A Practical Method for Measuring Macular Pigment Optical Density

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Purpose. Increasing evidence indicates that the macular pigments (MP) protect the central retina and may retard macular disease. For that reason, a practical method for measuring MP that does not require elaborate optics and can be applied to diverse populations by operators with a modest amount of experience was developed and validated.

Methods. A small tabletop device based on light-emitting diodes (LEDs) as the light source with electronic controls was constructed. Macular pigment was measured with the tabletop device with a 1° test stimulus at 460 nm using heterochromatic flicker photometry, and the results were compared with measurements using a traditional three-channel Maxwellian view system with a xenon-arc source.

Results. Macular pigment density of 30 subjects (age range, 16–60 years) was measured with both stimulus systems. Macular pigment measured with the LED tabletop device in free view was highly correlated with MP measured in Maxwellian view ($y = -0.03 + 1.06x$, $r = +0.95$). The average absolute difference between the two techniques was 0.04 (SD, 0.03). The new technique was not significantly affected by variations in lens optical density, pupil size, or small head movements.

Conclusions. Psychophysical measurement of MP provides a unique opportunity to make repeated noninvasive assessment of the concentration of a protective nutrient in the retina. The availability of this new device should make this measurement technology accessible to a wide variety of investigators for application to diverse populations. (*Invest Ophthalmol Vis Sci.* 1999;40:2481–2489)

Several epidemiologic studies have found associations between dietary intake or blood concentrations of the carotenoids lutein and zeaxanthin and protection from age-related macular degeneration.[1–4] However, other populations did not show these relationships.[5,6] Although differences between the populations may have contributed to the conflicting results, an important caveat is that all these studies used measures of lutein and zeaxanthin status that may not adequately reflect the concentrations of these pigments within the macular retina itself. Because the relationship between measures of dietary intake and blood concentrations of lutein and zeaxanthin and retinal concentrations of these pigments is weak,[7] using diet and blood values to predict retinal concentrations of these nutrients is not optimal.

It seems likely that lutein and zeaxanthin protect the retina locally.[1] Lutein and zeaxanthin are most dense in the inner retinal layers of the foveal retina and are referred to as the macular pigments (MP) that create the yellow color of the macula lutea. A growing body of evidence indicates that lutein and zeaxanthin (as measured in the macula) protect the retina from oxidative damage that accrues with age.[8,9] This protection may be mediated through passive filtering of short-wave light[10] or actively by quenching photosensitizers or reactive oxygen species.[1] Evidence supporting a protective role for MP comes largely from experimental data on nondiseased subjects (reviewed in Ref. 9; a noted exception is data by Landrum et al.[11]). To date, no epidemiologic data are available on the relationship between retinal concentrations of lutein and zeaxanthin and risk of age-related macular degeneration.

The lack of clinical data on this relationship is partially due to difficulties in measuring MP in vivo. Macular pigment has traditionally been measured using complex optical systems.[12] These optical systems require extensive training to operate and cannot be easily moved from the laboratory. Thus, most studies on MP have been conducted in university laboratories using populations composed of students and faculty rather than samples drawn from the general public. To obtain data from a wider more representative sample, a simple, more accessible method of measurement was needed.

In the present article, we describe a simplified device for measuring MP optical density that can be applied to diverse populations. This device implements noninvasive procedures that are similar to past studies (see review in Ref. 12) but uses a design that is less expensive, physically robust, and easy to use. Furthermore, it is more comfortable for the subject because the stimulus is presented in free view and the subject does not need a bite-bar for head stabilization.

As a first step in validating the new device, we compared MP density measured using a traditional Maxwellian view optical system with MP density measured with the new device. Measurement of MP with the Maxwellian view system has been validated previously by varying the test wavelength to derive

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Submitted for publication January 7, 1999; revised May 18, 1999; accepted June 3, 1999.

Commercial relationships policy: P.

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the MP density spectrum and demonstrating that it is nearly identical to the MP measured ex vivo.[13]

METHODS

Measurement of MP Optical Density

Subjects. Macular pigment density of 14 men and 16 women (age range, 16–60 years) was measured using the two different techniques. The ordering of the measurements was counterbalanced to avoid possible order effects. Twenty-six of the subjects in our sample had never participated in a psychophysical task before this study and were experimentally naive. The remaining four subjects were experienced in psychophysical tasks and were aware of the purpose of the study. Because this study involved direct comparisons between two methods, no exclusion criteria were used in sample selection. Informed consent was obtained from all subjects, and the tenets within the Declaration of Helsinki were followed.

Procedure. All measurements were made with the right eye only. Macular pigment density is highly similar in the left and right eyes.[13] We selected the right eye to maintain consistency with past studies, but the device can be used to measure the MP density of either eye with only minimal adjustments. The procedure for measuring MP was the same whether measured with the conventional Maxwellian view system or with the new device. For an expanded discussion of the procedure see Snodderly and Hammond.[12] In brief, visual sensitivity was measured using a test wavelength that is maximally absorbed by MP, 460 nm, and a reference wavelength that is not absorbed by MP, 550 or 570 nm. These measurements were made at a retinal locus where MP is most dense, the center of the fovea, and in an area where MP density is minimal, 4° or 6° in the temporal retina. Sensitivity was measured using flicker photometry, which presented the two test stimuli in temporal square wave alternation at 12 to 15 Hz for the foveal condition, and 6 to 7 Hz for the parafoveal condition. Temporal resolution for small stimuli presented as described above is higher in the fovea than in the parafovea[14] and therefore requires the flicker rate to be lowered when making parafoveal measurements. If a range of test wavelengths is used, this procedure for measuring MP in vivo yields an optical density spectrum for the pigments that matches the extinction spectrum of MP measured ex vivo.[13]

Maxwellian View Measurement

A conventional three-channel Maxwellian view system with a 1000-W xenon arc light source (power source: Raytheon, Lexington, MA; housing: Kratos Analytical, Ramsey, NJ) was used for the measurements. A schematic of this system is shown in Figure 1. The exit pupil of the system was 2 mm. One channel provided a 460-nm background field, and two other channels were combined to produce the flickering measuring stimulus. The second channel provided the test wavelength, the intensity of which was adjusted by the subject via a 2.0–log unit circular neutral density wedge. The third channel provided the reference wavelength, the intensity and wavelength composition was constant. Light from the second and third channels was presented in square wave alternation for the purpose of flicker photometry. The alternation was accomplished by using a sectored first-surface mirror rotated by a highly regulated Bodine motor (Electro Sales, Somerville, MA). The wavelength of the test field was produced by a grating monochromator with a nominal half bandwidth of 7 nm (model H-20; Instruments SA, Metuchen, NJ); blocking filters eliminated stray light and higher-order spectra. The wavelength of the background and the reference fields was produced by Ditric Optics interference filters (half-bandpass = 7 nm). Subjects were positioned for Maxwellian view by using an auxiliary pupil viewer (made with a comparator/reticle used in conjunction with a beam splitter placed immediately before the final focusing lens). Stabilization of the head was maintained by use of an adjustable dental impression bite-bar and headrest assembly.

The circular 1° measuring stimulus alternated between a 2.7-log Troland (Td), 550-nm reference field, and a 460-nm test field. The intensity of the test field was adjusted by the subject. The circular measuring field was presented near the center of a 10°, 2.5-log Td, 460-nm background. A small black dot placed on a slide transparency in the background channel provided a fixation point on the edge of the background in the nasal visual field. This stimulus configuration is shown in Figure 2. To make the foveal setting, the subject looked directly at the small measuring field. For the parafoveal setting, the subject looked at the fixation point.

Measurement with the New Tabletop Device

Figure 3 is a diagram of the optical system that we used to measure MP density in free view. The light for the background is provided by a source (S1) that consists of three LEDs with peak energy at approximately 470 nm and half-widths of approximately 20 nm. For the sake of simplicity, S1 is diagrammed as a linear array in Figure 3. In fact, S1 was constructed by packing the three LEDs (3 mm in diameter) as tightly as possible in a triangular array. This was done by first grinding off the lens of each LED and then embedding them within a brass tube with an inner diameter of 0.258 inches filled with an epoxy resin. After curing, the front surface of the resin was ground flat and polished.

Light from S1 was collimated with a planoconvex lens (L1, 10-cm focal length). A 1.75-inch circular aperture (A1), which defined the 6° background field, was located approximately 2 inches beyond L1 at a position where collimated light from the three LEDs overlapped. A1 was constructed by exposing high-density photographic mylar film with a computer-generated image of the aperture. The mylar film was then affixed with optical grade glue to the smooth side of a diffuser, D1. The polycarbonate diffuser is a high-efficiency holographic type (Physical Optics Corporation, Torrance, CA) with a circular diffuser angle of 20°. A1 was viewed by the subject reflected through a beam splitter (BS) and appeared evenly filled with the light transmitted through the diffuser. The subject's eye was located approximately 16 inches from the front surface of the beam splitter.

Light from S2 was collimated with a planoconvex lens (L2, 10-cm focal length). S2 was composed of two LEDs with peak wavelengths of 458 nm and one LED with a peak at 570 nm (half-bandwidths of 20 nm). Construction was as for S1. A 0.3-inch aperture (A2), defining the 1° measuring field was placed as for L1. The construction and composition of the aperture-diffuser sandwich were identical to that of the S1 channel. The subject viewed A2 directly through the beam splitter, which combined the two beams.

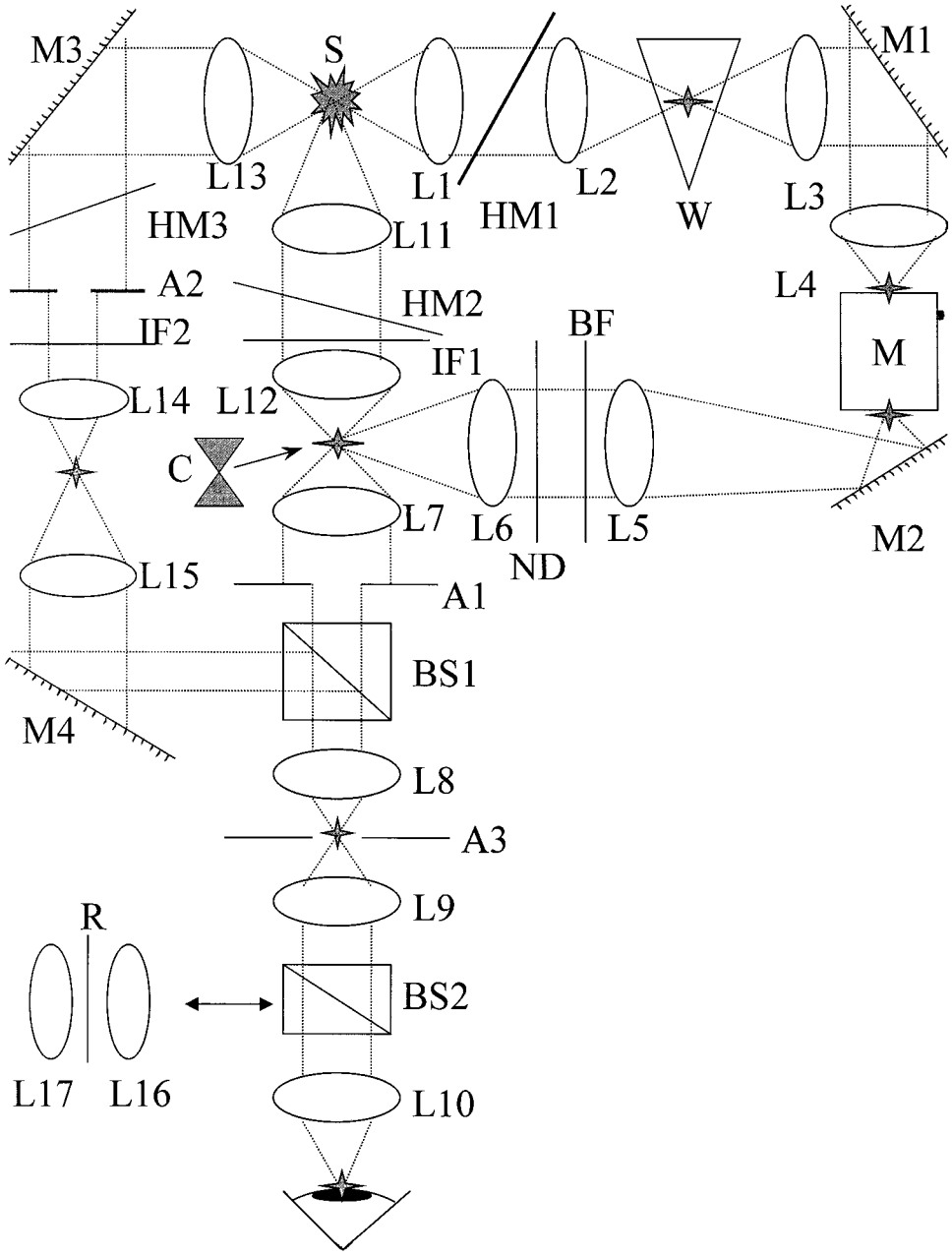

FIGURE 1. A schematic of the optical system used to measure MP optical density in Maxwellian view. A1 through A3, Apertures 1 through 3; BF, blocking filters remove extraneous spectra from prism used within M; BS1 and BS2, Beam splitters 1 and 2; C, flicker vanes with a first surface mirror used for alternating the standard and measuring stimulus; H1 through H3, hot mirrors used to reduce heat transmission; IF1 and IF2, interference filters (used in conjunction with neutral density filters, ND1 through ND3) render the standard and background stimulus monochromatic; L1 through L17, planoconvex achromatic lenses; M, monochromator renders the measuring light monochromatic; M1 through M4; right angle, first surface mirrors; R, Reticle; S, xenon arc light source; W, Wedge.

The entire optical system was enclosed within an opaque, Plexiglas box. The subject peered into the system through a 1-inch circular hole (H) that was centered on the optical axis. When properly positioned, the subject saw the 1° target superimposed on the 6° background with the slightly larger and out-of-focus edge of the hole (H) concentric with the background. A tiny (5-minute) opaque spot was located on the extreme left side of the background to serve as a fixation point for the parafoveal measuring condition. Another spot (also 5 minute) was located in the center of the target to serve as a fixation point for the foveal condition.

The configuration of the stimulus is illustrated in Figure 2. The 1° target was located within the background such that its center was displaced 4° from the fixation point located on the extreme left. A photocell (model PIN-10; UDT Sensors, Hawthorne, CA) was used to measure the relative radiance of the target and background.

Because of the 20° diffusing angle, head position was not critical. The subject was merely instructed to make sure that the viewing hole was concentric with the background. A chin and forehead rest were sufficient to help the subject maintain position, and a bite bar was unnecessary.

Calibration and Stimulus Control

In preliminary tests we found that the peak spectral energy of individual LEDs varies considerably within a category defined as the manufacturer's catalog number. We chose each LED with the desired spectral energy distribution in mind. For the shortwave component of the measuring field we wanted peak energy to be within 2 nm of 460 nm, which is close to the peak

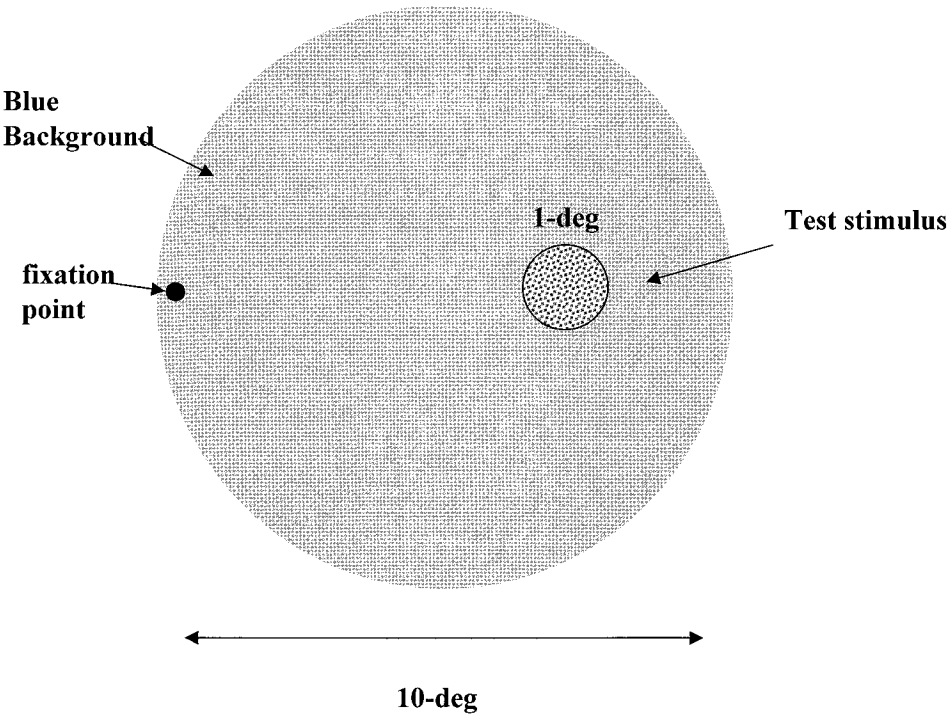

FIGURE 2. A schematic of the stimulus configuration that was used for measuring MP optical density in free and Maxwellian views.

absorption of MP. The long-wave component of the measuring field is less critical because it only needs to be outside the main absorption band of MP (greater than 520 nm) and of reasonable luminance. We chose 570 nm for that value. For the peak energy of the background, we chose a value of 475 nm as the best compromise, considering such factors as luminous efficiency and the spectral absorption of rods and shortwave cones. We decided to use a maximum of three LEDs for each source, because a triangular packing gave a good compromise between maximum radiance and compactness. Thus, we used three LEDs (model NSPB300A; Nichia, Mountville, PA) for S1, each with peak wavelength near 470 nm. For the measuring source, S2, we used two LEDs (Nichia Corp., Model NSPB300A) with peak wavelengths near 460 nm, leaving the third position for the LED peaking at 570 nm. We should emphasize that by combining both measuring lights into one compact source, the necessity of combining them with a light-losing beam splitter is avoided.

The stimuli were calibrated by placing a spectroradiometer-photometer (model 650; Photo Research Inc., Chatsworth, CA) at the position of the subject's eye. Figure 4 shows the relative spectral energy of the background (squares) and two measuring components (diamonds for the shortwave components; triangles for the long-wave component). The radiance of the background was set at 1.5-log Tds, the highest value that allowed a good adjustment range for the small superimposed measuring field. The 570-nm reference wavelength was set at 1.7-log Tds, the highest value that allowed a wide range of settings for the 460-nm test wavelength. The radiance of the 460-nm test wavelength is adjusted by the subject to minimize flicker (i.e., it is the dependent variable).

The LEDs are driven by constant current supplies. Radiance is controlled by delivering brief (1.5 μsec), rectangular pulses at a rate that can be varied from approximately 300 to 300,000 Hz. The pulse rate for each LED is individually adjust-

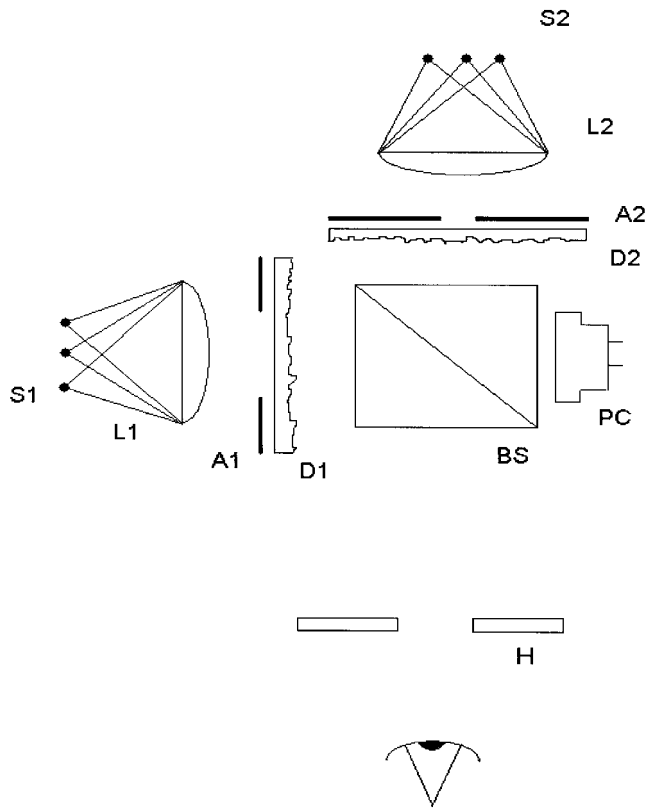

FIGURE 3. A schematic of the optical system used to measure MP optical density in free view. A1 and A2, Apertures 1 and 2; BS, beam splitter; L1 and L2, planoconvex achromatic lenses; PC, photocell; H, hot mirror used to reduce heat transmission; S1 and S2, light sources; D1 and D2, optical diffusers.

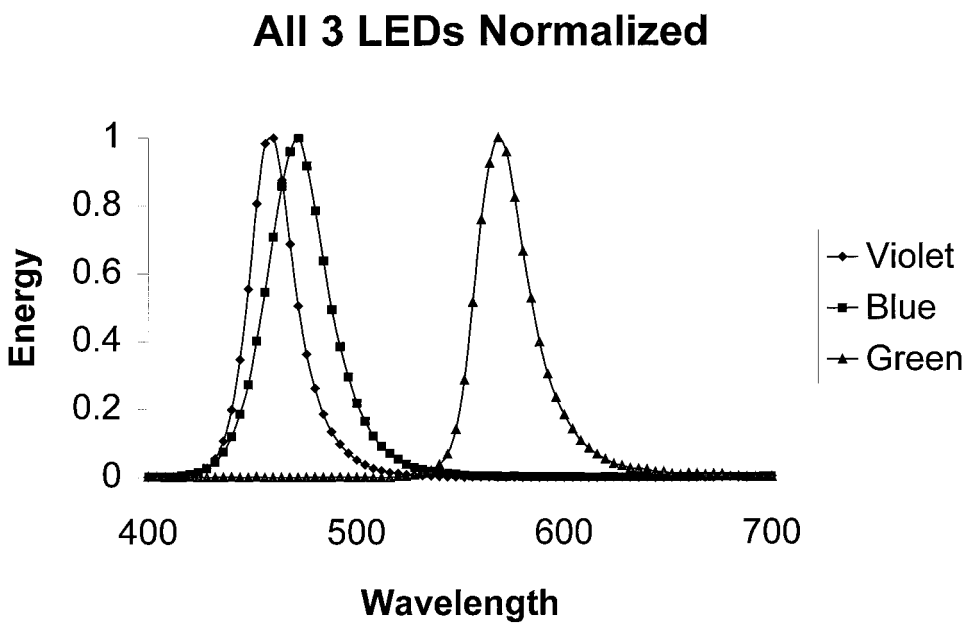

FIGURE 4. The relative spectral energy curve of the LEDs that were used to create the background (*squares*), measuring (*diamonds*), and reference (*triangles*) fields for the measurement of MP with the tabletop device.

able, with the radiance levels being monitored by a photocell and the relative values displayed on a digital display. Thus, the predetermined radiance values of each field can be precisely set at the beginning of each experimental session. In addition, the radiance of the 460-nm measuring beam can be varied by a current adjustment that allows pulse frequency values to correspond to absolute radiance values across experimental sessions.

There is a major advantage to using pulse frequency to control radiance in the particular way that we measure MP density. Our calculation of the pigments' optical density (OD) is simple, as shown below:

$$\text{O.D.} = \text{Log10}\ R_f/R_p$$

where R_f and R_p are simply the radiances of the 460-nm test beam that results in minimum flicker with respect to the 570-nm reference at the foveal and parafoveal loci, respectively. Because the pulse frequency should be proportional to the radiance, with a slope of 1.0 and origin of 0.0, it follows that:

$$\text{MP O.D.} = \text{Log10}\ F_f/F_p$$

where F_f and F_p refer to the frequency of the 460-nm test beam at the foveal and parafoveal positions, respectively. We tested this conclusion by placing a spectroradiometer at the eye's position and measuring the actual integrated radiance as pulse frequency was varied over a large range. The range of energies we explored was a conservative estimate of a good working region for the measurement of MP. Figure 5 shows that log energy ratio of the radiance values plotted against the log frequency ratio of the corresponding pulse frequency values in the range of adjustment needed to make MP measurements. Notice that the data points fall very close to the straight line, which has a slope of 1.0 and an intercept of 0.0. The conclusion is that frequency ratios can substitute for radiance ratios. This greatly simplifies the use of the instrument because pulse frequency is easily determined with inexpensive and accurate electronic devices, whereas an accurate energy calibration requires a fairly expensive and elaborate analogue device. The use of pulse frequency is a major simplifying aspect of the device.

Measurement of Lens Optical Density

For a subset of subjects (n = 10), we also measured lens optical density (see Table 1). For a discussion of the technique, see the recent chapter by Snodderly and Hammond (1999).[12] Scotopic thresholds were obtained using two channels of the Maxwellian view system. One channel provided a dim blue 20-minute fixation point. A second channel provided a 2.8° test stimulus. The test stimulus was either a 410-nm (high lens absorbance) or 550 nm (minimal lens absorbance[14]) light. These wavelengths were selected because of equal absorption values on the rhodopsin curve.[15] Lens optical density was calculated by directly subtracting the log relative sensitivity value at 410 nm from the log relative sensitivity value at 550 nm without referring to the rhodopsin curve itself. For a discussion of the rationale behind using the "balanced rhodopsin" technique see the original description by van Norren and Vos (1974).[16] Test field exposures were determined by a Uniblitz electromagnetic shutter (Rochester, NY). The test stimulus was always presented at 6° in the nasal visual field. Scotopic thresholds were obtained after subjects were dark adapted for 40 minutes. For a discussion of the procedure used for measuring these absolute thresholds see Hammond et al. (1997)[17] and Snodderly and Hammond.[12]

RESULTS

Macular pigment optical densities measured with the Maxwellian system and the free-viewing LED tabletop device are presented in Table 1. As shown in Figure 6, the two methods provide highly similar individual and mean values and are

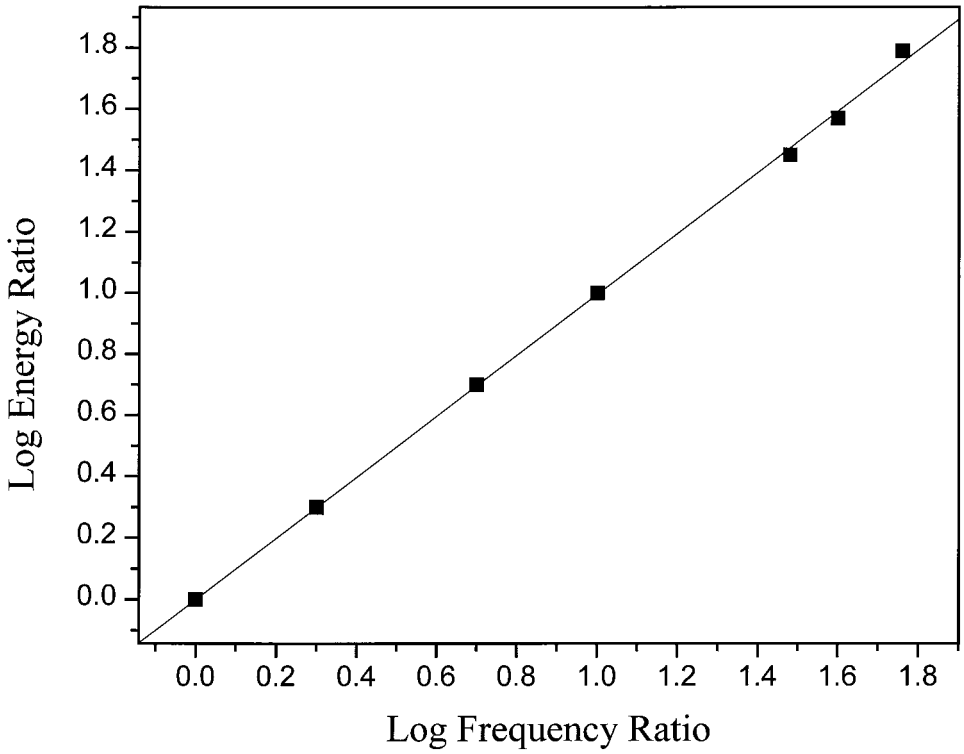

FIGURE 5. A comparison of the log frequency values of the pulses driving the LEDs with the measured log ratio energy output produced by these changes in frequency.

highly correlated ($y = -0.03 + 1.06x$, $r = +0.95$). Note that the intercept of the line is near 0 and the slope is near 1, showing that there is no systematic difference between the two techniques. In fact, the average absolute change in these values is lower (mean = 0.04; SD = 0.03) than the differences reported for studies that obtained repeated measures using a single method on different days.[18] To obtain a reliability estimate for MP measured with the new device, the MP density of four subjects was measured 10 times over a period of 2 to 4 weeks. An analysis indicated that the data were reliable (Cronbach's $\alpha = 0.89$). This level of reliability is comparable to that obtained measuring MP on different days using naive subjects but Maxwellian view optics (e.g., Cronbach's $\alpha = 0.85$[13] and 0.68[19]).

As shown in Table 1, of the subjects whose lens density was measured, differences between the MP values obtained with the two techniques were not related to individual differences in lens density. The lack of influence of lens optical density on the MP measures is further indicated by the observation that the difference in MP measured with the two techniques is unrelated to age ($r = -0.02$). If one method was more influenced by lens optical density, then one would expect the differences in measured MP to increase with age because lens optical density increases with age.

To confirm that differences in retinal illuminance associated with differences in lens optical density should have little effect, we conducted a small control experiment varying retinal illuminance. To this end, we used the LED tabletop device to measure MP density while changing the radiance of the background field in 0.25 intervals over a 1-log unit range. For the three subjects that were tested, MP density averaged 0.60 ± 0.14, 0.61 ± 0.14, 0.55 ± 0.17, 0.61 ± 0.21, and 0.61 ± 0.25, respectively. Thus, in effect, changing the background from dim (simulating a dense lens) to bright (simulating a clearer lens) does not significantly change the measured MP values.

For two subjects, we also tested the effects of pupil size by measuring MP with the LED tabletop device before and after pupil dilation with a mydriatic. The MP values when measured with nondilated pupils (0.16 and 0.42) were very similar to the values obtained during dilation (0.11 and 0.43, respectively).

We also tested the effects of head movement on the MP values of two subjects, using the tabletop device. The limiting factor in the lateral direction is the ability to see the stimulus. A subject can only move approximately 1.5 cm to the right or left before the stimulus is occluded by baffling. However, when subjects were misaligned to the allowable limit, no differences were found in their MP values (range of differences = 0.02). In the Z direction, subjects can move at least 10 cm forward or backward without any change in their MP values (range of differences = 0.04).

Individual differences in the average MP density of the individuals in this small sample tended to be consistent with our past observations on determinants of individual differences in MP density in different populations. For example, the average MP of the women (mean = 0.21, SD = 0.123, $n = 16$) was lower than the average MP of the men (mean = 0.30, SD = 0.20, $n = 14$). The average MP of the smokers (mean = 0.215, SD = 0.24, $n = 4$) was lower than the MP density of the nonsmokers (mean = 0.26, SD = 0.13, $n = 26$). Finally, the MP of the blue-eyed subjects (mean = 0.199, SD = 0.139, $n = 7$) was lower than the MP of the green/hazel-eyed subjects (mean = 0.29, SD = 0.21, $n = 5$) or the brown/black-eyed subjects (mean = 0.35, SD = 0.14, $n = 16$). The sample sizes of these groups were too small to assess the statistical significance of these differences, but the trends indicate that this population is representative of other groups whose MP has been studied.

TABLE 1. Descriptive Statistics

Subject	Iris Color	Age	Sex	MPOD-free	MPOD-Maxwell	OD Change	Smoking Status	Lens OD
LJ	Brown	46	M	0.34	0.26	0.08	NS	
PJ	Black	23	M	0.39	0.46	−0.07	NS	
AT	Blue	24	F	0.34	0.46	−0.12	NS	
RC	Brown	30	F	0.42	0.505	−0.09	NS	1.69
HJ	Blue	22	M	0.32	0.38	−.06	NS	1.5
ML	Black	48	F	0.39	0.32	0.07	NS	
HC	Brown	19	M	0.11	0.02	0.09	NS	
WA	Brown	25	M	0.46	0.47	−0.01	NS	1.63
EA	Brown	31	F	0.07	0	0.07	NS	1.37
ZL	Black	30	M	0.47	0.41	0.06	PS	
VM	Brown	23	F	0.314	0.25	0.06	NS	1.51
VN	Brown	16	F	0.27	0.22	0.05	NS	
RC	Brown	41	F	0.219	0.178	0.04	PS	
HR	Green	34	M	0.43	0.42	0.01	NS	1.65
LM	Brown	30	M	0.223	0.2	0.02	NS	1.46
GA	Blue	31	M	0.103	0.03	0.07	NS	
SJ	Blue	28	M	0.19	0.15	0.04	NS	
TM	Brown	23	F	0.15	0.13	0.02	CS	
BB	Blue	58	F	0.222	0.214	0.01	NS	
KM	Brown	28	M	0.164	0.111	0.05	NS	
RB	Blue	33	M	0	0.03	−0.03	CS	
TC	Brown	51	F	0.188	0.142	0.04	PS	
MM	Hazel	49	F	0.292	0.267	0.03	PS	
WB	Brown	56	M	0.595	0.594	0	NS	
RR	Brown	29	M	0.59	0.55	0.04	CS	1.71
WA	Green	51	F	0.24	0.3	−0.06	NS	1.64
AT	Brown	29	F	0.02	0	0.02	NS	
SS	Blue	20	F	0.17	0.19	−0.02	NS	1.1
BJ	Hazel	21	F	0.12	0.12	0	NS	
VJ	Green	60	F	0.141	0.138	0.00	NS	
Mean ± SD		33.6±12.7		0.265±0.15	0.251±0.17	0.01±0.05		

CS, current smokers; NS, never smokers; PS, past smokers; MPOD, macular pigment optical density.

DISCUSSION

Past techniques for measuring MP in vivo include fundus reflectometry,[20,21] fluorophotometry,[22,23] and psychophysical methods using Maxwellian View Optics.[17–19,24–30] Of these techniques, the psychophysical technique has been used the most often and provides reliable data.[18] Nonetheless, a limited number of laboratories have Maxwellian View systems, and skilled operators are necessary to obtain reliable data. Moreover, these systems are not typically mobile and therefore not amenable to field study. In this report we have described the development of a technique for measuring MP in vivo that is easy to use, is relatively inexpensive, and can therefore be more widely disseminated.

The major difference between stimuli viewed in Maxwellian view and free view, is that in the Maxwellian view the stimuli enter the eye as a narrow pencil-shaped beam centered in the pupil. In contrast, free view utilizes the whole area of the pupil. Thus, an advantage of Maxwellian view optics is that variations in pupil size have no effect on the final retinal illuminance because the exit pupil of the optical system is smaller than the smallest diameter that can be obtained by the pupil. Moreover, free view cannot obtain the higher retinal illuminance levels obtainable with Maxwellian optics.

In this study we tested whether measurements of MP were affected by variations in pupil size or retinal illuminance by comparing MP measured using Maxwellian view optics with MP measured using a free viewing situation. Subjects of different ages and lens optical densities were tested. As shown in Table 1, a high correlation was found between MP measured using the different techniques, and no systematic differences were found between the two techniques. In fact, variation across the two techniques is equal to what would be expected given the across-session variance using the Maxwellian view system alone.[18] The similarity in the MP values derived from the two systems underscores the basic hardiness of this psychophysical procedure for measuring MP. For example, the test stimulus in Maxwellian view was composed of wavelengths with a narrower band-pass (7 nm as opposed to 20 nm) and higher retinal illuminance, which were referenced to a peripheral point located at 6° as opposed to 4°. The similarity in the derived values indicates that a wide number of conditions can be altered without affecting the ultimate reliability of the measurements. This suggests that we may be able to use the method on a variety of subjects under varying conditions.

A simple method of measuring MP is useful for a variety of applications. As outlined in the introduction, measuring MP would provide a direct assessment of the relationship between

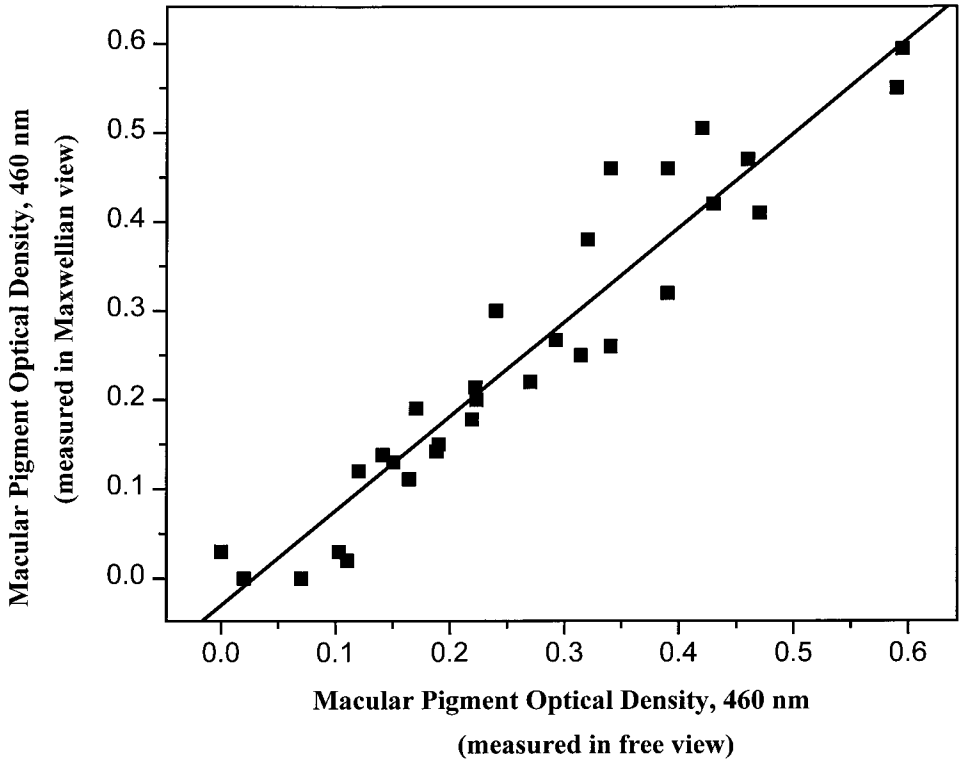

FIGURE 6. The relationship between MP optical density measured in Maxwellian view and MP density measured with the tabletop LED device in free view.

local concentrations of lutein and zeaxanthin and macular disease. Before this assessment is made, however, future work must focus on validating the psychophysical method of measuring MP on patients with eye disease. Our preliminary investigations (unpublished) indicate that even individuals with cataractous lenses can perform the task, but no information is currently available showing what types of patients with retinal pathology can perform the task, and whether data from patients will be valid measures of MP. Currently, the most convincing validation is to show that it is possible, using the task, to derive an optical density spectrum for MP that matches the extinction spectrum of MP measured ex vivo.[13] For rigorous application of the psychophysical methodology to patients, it will be necessary to repeat the validation experiments that have previously been performed with normal subjects using carefully characterized clinical populations.

Measuring an individual's MP density over time with our methodology is practical due to the nondestructive nature of the measurement. Unlike other techniques for measuring tissue concentrations of a nutrient (e.g., adipose biopsy), our technique is noninvasive and therefore does not alter the tissue itself. Thus, repeated measurement of the concentrations of lutein and zeaxanthin in the retina can be made. Tissue measures of this type would be a significant addition to the repertoire of techniques available to nutritional scientists studying the metabolism of nutrients within the body. The ability to obtain repeated measures of the macular pigments also allows for the assessment of interventions without the added variability of measuring different biopsies of tissue. Finally, to the extent that this technology can be made widely available, clinicians may be interested in adding this measurement to their usual assessment procedures. Accurate information regarding the nutritional status of the retina is of interest to patients with early signs of disease but also to nondiseased individuals concerned with maintaining their health.

Acknowledgments

The authors thank Robert K. Moore for designing the electronics and Ken DeLucia for technical assistance.

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