Mapping cone photopigment optical density

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The distribution of cone photopigment across the retina affects the amount of light captured by cones at each retinal location. Cone photopigment optical density is measured in two ways, with reflectometry and/or with color matching. Color matching measures a higher optical density than does reflectometry. Control experiments confirm that large-field color matches measure photopigment optical density toward their outer edge. There is qualitative agreement as to photopigment distribution from both techniques near the fovea. Beyond 1 deg, color matching indicates little decrease in photopigment with increasing eccentricity, whereas retinal densitometry shows a steep decline in photopigment. The decrease in perifoveal optical density measured with reflectometry is attributed to the decrease in cone coverage from fovea to perifovea as rods and interphotoreceptor spaces increase. Differences among subjects in photopigment distribution near the fovea, measured with both techniques, reflect differences in the specialization of the foveal center for cone length and/or photopigment concentration per cone, which are factors influencing results from both techniques.

1. INTRODUCTION

The first step in vision, and hence an important limiting step, is the capture of light by the photopigments and their isomerization.[1] In daylight, when humans have their best visual acuity and color vision, the spatial arrangement of cone photopigment affects the sampling for all later stages of vision. This includes spatial sampling by individual cones, signal transduction, signal coding, and eventually image interpretation. The amount of photopigment per unit area on the retina is part of a spatial sampling function that has several components: the percentage of the retina covered by cones (cone coverage), cone orientation, photopigment extinction spectra, cone length, and photopigment concentration per cone. Human cone coverage and packing estimates from histology on human tissue[2–4] and psychophysical methods[5] indicate that cones are most densely packed in the fovea, and the inner segments are tightly packed with individual variability in the number of cones per unit area in the central 1 deg. Cones are longest in the central fovea and decrease in length with increasing retinal eccentricity.[3] Recent studies[3–5] have not examined cone length, which is crucial to determine the volume of photopigment at each retinal location available to capture light, but evidence exists that there is considerable individual variability[6–11] in cone optical density at the center of the fovea. In this paper we use two techniques to measure the distribution of human cone photopigments *in vivo,* color matching[6–10] and imaging retinal densitometry.[11–17] Both describe cone photopigment optical density as an equivalent layer of cone photopigment and measure the amount of light absorbed. However, each technique is influenced by some factors that do not influence the other, and both are sensitive to differences in cone length.

A. Color Matching

The first technique is color matching, a psychophysical technique that measures only functioning cones. In color matching,[6–10] a standard light is matched in color and luminance by a mixture of two or three other lights. The subject adjusts the ratio and total amount of the mixture lights to match the standard. When there is a match, the cones of a given spectral sensitivity type catch the same number of quanta (photons) for the mixture as for the standard. Thus color matching equates the number of quanta absorbed by each cone type. For each match, the subject must compare the responses of functioning cones over time or space. At modest light levels, color-matching findings from three laboratories[6,7,9] indicate ~0.1-log-unit difference in measured optical density between 1- and 8-deg field sizes, with 0.27 typical for a 4-deg stimulus. Most of the variation is between 1 and 2 deg, and there is considerable variability among subjects in the shape of the change with field size in addition to variation at a single field size.[18]

When the eye is exposed to a strong light, the photopigment bleaches and the color matches change, according to the Beer–Lambert relation[7]

$$F(\lambda) = 1 - \exp(-\alpha_\lambda c l), \quad (1)$$

where $F(\lambda)$ is the fraction of light absorbed, α_λ is the extinction spectrum of the pigment, c is the chromophore concentration of the photopigment, and l is the path length of the light through the pigment. By using this equation together with the fundamentals of Smith *et al.*[19] and solving for the conditions that satisfy a color match,[7] we can compute the density of the cone photopigments. The optical density of cone photopigment is computed from the change between color matches at high and low light intensities, as explained elsewhere.[7,8] Thus extinction spectra, photopigment concentration, and optical-path length of functioning cones affect the color matches. Because all the color matches performed in this study are made at a steady state of photopigment concentration and at wavelengths longer than 540 nm, bleaching products, rods, and short-wavelength-sensitive cones do not contribute to the matches. Color matches are insensitive to cone coverage, number of cones, and cones that do not send a neural response. We have previously shown[10] that the color-

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matching technique gives similar estimates of the half-bleach illuminance and the regeneration of cone photopigments to retinal densitometry, although there are differences in the bleaching versus retinal illuminance function that may be due to methodology.

B. Retinal Densitometry

A second way to measure cone photopigment optical density is with the use of reflectometry, also called retinal densitometry.[11–17,20–29] Retinal densitometry measures the difference in amounts of light returning from the ocular fundus between two conditions or locations and expresses it as a density difference. The amount of light returning changes as the photopigment bleaches. First the eye is dark adapted to allow as much photopigment as possible to regenerate. Next the eye is illuminated, and the amount of light returning to a detector is recorded. Then the eye is exposed to light bright enough to bleach most of the photopigment, and the measurement is repeated. The logarithm of the ratio of the two measurements is the density difference. We use a specially modified scanning laser ophthalmoscope (SLO) as an imaging retinal densitometer.[17] The SLO has the advantage that at any instant only ~20 mm of retina is illuminated for the field size used (thereby reducing light scattered from other retinal regions.) In addition, it has a confocal design, permitting careful control of the sampling of light returning to the detector from the retina. This includes all light returning to the detector, whether it passed through the cones once (going in or out), twice (going both in and out), or not at all (reflected or scattered back, from structures other than the cones). An optical-density measurement from retinal densitometry is called two-way density or density difference. This distinguishes it from the optical-density estimate obtained by color matching, which is assumed to represent a one-way path through the photoreceptors. Unlike color matching, retinal densitometry can be confounded by scattered light returning to the detector. Scattered light reduces the measured density. Our typical density difference measurement in the fovea ranges from 0.2 to 0.45 log unit,[16,17] depending on the subject, imaging conditions, and wavelength. Retinal densitometry represents the volume of photopigment across the retinal surface that is available to absorb photons of the appropriate wavelength. Thus, in addition to factors that contribute to the color-matching measurements of photopigment density, cone coverage and scattered light are also important for retinal densitometry. Cones that are not sending a neural signal but are absorbing light are included in the measurement of cone-density difference. In retinal densitometry, the subject makes no judgments.

In this paper we investigate foveal organization by comparing the cone photopigment distributions measured by each technique. Features measured with both techniques are assumed to arise from factors that are common to both techniques: the photopigment extinction spectra, path length (including photoreceptor orientation), and photopigment concentration within a cone. Cones not oriented along the optical path produce a lower optical-density measurement.[24] Features that differ between techniqes are assumed to arise from factors that differ in importance and consequences between techniques: cone coverage and stray-light artifacts.

2. METHOD

A. Subjects

The seven subjects tested with the use of both techniques were males with normal color vision, aged 21–55 years. Informed consent was obtained after the nature and possible consequences of the studies were fully explained. Two subjects (3 and 6) are known to have photoreceptors that point more than 2 mm away from the center of the pupil of the eye,[30] which lowers the measured optical density.[31] Nine additional subjects were tested by using only the color-matching technique (4 males and 5 females, aged 26–54 years).

B. Color Matching

For color matching, the apparatus and the method we used were those described previously.[7,8] The subject adjusted the ratio and intensity of 650- and 546-nm lights in one hemifield of a circle to match the other hemifield, which was 589.6-nm light, plus enough 480-nm light to balance the saturation at high retinal illuminances. A 2.1-mm entry pupil, centered in the natural pupil, was used. Pupil entry position was monitored continuously during an experimental session by using an infrared-sensitive monitoring system. Four field sizes (8, 4, 2, and 1 deg) were tested in order, first at low retinal illuminance (3.44 log Td). Then, after at least 3 min of adaptation to a higher retinal illuminance (4.95 log Td), matches were again made in the same order. By working at long wavelengths (>540 nm) and adding a small amount of a 480-nm desaturant, we minimized short-wavelength-sensitive cones and rod contributions to the measurements.

In a control experiment, we examined how the responses from cones at different locations sum over space for color matching. To determine how photoreceptors from each retinal eccentricity (4, 2, 1, or 0.5 deg eccentric from the fovea, corresponding to matches of 8, 4, 2, and 1 deg, respectively) affect color matches, we used annular stimuli to limit the illumination to only a given population of photoreceptors. Two authors, experienced observers with careful fixation, were tested. Annuli had outer diameters of 8, 4, 2, and 1 deg and inner diameters that were selected from 4, 2, and 1 deg. The centers appeared black. The difference between high- and low-illuminance color matches is a measure of cone photopigment optical density.[4,14] Results are plotted as a symmetric function of eccentricity of the outer diameter, which should be viewed as an average rather than implying symmetry.

C. Retinal Densitometry

For reflectometry, the apparatus is a five-laser SLO[17,32] operated in indirect mode. This means that, for each point in the retinal image, an annular aperture optically conjugate to the retina blocks light from the central 200-μm locus and passes light that has been scattered beyond this. The choice of a retinal annulus biases the detection of light toward that scattered in the deeper retinal layers[15–17,26] and thus has passed at least once through the photoreceptor layer. This approach decreases direct specular reflections from the fovea and foveal crest but also decreases the maximum possible measured optical density because it also selects less of the light that passes twice through the cones. The SLO entrance pupil is also

annular, formed by a beam separator near the pupil plane.[17] Light from a laser enters through the central portion, and the returning light is reflected into the detection pathway by the outer portion of the pupil. The center of the subject's pupil is aligned with the center of the SLO entrance pupil. This arrangement rejects specular reflection from the optics and anterior portions of the eye, efficiently collects light from the eye, and is determined by the current optics of our SLO.[17] After pupil dilation, the subject is dark adapted for 15 min. Alignment to the instrument is

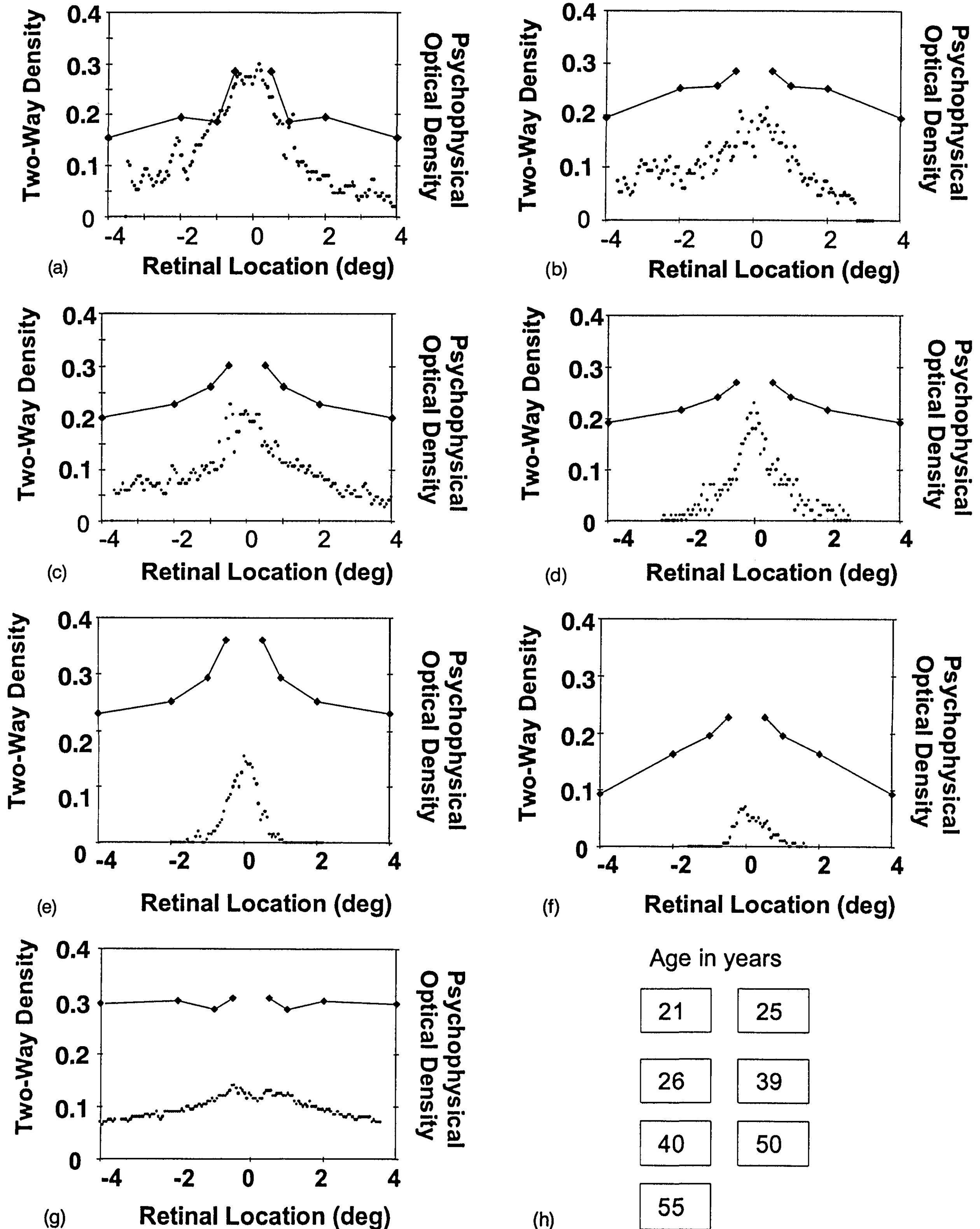

Fig. 1. (a)–(g) Cone photopigment distribution across the horizontal meridia of seven subjects for density difference from reflectometry (two-way density) compared with optical density from color matching (psychophysical optical density). (h) Ages of the subjects. The data sets were truncated for convenience in plotting when the density difference did not differ from zero (d)–(f). Full-density maps are shown in Ref. 17.

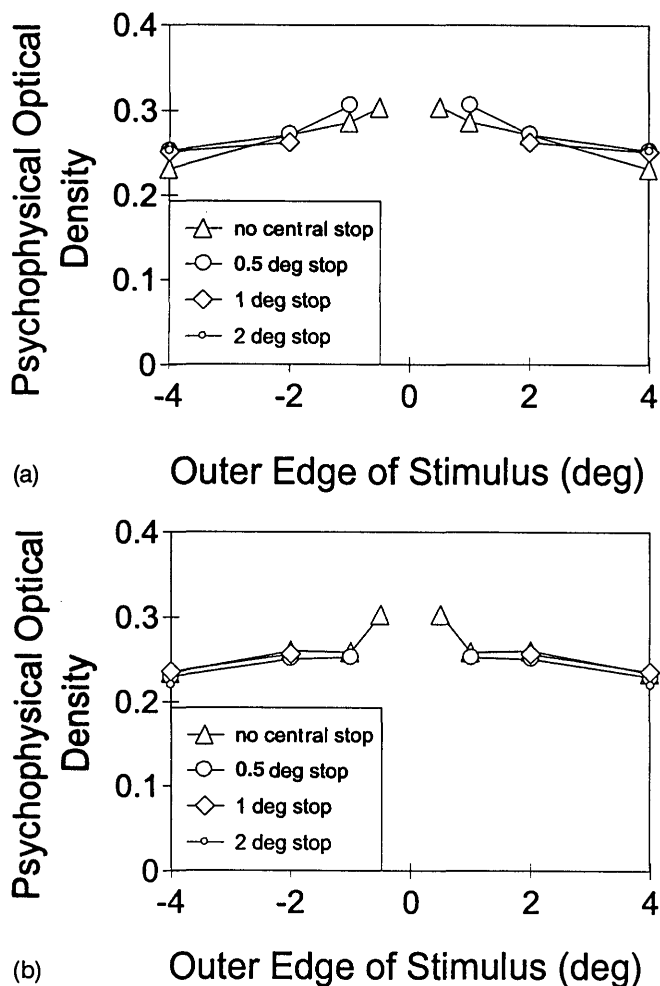

Fig. 2. Psychophysical optical density comparing (a) circular with (b) annular test fields for two subjects. The retinal location for the psychophysical optical density is the outer diameter of the test field. The similarity between the data from test fields with the center black (annulus) and the data from test fields with each half-field a uniform color (circular field) indicates that the color matches are influenced more by the outer portion of the field. The data are duplicated symmetrically about the fovea.

performed with invisible 830-nm light. The subject then views a bright (5.2-log-Td) 594-nm light covering 28.6 × 23 deg. Four images are digitized at video rates at initial exposure, and then additional images are digitized at ~1-min intervals until no further pigment bleaching is observed. Images are 512 × 480 pixels, digitized at 256 linear gray levels. Each pixel represents 3.5-arcmin[5,17,32] visual angle. Density difference, or two-way density, is calculated from an average of two or more selected images for both the dark-adapted condition (initial exposure) and the bleached condition (at least 2 min after exposure). These two images are aligned, and the difference of the logarithms of the intensity at each pixel location is calculated to generate a density difference map of cone photopigment.[17]

3. RESULTS

A. Color Matching

Cone photopigment optical density, as measured by color matching [Fig. 1, where the psychophysical optical density is shown by the diamonds and lines, and Figs. 2 and 3(a)] peaks in a central region and then decreases somewhat with eccentricity. The decrease with eccentricity averages 0.096 for 0.5–1-deg retinal eccentricity or 1–2-deg field size, with large individual differences[6,7,9] [Figs. 3(b) and 3(c)]. The individual differences in the increased density measured for small versus large fields are not related to the optical density of the perifoveal cones [Fig. 3(c)]. That is, the size of the color-match area effect is not an indication of the total density of the cones for normals.

In the control experiment, the optical density estimated from circular and annular stimuli with the same outer diameter agreed to within experimental variability for both observers (Fig. 2). For instance, color matches made with

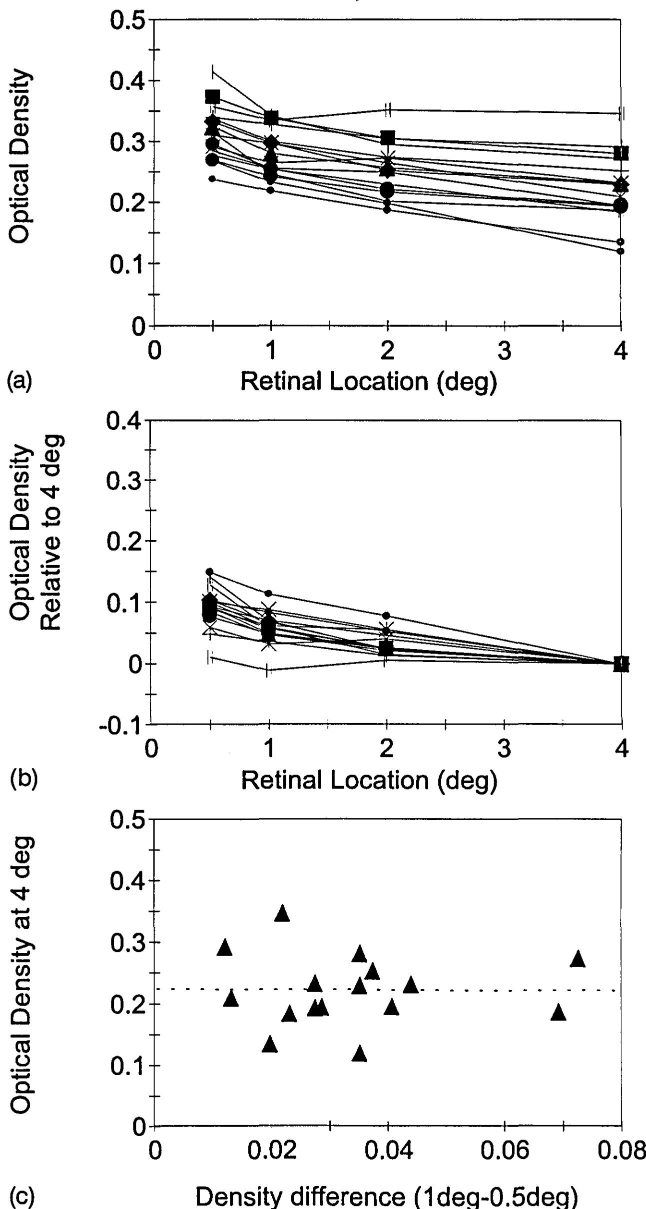

Fig. 3. (a) Dependence of color-matching measurement of optical density on field size for 16 subjects. (b) Size of the color-match area effect relative to matches made with an 8-deg field size (4-deg retinal eccentricity). (c) Change in optical density at the fovea is not dependent on the optical density in the perifovea. The filled triangles compare the optical density measured at 4 deg, by using color matching, with the change in optical density between matches made with a 1- and a 2-deg field ($r = -0.01$).

an 8-deg circular stimulus and an annular stimulus with an outer diameter of 8 deg were similar, even though the center was not illuminated for any annular stimulus.

B. Retinal Densitometry

Cone photopigment optical density, as measured by reflectometry, is highest in a central region and decreases with increasing eccentricity (Fig. 1; see also Ref. 17). For some subjects a horizontal profile through the center indicates that the decrease (Fig. 1, where the two-way density is shown as individual points) has a steep slope; for others (subjects 2 and 7) the decrease is more gradual.[33] For some subjects the density difference in a region near the center of the fovea decreases slightly because of a foveal reflex, which is visible in both the original images and the cone photopigment-density maps. We minimized this reflection by using optical techniques.[17] For most subjects the optical density has dropped to less than half its peak value by 2-deg eccentricity.[34] The data from subjects 4–6 are truncated at eccentricities where the density difference was indistinguishable from noise.[17]

4. DISCUSSION

A. Comparison of Data from Color Matching and Retinal Densitometry

For the central 1 deg of the retina, the two techniques give qualitatively similar estimates of the changes in optical density of cone photopigment with retinal eccentricity, although color matching always gives a higher estimate of the optical density. That is, subjects with a steep density gradient at the fovea as measured by color matching have a steep gradient of two-way density when measured by retinal densitometry (Fig. 4). Farther from the center of the fovea there is a major qualitative difference between the two techniques. With color matching there is always measurable optical density from the fovea out to at least 4-deg retinal eccentricity, and, for most subjects, the color-matching optical density at 4 deg is at least half that measured at the peak. With reflectometry, on the other hand, the optical density at 4 deg is too low to measure for most of our subjects.

There are alternative explanations for the difference in results for the two techniques. One explanation is that reflectometry is affected by the decreased coverage of the retina by cones with increasing retinal eccentricity but that color matching is not. Thus, at increasing retinal eccentricities, the volume of photopigment decreases because the cones shorten and become sparser. Cone length influences both measurements; thus the optical density from both measurements should decrease somewhat with increasing eccentricity. However, the number of cones affects only reflectometry; thus only the density difference measurements should mirror the cone coverage found in anatomical studies.[2,4,35] The more rapid decrease in the density difference measurements of reflectometry as compared with the optical-density measurements of color matching is consistent with this hypothesis.

Another explanation is that the color-matching measurements are so heavily influenced by the central cones that the effects of decreasing cone lengths in the perifovea are minimal. This explanation is ruled out by the annulus experiments. That is, if color matches made with 8-deg stimuli are disproportionately influenced by the extra-long and tightly packed central cones rather than mainly by the shorter cones at 4-deg eccentricity, there should be a large difference between matches made with annular and circular stimuli. There is not. This should not be surprising; although there are fewer cones per unit area in the perifovea, their total numbers are greater because they cover a larger area. Thus the lower optical densities at eccentric locations found with the use of reflectometry are due to decreased cone coverage per unit area on the retina. The optical density of individual cones drops slowly from 1- to 4-deg retinal eccentricity.

B. Comparisons across Individuals

Within 1 deg of the fovea, cone photopigment optical density varies with eccentricity among subjects in qualitatively similar ways for both techniques (Fig. 4). Individuals with a steep foveal density gradient as measured by color matching also have a steep gradient when measured by retinal densitometry ($r = 0.89, P < 0.001$). Artifacts that have plagued reflectometry affect the measurement of absolute optical density but not of density distribution. Reflexes from the foveal pit and crest are an exception because these contribute light to the detector that has not passed through the photoreceptors. Our technique is designed to limit the contribution of these reflexes both by optical means and by visually rejecting areas of images containing a reflex.[17] Also, if these reflexes were dominating our reflectometry measurements, we would expect the two techniques to differ most in the central fovea, where the foveal reflex is strongest. Instead, this is the region in which the agreement is best. The two techniques taken together imply that there are important individual differences in the factors that influence measurements with both techniques. These factors include cone orientation, extinction spectra, and optical density per cone, determined by the product of cone length and photopigment concentration per cone. Cone orienta-

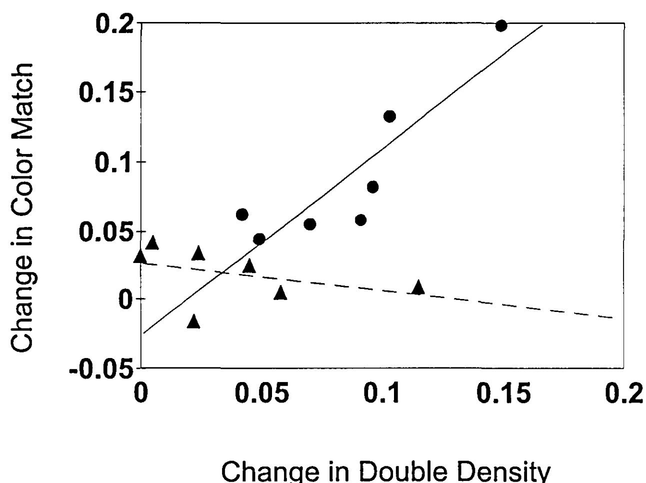

Fig. 4. Change in color match with field size plotted against the change in the reflectometry measurement of double density with field size for the seven subjects. The circles show the change from 0.5 to 1 deg, and the solid line is the best-fit line to these data. The triangles show the change from 1 to 2 deg, and the dashed line is the corresponding best-fit line. There is no significant correlation for the two techniques away from the central 1 deg of the fovea, but they agree well nearer the fovea ($r = 0.89, P < 0.001$).

tion, which affects optical path length through the cones and thus optical density, varied for those of our subjects who were tested,[31,36] with the individual lowest in optical density having cones pointed farther from the entrance pupil than most subjects. This affects absolute optical-density measurements for both techniques, but the orientations would have to differ across the retina to affect the density distribution. Cone photopigment extinction spectra vary among subjects,[31,37,38] which should affect the absolute optical-density measurements for both techniques. However, attributing the optical-density distribution differences to differences across the retina in the photopigment extinction spectra requires that an X-chromosome-linked trait be expressed differently in different parts of a given male retina.[39] Thus the most plausible explanations of the individual variations in optical-density distribution near the foveal center include individual variations in cone length and photopigment concentration per cone, which are one term in the Beer–Lambert law [Eq. (1)]. Given the known increase in cone length at the fovea, this is the most likely source of the individual differences. This individual variation is not simply related to the optical density of the cones (Fig. 4).

Away from the fovea, there is also a large individual variation in density measured by retinal densitometry. This could be age related, although we have found no consistent effect of age on cone optical density measured by color matching,[18] and studies of photoreceptor populations as a function of age suggest that rods are more severely affected than cones.[40] Clearly, ascertaining whether there is evidence for a change in cone coverage with age will require more subjects.

5. CONCLUSIONS

A. Individual variations in optical-density distribution, measured with two separate techniques, indicate important spatial differences between individuals in the first, and limiting, step of vision: the capturing of quanta.

B. Color matching and retinal densitometry are complementary techniques for measuring the distribution of the cone photopigments in the retina. They provide similar results as to the variation of the amount of cone photopigment across the central 1 deg of the retina but give differing results at more peripheral locations.

C. Rayleigh color matches made with a large circular field give the same estimate of optical density as do matches made with an annular field of the same outer diameter.

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REFERENCES AND NOTES

1. R. W. Rodieck, *The Vertebrate Retina* (Freeman, San Francisco, Calif., 1973).
2. G. A. Osterberg, "Topography of the layer of rods and cones in the human retina," Acta Ophthalmol. **13,** Suppl. 6, 1–103 (1935).
3. C. A. Curcio, K. R. Sloan, Jr., O. Packer, A. E. Hendrickson, and R. E. Kalina, "Distribution of cones in human and monkey retina: individual variability and radial asymmetry," Science **236,** 579–582 (1987).
4. C. A. Curcio, K. R. Sloan, R. E. Kalina, and A. E. Hendrickson, "Human photoreceptor topography," J. Comp. Neurol. **292,** 497–523 (1990).
5. D. R. Williams, "Topography of the foveal cone mosaic in the living human eye," Vision Res. **28,** 433–454 (1988).
6. J. Pokorny and V. C. Smith, "The effect of field size on red–green color mixture equations," J. Opt. Soc. Am. **66,** 705–708 (1976).
7. S. A. Burns and A. E. Elsner, "Color matching at high illuminances: the color match area effect and photopigment bleaching," J. Opt. Soc. Am. A **2,** 698–704 (1985).
8. S. A. Burns, A. E. Elsner, L. A. Lobes, Jr., and B. H. Doft, "A psychophysical technique for measuring cone photopigment bleaching," Invest. Ophthalmol. Vis. Sci. **28,** 711–717 (1987).
9. A. Eisner, "Comparisons across age of selected visual functions," in *Colour Vision Deficiencies VIII,* G. Verriest, ed. (Junk, Dordrecht, The Netherlands, 1987), pp. 99–109.
10. S. A. Burns and A. E. Elsner, "Localizing color vision deficiencies in eye disease," in *Colour Vision Deficiencies,* B. Drum and G. Verriest, eds., Documenta Ophthalmologica Proceedings Series (Kluwer, Dordrecht, The Netherlands, 1989), pp. 167–180.
11. P. E. Kilbride and K. M. Keehan, "Visual pigments in the human macula assessed by imaging fundus reflectometry," Appl. Opt. **29,** 1427–1435 (1990).
12. P. E. Kilbride, J. S. Read, G. A. Fishman, and M. Fishman, "Determination of human cone pigment density difference spectra in spatially resolved regions of the fovea," Vision Res. **23,** 1341–1350 (1983).
13. D. van Norren, "Towards improved instrumentation for retinal densitometry," in *Research in Retinitis Pigmentosa,* E. Zrenner, H. Krastel, and H.-H. Boebel, eds., Vol. 62 of Advances in the Biosciences (Pergamon, Oxford, 1987), pp. 177–178.
14. D. van Norren and J. van de Kraats, "Imaging retinal densitometry with a confocal scanning laser ophthalmoscope," Vision Res. **29,** 1825–1830 (1989).
15. A. E. Elsner, S. A. Burns, and R. H. Webb, "Photopigment densitometry with a scanning laser ophthalmoscope," in *Annual Meeting,* Vol. 11 of 1988 OSA Technical Digest Series (Optical Society of America, Washington, D.C., 1988), paper WY3.
16. A. E. Elsner, S. A. Burns, F. C. Delori, and R. H. Webb, "Quantitative reflectometry with the SLO," in *Laser Scanning Ophthalmoscopy and Tomography,* J. E. Nasemann and R. O. W. Burk, eds. (Quintessenz, Munich, 1990), pp. 109–121.
17. A. E. Elsner, S. A. Burns, R. W. Webb, and G. H. Hughes, "Reflectometry with a scanning laser ophthalmoscope," Appl. Opt. **31,** 3697–3710 (1992).
18. A. E. Elsner, L. Berk, S. A. Burns, P. R. Rosenberg, "Aging and human cone photopigments," J. Opt. Soc. Am. A **5,** 2106–2112 (1988).
19. V. C. Smith, J. Pokorny, and S. S. Starr, "Variability of color matching data. I. Interobserver variability in the unit coordinates," Vision Res. **16,** 1087–1094 (1976).
20. H. Ripps and R. A. Weale, "Cone pigments in the normal human fovea," Vision Res. **3,** 531–543 (1963).
21. H. Ripps and R. A. Weale, "Photo-labile changes and the directional sensitivity of the human fovea," J. Physiol. **173,** 57–64 (1964).
22. W. A. H. Rushton and G. H. Henry, "Bleaching and regeneration of cone pigments in man," Vision Res. **8,** 617–631 (1968).
23. M. Alpern, F. Maaseidvaag, and N. Ohba, "The kinetics of cone visual pigments in man," Vision Res. **11,** 539–549 (1971).
24. D. van Norren and J. van der Kraats, "A continuously recording retinal densitometer," Vision Res. **21,** 897–905 (1981).
25. V. C. Smith, J. Pokorny, and D. van Norren, "Densitometric measurement of human cone photopigment kinetics," Vision Res. **23,** 517–524 (1983).
26. A. E. Elsner, S. A. Burns, and R. H. Webb, "Photopigment

concentration in normal and photocoagulated retina," Invest. Ophthalmol. Vis. Sci. Suppl. **30,** 370 (1989).

27. H. D. Baker, R. Henderson, and L. P. O'Keefe, "An improved retinal densitometer: design concepts and experimental applications," Visual Neurosci. **3,** 71–80 (1989).
28. D. van Norren and J. van de Kraats, "Retinal densitometer with the size of a fundus camera," Vision Res. **29,** 369–374 (1989).
29. A. E. Elsner, S. A. Burns, M. R. Kreitz, and R. H. Webb, "Sensitivity maps vs. cone pigment density distribution," Invest. Ophthalmol. Vis. Sci. **31,** 108 (1990).
30. J.-M. Gorrand and F. C. Delori, "A method for assessing the photoreceptor directionality," Invest. Ophthalmol. Vis. Sci. **31,** 425 (1990).
31. S. A. Burns and A. E. Elsner, "Color matching at high illuminances: photopigment optical density and pupil entry," J. Opt. Soc. Am. (to be published).
32. A. Plesch and U. Klingbeil, "Optical characteristics of a scanning laser ophthalmoscope," in *New Methods in Microscopy and Low Light Imaging*, J. E. Wampler, ed., Proc. Soc. Photo-Opt. Instrum. Eng. **1161,** 390–398 (1989).
33. Gradual slopes were found on retest.
34. Higher optical densities are found by us and others with 514 or 543 nm and a circular aperture without a central stop instead of the conditions used in this paper. However, reflection artifacts from the inner limiting membrane, nerve fiber layer, and foveal reflex are typical. The rod contribution must be eliminated by a multiwavelength method, and then the optical-density distribution is calculated by assuming (a) the spectral sensitivities of rods versus cones and (b) the spectral differences in the reflectivity of the fundus.
35. If we assume that the inner segments form the "aperture" for the cones, then the cone coverage probably varies across individuals differently from the cone sampling density. That is, the coverage decreases only when spaces begin to appear between the inner segments. This seems to occur approximately when rods start to appear in the photoreceptor matrix.[2–4]
36. S. A. Burns, A. E. Elsner, J. M. Gorrand, and M. R. Kreitz, "Photopigment variability and pupil entry position," in *Noninvasive Assessment of the Visual System*, Vol. 1 of 1992 Technical Digest Series (Optical Society of America, Washington, D.C., 1992), pp. 160–163.
37. M. Neitz, J. Neitz, and G. H. Jacobs, "Spectral tuning of pigments underlying red–green color vision," Science **252,** 971–974 (1991), have shown that shifts in wavelength of photopigment extinction spectra are due to replacement of nonpolar with hydroxyl-bearing amino acids.
38. A. E. Elsner, S. A. Burns, and J. Waldvogel, "High illuminance color matching in anomalous trichromacy," in *Colour Vision Deficiencies*, B. Drum and G. Verriest, eds. Vol. 62 of Advances in the Biosciences (Junk, The Hague, The Netherlands, 1988), pp. 57–68.
39. Color matches do not vary with field size at high illuminances.[4] If an observer had difficulty with small, high-illuminance color matches, an average of the two larger field sizes was taken.
40. H. Gao and J. G. Hollyfield, "Aging of the human retina," Invest. Ophthalmol. Vis. Sci. **33,** 1–17 (1992).