the colour normals.

Table 4. Significant correlation coefficients (r) between the parameters given in Table 3

	r	P
Visual pigment density–ρ	0·54	0·003
Visual pigment density–outer segment reflectance	0·36	0·061
Outer segment reflectance–ρ	0·39	0·037
Age–lens density	0·69	< 0·0001

to minimize χ^2. Since the model was developed for colour normals with a fixed number of cones, it is consistent with the replacement theory, and a long wavelength-sensitive (LWS) cone fraction is calculated. Results from fits with the Van de Kraats model are shown as lines in Figs 1*A*, 2*A* and *B*.

Table 2 shows parameter values derived from the data-fitting process to the mean reflectance spectra of the different groups. Due to inter-individual differences, the parameters also vary within the group of colour normals and dichromats themselves. Table 3 shows mean values of the individual parameters calculated with the model. Data indicated with an asterisk differ significantly ($P < 0{\cdot}05$) from those of colour normals. No significant differences between colour normals and dichromats were found for lens density, melanin density, effective blood layer thickness, outer segment discs reflectance, inner limiting membrane (ILM) reflectance, and choroid scatter loss. Visual pigment density for colour normals was higher than for colour defectives. The difference in macular pigment density, so clearly seen in Fig. 1*B*, was not significant for protanopes and deuteranopes separately. However, if we treat protanopes and deuteranopes as one group of dichromats, the difference is significant ($P < 0{\cdot}05$). Further, differences between colour normals and dichromats were found, as expected, for the wavelength at the peak of the double density, interpolated from the data-fitting process, and for the LWS cone fraction. The wavelength at which the double density peaks, λ_D, was determined as $552{\cdot}1 \pm 3{\cdot}8$ nm for colour normals. For protanopes λ_D is $535{\cdot}7 \pm 3{\cdot}2$ nm, close to the wavelength of 534 nm at which the absorption of medium wavelength-sensitive (MWS) cones peaks (Bowmaker & Dartnall, 1980; DeMarco *et al.* 1992). For deuteranopes λ_D is $558{\cdot}7 \pm 3{\cdot}0$ nm, in the range of the LWS cone absorption peak of 556–563 nm (Bowmaker & Dartnall, 1980; DeMarco *et al.* 1992). The LWS cone fraction was calculated as $-0{\cdot}036 \pm 0{\cdot}089$ for protanopes and $0{\cdot}983 \pm 0{\cdot}058$ for deuteranopes.

To obtain a measure for the optical quality of the photoreceptor layer, Van de Kraats *et al.* (1996) introduced a parameter SC, fixed at 1 for perpendicular and between 0 and 1 for oblique retinal angle. The parameter describes the transmission of the total outer segment as a function of retinal angle of the incident light. To induce a change in retinal angle the position of illumination and detection beam in the pupil plane is varied. In the present experiments a shift in the pupil plane of $x = 2{\cdot}5$ mm was chosen for the oblique condition. For some subjects this value had to be smaller because of restrictions in the size of the pupil. To normalize, we converted SC to a parameter ρ, defined as $\log(\mathrm{SC}) = -\rho x^2$, in analogy with the conventional description of the psychophysical Stiles–Crawford effect (Enoch & Lakshminarayanan, 1991). Our analysis showed no significant differences for ρ between colour normals and dichromats. Age is included in Table 3, although this is not a model parameter.

We searched for correlations between the different parameters. Significant correlations were found between visual pigment density, ρ and the outer segment reflectance. Although the ranges for visual pigment density, ρ and the outer segment reflectance are different for colour normals and dichromats, they show similar correlations. Therefore, we considered all twenty-seven subjects as one group to obtain correlation coefficients. These are presented in Table 4.

The density of the lens increases with age, which is confirmed by a positive correlation. No other parameter correlated with age, which ranged from 18 to 57 years.

If we assume equal visual pigment densities for dichromats and colour normals the replacement type Van de Kraats model cannot explain the observed reduction in double density in dichromats. Reflectance of empty cones will have a diluting effect on the measured visual pigment double density. To estimate this effect, we modified the Van de Kraats model to include empty cones, having the normal

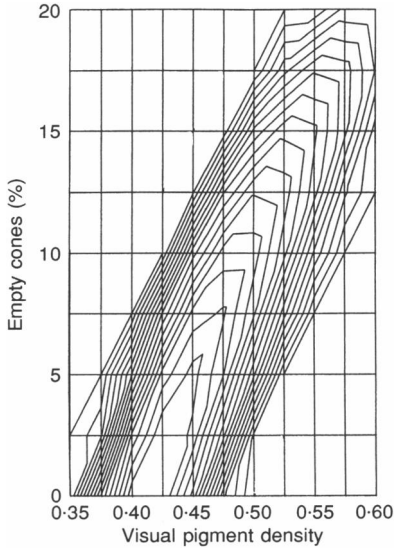

Figure 3. Confidence intervals for the mean reflectance spectra of the deuteranopes

Intervals are shown as a function of the visual pigment density and the fraction of empty cones for the modified Van de Kraats model. Lines indicate regions with $\Delta\chi^2 = 1$.

number of discs in their outer segments, but no visual pigment. The LWS fraction was fixed at 0·0 for protanopes and 1·0 for deuteranopes. The simultaneous fitting of both visual pigment density and number of empty cones in dichromats was difficult, since their covariance was high. Therefore, we calculated χ^2 fixed values of both visual pigment density and fraction of empty cones, while all other parameters were left free. Results for the mean reflectance spectra of the deuteranopes are shown in Fig. 3. With a visual pigment density fixed to the value of colour normals of 0·587, an empty cone fraction of 17% results. However, best fits were obtained assuming no empty cones and a lower visual pigment density than for colour normals. For protanopes a similar conclusion could be drawn.

The empty spaces model for dichromacy predicts a reduction in apparent outer segment reflectance, since fewer cones are available as possible reflectors for the incoming light. This was not found; only a small, insignificant decrease was observed in dichromats (see Table 3). For wavelengths shorter than 600 nm, light travelling through empty spaces in the cone mosaic would be absorbed efficiently by the blood-rich deeper layers. Thus, inducing only a small diluting effect in the measurement of the visual pigment double density, an additional assumption would be required to account for the observed reduction in dichromats. The empty spaces model was therefore rejected.

DISCUSSION

Model analysis of spectral fundus reflectance favours the replacement model to explain the effect of the lack of one of the visual pigments in dichromats on the cone mosaic. This is consistent with psychophysical experiments, based on frequency of seeing curves (Cicerone & Nerger, 1989*b*; Wesner *et al.* 1991). Differences in visual pigment density are not critical in their analysis. Therefore, the replacement model can explain both their frequency of seeing curves and our optical reflectance spectra, assuming a lower visual pigment density in dichromats than in colour normals (Tables 2 and 3). Vos & Walraven (1970) concluded, on deriving foveal receptor primaries, and analysing the Bezold–Brücke effect, that the total number of cones in dichromats is about 40% lower than in normals. However, one of their main assumptions was an equal visual pigment density for colour normals and dichromats. Assuming lower visual pigments in dichromats could probably also explain their data.

Visual pigment densities differed significantly between colour normals and colour defectives (cf. Table 3). The range, yielded by the Van de Kraats model, was 0·41–0·80 for colour normals and 0·26–0·54 for colour defectives. For colour normals a value of about 0·4 is found with colour-matching techniques and densitometry. (For a summary see Van de Kraats *et al.* 1996.) Higher densities, up to 1·0, are predicted by analysis of the Stiles–Crawford II effect (Walraven & Bouman, 1960; Enoch & Stiles, 1961). Note that smaller measuring fields yield higher densities, clearly visualized by Van Norren & Van de Kraats (1989*b*). Only limited data on the density of visual pigments in dichromats are available in the literature. With the technique of densitometry Rushton (1963*b*) and King-Smith (1971) calculated a density of 0·35 for a single protanope using a 2 deg measuring field. King-Smith (1973) measured two deuteranopes, two deuteranomalous and one colour normal and calculated for these five subjects a mean density of 0·4 for a 2 deg field. Alpern & Wake (1977) measured the visual pigment density of fifteen deuteranopes. An analysis of their results, neglecting the influence of stray light, gives a density of 0·12 for a 2 deg measuring field, which is even lower than the uncorrected result for protanopes measured by Rushton (1963*b*). Using heterochromatic flicker photometry, Miller (1972) obtained a density of 0·4–0·5 for one protanope and a density of 0·5–0·6 for one deuteranope using a 1·6 deg test field. Smith & Pokorny (1973) found 0·3 for three protanopes and 0·4 for four deuteranopes for a 2·5 deg test field.

The LWS cone fraction was calculated as $-0{\cdot}036 \pm 0{\cdot}089$ for protanopes and $0{\cdot}958 \pm 0{\cdot}050$ for deuteranopes, a satisfactory result consistent with the classical idea of absence of one of the visual pigments in dichromats. There is some dispute about the ratio of the peak densities of the visual pigment in the LWS and MWS cones for colour normals. Microspectrophotometric measurements support an equal peak density (Bowmaker & Dartnall, 1980). Colour-matching data suggest a ratio of $\pm 1{\cdot}33$ for the LWS to MWS peak density (Pokorny, Smith & Starr, 1976; MacLeod & Webster, 1988; Burns & Elsner, 1993). Two phenomena can possibly explain this discrepancy. First, short-wavelength-absorbing photo-products can reduce the density inferred from the colour-matching experiments. This effect is stronger for the MWS than for the LWS cone (Stockman, MacLeod & Johnson, 1993). Further, at long wavelengths, light is reflected from the deeper, blood-rich layers more efficiently than at shorter wavelengths. This might make the LWS cones more efficient for visual perception (Van de Kraats *et al.* 1996). Since for protanopes and deuteranopes we have found similar densities, we assumed the density to be equal in the LWS and MWS cones for colour normals. An LWS cone fraction of $0{\cdot}56 \pm 0{\cdot}11$ is then found for colour normals, with a range of 0·41–0·71. Vos & Walraven (1970) estimated an LWS cone fraction of 0·62 from the Stiles–Weber fractions, within the framework of the fluctuation theory for contrast detection. Jacobs & Neitz (1991) found, with flicker photometry, an average value of 0·67 with a range from 0·4 to 0·9. Vimal, Pokorny, Smith & Shevell (1989) used frequency of seeing curves to explore the retinal mosaic of two colour normals and obtained values of 0·52 and 0·77 and corresponding flicker photometric values of 0·61 and 0·80. Wesner *et al.* (1991) found for three colour normals

0·60, 0·82 and 0·87 from frequency of seeing curves and corresponding flicker photometric values of 0·53, 0·71 and 0·82. Cicerone & Nerger (1989*a*) also used frequency of seeing curves and obtained a mean value of 0·67 for the LWS cone fraction, with a range of 0·59–0·70. Thus, the majority of psychophysical measurements point to an LWS cone fraction of about 0·6. Mollon & Bowmaker (1992) made microspectrophotometric measurements of patches of the foveal retina from Old World monkeys and reported an equal number of MWS and LWS cones. Our results with fundus reflectometry, with the advantage of being an *in vivo* and objective technique, are consistent with most psychophysical studies.

Even a simple analysis showed the surprising result that the density of the macular pigment in dichromats is lower than in colour normals (Fig. 1*B*). This was confirmed in the extended model calculation. Macular pigment density is greatest at the centre of the fovea, along the path of the photoreceptor axons, and can be viewed as a homogeneous filter that lies between the photoreceptors and the inner limiting membrane (Snodderly, Auran & Delori, 1984). Apparently, a lower visual pigment density is accompanied by a lower macular pigment density. Moreland & Ruddock (1993) estimated the optical density of macular pigment from foveal and non-foveal colour-matching data. They found no difference between a group of fifty-one normals and ten deuteranopes.

An alternative explanation of our findings would be parafoveal fixation in dichromats. This would lead to a decrease in both visual pigment density (Van Norren & Van de Kraats, 1989*b*) and macular pigment density (Snodderly *et al.* 1984). This explanation can be rejected, because with the Utrecht Retinal Densitometer the fixation can be monitored.

The range for the measure of the optical quality of the photoreceptor layer, ρ, differed between colour normals and dichromats. A high visual pigment density is accompanied by a high outer segment reflection and a high ρ. With an optical technique to visualize the Stiles–Crawford effect, Burns, Elsner, Gorrand, Kreitz & Delori (1992) demonstrated a similar correlation between the density of the visual pigment and the optical quality of the cones. In the model calculation we assumed that for perpendicular measurement angle all the incoming light is captured in the photoreceptors. For low values of ρ this may be an overestimation. According to the Van de Kraats model, the reflectance of the photoreceptor layer and visual pigment density originates from the summation of small contributions from discs in the outer segment. Allowing some leakage of light into the interspace for perpendicular measurement angle at low ρ values implies an apparent decrease in outer segment reflectance and visual pigment density and could therefore explain the observed correlations.

ALPERN, M. & WAKE, T. (1977). Cone pigments in human deutan colour vision defects. *Journal of Physiology* **266**, 595–612.

BOWMAKER, J. K. & DARTNALL, H. J. A. (1980). Visual pigments of rods and cones in the human retina. *Journal of Physiology* **298**, 501–512.

BROWN, J. L., PHARES, L. & FLETCHER, D. E. (1960). Spectral energy thresholds for the resolution of acuity targets. *Journal of the Optical Society of America* **50**, 198–204.

BURNS, S. A. & ELSNER, A. E. (1993). Color matching at high illuminances: photopigment optical density and pupil entry. *Journal of the Optical Society of America* **A10**, 221–230.

BURNS, S. A., ELSNER, A. E., GORRAND, J., KREITZ, M. R. & DELORI, F. C. (1992). Comparison of reflectometric and psychophysical measures of cone orientation. In *Digest of Topical Meeting on Noninvasive Assessment of the Visual System*, vol. **5**, pp. 160–163. Optical Society of America, Washington, DC, USA.

CICERONE, C. M. & NERGER, J. L. (1989*a*). The relative numbers of long-wavelength-sensitive to middle-wavelength-sensitive cones in the human fovea centralis. *Vision Research* **29**, 115–128.

CICERONE, C. M. & NERGER, J. L. (1989*b*). The density of cones in the fovea centralis of the human dichromat. *Vision Research* **29**, 1587–1595.

DARTNALL, H. J. A., BOWMAKER, J. K. & MOLLON, J. D. (1983). Human visual pigments: microspectrophotometric results from the eyes of seven persons. *Proceedings of the Royal Society* B **220**, 115–130.

DEMARCO, P., POKORNY, J. & SMITH, V. C. (1992). Full-spectrum cone sensitivity functions for X-chromosome-linked anomalous trichromats. *Journal of the Optical Society of America* **A9**, 1465–1476.

ENOCH, J. M. & LAKSHMINARAYANAN, V. (1991). Retinal fibre optics. In *Vision and Visual Dysfunction* 1: *Visual Optics and Instrumentation*, ed. CHARMAN, W. N. & CRONLY-DILON, J. R., pp. 280–309. MacMillan Press Ltd, London.

ENOCH, J. M. & STILES, W. S. (1961). The colour change of monochromatic light with retinal angle of incidence. *Optica Acta* **8**, 329–358.

FRANÇOIS, J. & VERRIEST, G. (1958). Relation entre l'éclairement et l'acuité visuelle dans un groupe de sujets normaux et dans différents groupes d'anomalies congénitales de la vision. *Ophthalmologica* **134**, 193–204.

GELLER, A. M. & SIEVING, P. A. (1993). How many cones are required to 'see'?: lessons from Stargardt's macular dystrophy and from modelling with degenerate photoreceptor arrays. In *Retinal Degeneration*, ED. HOLLYFIELD, J. G., ANDERSON, R. E. & LA VAIL, M. M., pp. 25–34. Plenum Press, New York.

HECHT, S. (1949). Brightness, visual acuity and colour blindness. *Documenta Ophthalmologica* **3**, 289–306.

JACOBS, G. H. & NEITZ, J. (1991). Electrophysiological estimates of individual variation in the L/M cone ratio. In *Colour Vision Deficiencies*, vol. XI, pp. 107–112. Kluwer Academic Publishers, The Netherlands.

KING-SMITH, P. E. (1971). The optical density of erythrolabe determined by retinal densitometry. *Journal of Physiology* **218**, 100–101*P*.

KING-SMITH, P. E. (1973). The optical density of erythrolabe determined by retinal densitometry using the self-screening method. *Journal of Physiology* **230**, 535–549.

MACLEOD, D. I. A. & WEBSTER, M. A. (1988). Direct psychophysical estimates of the cone-pigment absorption spectra. *Journal of the Optical Society of America* **A5**, 1736–1743.

MILLER, S. S. (1972). Psychophysical estimates of visual pigment densities in red–green dichromats. *Journal of Physiology* **223**, 89–107.

MOLLON, J. D. & BOWMAKER, J. K. (1992). The spatial arrangements of cones in the primate fovea. *Nature* **360**, 677–678.

MORELAND, A. B. & RUDDOCK, K. H. (1993). Variations in colour matching data associated with light losses in the macular pigment. In *Colour Vision Deficiences*, vol. XI, pp. 427–435. Kluwer Academic Publishers, The Netherlands.

NATHANS, J., PIANTANIDA, T. P., EDDY, R. L., SHOWS, T. B. & HOGNESS, D. S. (1986). Molecular genetics of inherited variation in human color vision. *Science* **232**, 203–210.

POKORNY, J., SMITH, V. C. & STARR, S. J. (1976). Variability of color mixture data-II. The effect of viewing field size on the unit coordinates. *Vision Research* **16**, 1095–1098.

PRESS, W. H., FLANNERY, B. P., TEUKOLSKY, S. A. & VETTERING, W. T. (1989). *Numerical Recipes in Pascal.* Cambridge University Press, Cambridge.

RUSHTON, W. A. (1963*a*). A cone pigment in the protanope. *Journal of Physiology* **168**, 345–359.

RUSHTON, W. A. (1963*b*). The density of chlorolabe in the foveal cones of the protanope. *Journal of Physiology* **168**, 360–373.

SEIPLE, W., HOLOPIGIAN, K., SZLYK, J. P. & GREENSTEIN, V. C. (1995). The effects of random element loss on letter identification: Implications for visual acuity loss in patients with retinitis pigmentosa. *Vision Research* **35**, 2057–2066.

SMITH, V. C. & POKORNY, J. (1973). Psychophysical estimates of optical density in human cones. *Vision Research* **13**, 1199–1202.

SNODDERLY, D. M., AURAN, J. D. & DELORI, F. C. (1984). The macular pigment. II. Spatial distribution in primate retinas. *Investigative Ophthalmology and Visual Science* **25**, 674–685.

STOCKMAN, A., MACLEOD, D. I. A. & JOHNSON, N. E. (1993). Spectral sensitivities of the human cones. *Journal of the Optical Society of America* **A10**, 2491–2521.

VAN BLOKLAND, G. J. & VAN NORREN, D. (1986). Intensity and polarization of light scattered at small angles from the human fovea. *Vision Research* **26**, 485–494.

VAN DE KRAATS, J., BERENDSCHOT, T. T. J. M. & VAN NORREN, D. (1996). The pathways of light measured in fundus reflectometry. *Vision Research* (in the Press).

VAN NORREN, D. & VAN DE KRAATS, J. (1989*a*). Retinal densitometer with the size of a funduscamera. *Vision Research* **29**, 369–374.

VAN NORREN, D. & VAN DE KRAATS, J. (1989*b*). Imaging retinal densitometry with a confocal scanning laser ophthalmoscope. *Vision Research* **29**, 1825–1830.

VIMAL, R. L., POKORNY, J., SMITH, V. C. & SHEVELL, S. K. (1989). Foveal cone thresholds. *Vision Research* **29**, 61–78.

VOS, J. J. & WALRAVEN, P. L. (1970). On the derivation of the foveal receptor primaries. *Vision Research* **11**, 799–818.

WALRAVEN, P. L. & BOUMAN, M. A. (1960). Relation between directional sensitivity and spectral response curves in human cone vision. *Journal of the Optical Society of America* **50**, 780–784.

WESNER, M. F., POKORNY, J., SHEVELL, S. K. & SMITH, V. C. (1991). Foveal cone detection statistics in color-normals and dichromats. *Vision Research* **31**, 1021–1037.

WILDNER, D. G. (1970). *The Photopic Spectral Sensitivity of Color Normal, Protanopic and Deuteranopic Observers.* University Microfilms, Ann Arbor, MI, USA.

Received 26 June 1995; accepted 27 October 1995.