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Stimulus edge effects in the measurement of macular pigment using heterochromatic flicker photometry

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Abstract. Heterochromatic flicker photometry (HFP) is the most common technique of measuring macular pigment optical density (MPOD). Some data strongly suggest that HFP samples MPOD specifically at the edge of center-fixated circular stimuli. Other data have led to the conclusion that HFP samples over the entire area of the stimulus. To resolve this disparity, MPOD was measured using HFP and a series of solid discs of varying radii (0.25 to 2.0 deg) and with thin annuli corresponding to the edge of those discs. MPOD assessed with the two methods yielded excellent correspondence and linearity: $Y = 0.01 + 0.98X$, $r = 0.96$. A second set of experiments showed that if a disc stimulus is adjusted for no-flicker (the standard procedure) and simply reduced in size, no flicker is observed despite the higher level of MPOD in the smaller area. Taken together, these results confirm that MPOD is determined at the edge of the measuring stimulus when using stimulus sizes in the range that is in dispute (up to a radius of 0.75 deg). The basis for this edge effect can be explained by quantitative differences in the spatial-temporal properties of the visual field as a function of angular distance from the fixation point. [DOI: 10.1117/1.JBO.20.11.115004]

Keywords: macular pigment; heterochromatic flicker photometry; edge effect.

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1 Introduction

Wald[1] first identified human macular pigment as a xanthophyllic carotenoid by showing that its absorption spectrum matched that of known xanthophylls. Approximately 40 years later, Bone et al.[2] definitively identified the components of the macular pigment as the specific xanthophylls (3R,3'R,6'R)-lutein (L), (3R,3'R)-zeaxanthin (Z), and (3R,3'S)-meso-zeaxanthin (M). The study of the macular carotenoids is of interest to both basic and clinical scientists because of its many putative effects on visual and cognitive function and its value as a biomarker.[3] Hence, there is much interest in accurate measurement of the pigments within the living eye.

Numerous methods have been developed for *in vivo* quantitative measurement of levels of macular pigment (MP), usually expressed in units of optical density (OD). Some are purely physical, e.g., fundus reflectance, lipofuscin fluorescence, and Raman spectroscopy. Others are psychophysical, e.g., color matching, motion photometry, detection threshold, and heterochromatic flicker photometry (HFP). Each method has its strengths and weaknesses related to ease of use, assumptions, efficiency, reliability, confounds, etc.[4] The purely physical methods tend to require complex instrumentation, intense lights, and pupillary dilation. The psychophysical methods are relatively simple and do not require intense lights or pupillary dilation, but do rely upon some kind of sensory-motor response. All, save one (Raman spectroscopy), employ normalization to a parafoveal locus where MP is known to be optically undetectable, thereby removing lens absorption and scatter as confounds.

The most commonly used method of measuring MP is HFP. It shares with the other psychophysical procedures simplicity, efficiency, relatively low cost, reliability, and, of course, a sensory-motor response. There is, however, one reported property that sets it apart from the other psychophysical and physical techniques: there is evidence showing that the MPOD values refer to the edge of center-fixated circular stimuli, at least for targets smaller than a diameter of 1.5 deg (where the issue has been examined). Whether or not this edge effect obtains with larger HFP stimuli, or for the other psychophysical methods, has not been examined. In contrast, the physical methods implicitly or explicitly assume that the derived values represent the simple sum over the volume of MP defined by the geometry of the target. Although this assumption may be true for some physical methods, it has never been experimentally tested. In fact, it is clearly not true for the Raman method since the backscattered signal is nonlinear for MPOD values $> \sim 0.30$;[5] i.e., the deeper layers of pigment are screened by the shallower layers. A potential strength, then, of the HFP method is that the MPOD value has a known retinal locus with reference to the fixated center of the circular target. Various loci can be sampled up to the limit of the edge effect, thus allowing the distribution of the MP to be defined. More peripheral loci can be evaluated either with smaller probes or thin annular targets. Reliance on the edge effect does, of course, depend upon it being generally true. Work by Bone et al.[6] (hereafter referred to as Bone) has questioned its validity.

The edge effect, within the context of the HFP measurement of MPOD, was first suggested in print by Werner et al.[7] The first experimental test was by Hammond et al.[8] They determined MPOD in 30 observers using a 1-deg-diameter centrally fixated disc and a 12-min spot positioned at 30 min from a fixation point. The edge effect would predict that the two measurements

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would be identical within experimental error. The mean values for the disc and spot measurements were 0.38 ± 0.25 SD and 0.34 ± 0.26 SD, respectively. A scatter plot (spot versus disc) yielded a regression equation of $Y = 0.89X + 0.08$ with $R = +0.91$. In addition, they measured MPOD for four subjects using a 1-deg disc and a 12-min-wide annulus concentric with the disc. The values were 0.44 ± 0.16 SD and 0.43 ± 0.14 SD for the disc and annulus, respectively. The authors concluded that the edge effect holds for a 1-deg disc. Data (less completely reported) for two subjects suggested validity up to a disc diameter of 3 deg. A later study by Hammond and Caruso-Avery[9] examined 171 subjects using 2-deg disc and annulus stimuli; they found MPOD values of 0.13 ± 0.10 SD and 0.10 ± 0.09 SD, respectively. In view of the small difference, they concluded support for the edge effect. A re-analysis of their data shows that the difference of 0.03 was statistically significant, which is perhaps not surprising in view of the large sample size, but 0.03 may not be theoretically or practically significant. In summary, the paper of Hammond et al.[8] strongly supports the edge hypothesis for 1-deg discs, while the one by Hammond and Caruso-Avery[9] less definitively supports it for 2-deg discs. The Bone study,[6] however, challenged these conclusions.

Bone utilized an HFP technique to measure MPOD in both the left and right eyes of 10 observers. They employed stimuli and procedures similar (but not identical) to those of Hammond et al.[8] or Hammond and Caruso-Avery.[9] In one experiment, they compared MPOD results derived from a disc of 1.17-deg diameter with an annulus of 0.92- and 1.17-deg inner and outer diameter, respectively. The edge effect would, of course, predict nearly identical values. Instead, they found that the discs consistently yielded ~0.10 larger MPODs than the rings. In a second experiment, they used a 1.5-deg-diameter disc and four thin annuli with average diameters of 0.49, 0.74, 1.05, and 1.44 deg. This condition allowed them to estimate the ring size that matched the mean value for the disc. Using an interpolation procedure, the authors concluded that discs and rings give equivalent MPOD values not at 100% of the disc radius, i.e., at the edge, but at about 50% of the radius, i.e., halfway between the center and the edge. Thus, the results of Bone directly contradict those of Hammond et al.[8] The implications of the disparate results are critically important for interpreting the meaning of HFP measures of MPOD.

If the edge effect held perfectly for all disc sizes, then the HFP procedure using disc stimuli would yield an unambiguous measure of the MPOD distribution: values derived from any center-fixated disc of a given radius would map to the point on the MP distribution at the radius's distance from the fixation point. Thus, the entire distribution could be defined by a series of disc sizes. This strategy, however, has been used only for discs up to 2-deg diameter corresponding to the limits of empirical confirmation of the edge effect. More extreme loci have been evaluated with appropriately positioned small spots or appropriately sized annuli. Bone et al. have now challenged the edge effect even for discs within the 1- to 1.5-deg range with results that suggest a radically different interpretation of what HFP values mean. They hypothesized that MPOD measurement with HFP, using discs, represents values averaged over the underlying retinal area. A model incorporating an estimate of the MPOD distribution from the annuli data was consistent with their hypothesis. Given the large number of published HFP studies of MPOD using disc targets in the 0.5- to 2.0-deg range, it is clearly important to resolve the discordant findings.

Frequently, contradictory results are attributable to differences in stimulus conditions or procedures. Although both the Bone and Hammond et al.[8] experiments involved HFP, they were not identical. Bone used more intense luminance levels than the Hammond et al.[8] and Hammond and Caruso-Avery[9] studies: ~1.0 and 2.0 log units higher than Hammond et al.[8] and Hammond and Caruso-Avery,[9] respectively. In addition, Bone employed a center-surround configuration, whereas Hammond et al.[8] and Hammond and Caruso-Avery[9] used an increment superposed on a background. Perhaps, the most significant difference, however, is that Hammond et al.[8] and Hammond and Caruso-Avery optimized flicker rates for their subjects, whereas Bone adjusted the flicker rate only for their ring stimuli; i.e., flicker rate was fixed for their disc stimuli. Perceived flicker is produced when two lights are continuously interleaved such that one goes on when the other goes off and vice versa. The radiance of one of the lights is adjusted such that its visual effectiveness equals the other. At the appropriate flicker rate, this is accomplished when the target appears fused for the subject. When used to measure MP, a normalizing wavelength (i.e., not absorbed by MP) of fixed radiance is used and the subject adjusts the measuring wavelength (i.e., absorbed by MP) radiance to match that standard. This task is exquisitely sensitive to flicker rate, e.g., Refs. 10 and 11. A fixed alternation frequency will lead to variable results across subjects and between retinal loci within subjects. This is due to individual differences in flicker sensitivity and differential temporal properties across the retina (this will be discussed at greater length below). As an example of the former, there are large individual differences in temporal vision within a given age cohort and systematic decrements with increasing age and retinal disease.[12] For instance, a flicker rate that produces a distinct, narrow no-flicker zone for a young person might produce an extremely large no-flicker zone for an elderly person, potentially leading to greater measurement variability. In order to create a narrow zone of no flicker that is equivalent across subjects and across retinal loci for a given subject, one must optimize flicker rates for individual subjects. (The no-flicker zone may be considered optimal when the upper and lower limits of the zone, as defined by the blue radiance settings made by the subject, are within a range of ~10% of blue radiance. This is achieved by systematically adjusting the flicker rate while observing the subject's blue radiance settings for each frequency setting.) This type of perceptual equivalency implies a similarly equivalent underlying neural response. A fixed flicker rate, in contrast, will lead to a variable perceptual experience with a similarly variable neural response. The fact that Hammond et al.[8] optimized flicker rates for each subject and Bone did not means that most subjects in those two experiments performed different tasks.

Given the widespread use of HFP to measure MP levels (most studies utilizing disc stimuli in the 0.8- to 1.5-deg range) and given the clear discrepancy between Bone's results and the earlier work by Hammond et al. demonstrating the edge effect, we felt a reexamination was needed. We conducted two experiments. The goal of experiment 1 was to gain insight into the reason for the discrepant results and conclusions concerning the validity of the edge effect. The goal of experiment 2 was to develop an explanation of the edge effect should we replicate the earlier positive results.

2 Methods

2.1 *Subjects*

Eight subjects (five females, three males, with ages ranging from 22 to 62 years) participated in experiment 1. The subjects were selected for and represented a wide range (0.10 to 0.66) of $MPOD_{460}$ at 30 min eccentric to the center of the fovea. The subjects included two authors (BW and WS) and one other experienced psychophysical observer (MB). All other subjects were novices and naïve to the purpose of the experiment. All subjects had normal or corrected-to-normal acuity. Three of the subjects also participated in experiment 2. The two male subjects, BW and WS, are practiced psychophysical viewers; the third subject was a novice female (AT) and naïve to the purpose of the experiment. The study followed the Tenets of the Declaration of Helsinki. All procedures were approved by the institutional review board at Brown University and informed consent was obtained from each subject prior to testing.

2.2 *Apparatus*

The apparatus was a light-emitting diode (LED)-based HFP apparatus [a macular pigment densitometer (MPD)] that was developed specifically for the measurement of MPOD and is fully described in Wooten et al.[13] Four blue LEDs with a center wavelength of 470 nm (half bandwidth = 20 nm, 2.75 cd/m^2) provided the 6 deg circular adapting background. Two blue LEDs with a center wavelength at 458 nm (half bandwidth = 20 nm) and one LED with a center wavelength at 565 nm (half bandwidth = 20 nm, 16.7 cd/m^2) provided the components of all test and reference stimuli. Twelve targets were presented, 6 disc targets and 6 ring targets, where the radius to the center of the ring corresponded with the radius of the corresponding disc stimulus. The test targets were all foveally fixated. The radii of the test (measurement) targets were 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 deg for the disc targets; the corresponding ring stimuli had annulus widths of 2, 5, 10, 15, 20, and 25 min, respectively. The distance from the center of each ring stimulus to the center of its annulus gap is equal to the radius of the corresponding disc stimulus. All targets were made with a high-definition, computer-generated lithographic method. The clear portions of the targets had an OD of ~0.05 and the dark portions had an OD of ~4.0. See Fig. 1 for a graphic rendering of the foveal stimuli.

The normalizing, parafoveal target was a 2.0-deg-disc that was viewed at 7-deg eccentricity in the temporal retina with the aid of a red LED fixation point that was 5 min in diameter. In this application, HFP is a comparison method: the measurements taken at the foveal test loci, where MP is most heavily deposited, are compared with the measurements taken at the parafoveal normalizing locus, where MP is optically immeasurable, to obtain the MPOD. The test and reference components of the target were alternated in a square-wave fashion, 180 deg out of phase. The MPD achieves flickering electronically, i.e., the target components are switched on and off. The subject turned a knob that adjusted the radiance of the test (458 nm) component relative to the radiance of reference (565 nm) component, which was held constant. Radiance levels were achieved by varying the density of 1.5-μs pulses from 300 to 300,000 Hz. Adjustment continued until the target stimulus was perceived as not flickering.

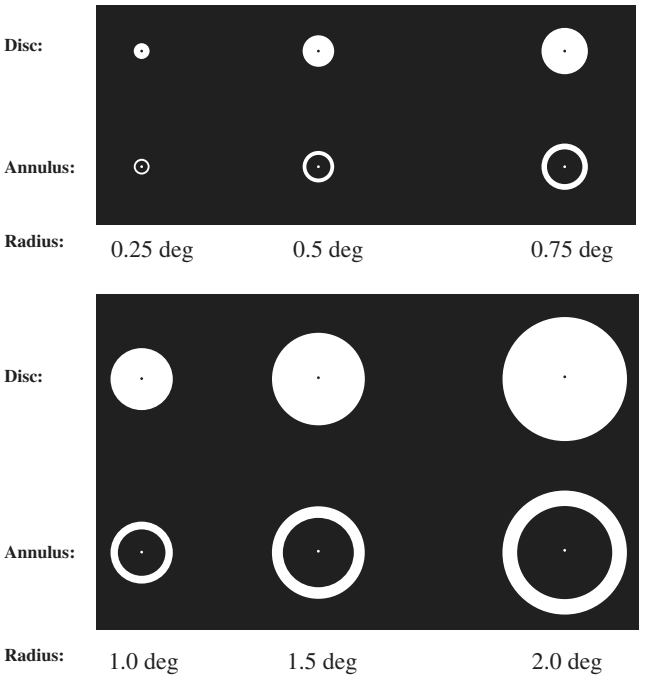

Fig. 1 The foveally fixated stimuli consisted of a series of six discs with radii ranging from 0.25 deg of visual angle to 2.0 deg of visual angle and annular rings of corresponding radii.

The MPD is a Newtonian (free) view apparatus. The targets were viewed at a distance of 18 in. with a 1.3-diopter lens (this was used to allow all of the subjects to comfortably accommodate). Thus, the targets were in sharp focus for emmetropes; for others, corrective lenses were allowed. Adjustable chin and forehead rests were utilized. Typically, the apparatus is configured for measuring MPOD in the right eye but is easily adjusted to measure the left eye. The alternate eye is always occluded by an adjustable block. Only the right eye was measured in the current study.

2.3 *Experiment 1*

2.3.1 *Procedure*

The purpose of this experiment was to examine the validity and limits of the edge effect for various sizes of stimuli. The retinal distribution of MPOD for eight subjects was measured. Flicker frequency was always adjusted for each stimulus prior to testing in order to get a narrow null zone, thereby reducing the potential for variability. The initial flicker rate was approximated using the subject's age and the retinal locus being tested. An optimal null zone was achieved by means of a bracketing method. This method was performed as follows. The blue radiance was set to a low value that provided a distinct percept of flicker. The subject was instructed to increase the blue radiance until no flicker was perceived. The blue radiance was then set to a high value that provided a distinct flicker percept. The subject was instructed to decrease the blue radiance until no flicker was perceived. The two settings gave the lower and upper limits of the null zone for that subject at the preset flicker frequency. A null zone was considered optimal when the range between the upper and lower blue radiance values was at or below 10% of the arithmetic mean of those blue radiance values. If the range was too wide, the flicker rate was decreased 1 Hz and the bracketing

procedure was repeated. Similarly, if the subject was unable to abolish flicker, the flicker rate was increased 1 Hz and the bracketing procedure was repeated. This procedure was repeated until an optimal null zone was achieved. The optimal null zone and resulting MPOD was assessed at six retinal loci from 0.25- to 2.0-deg eccentricity using the disc and annuli described in Sec. 2.2. For each subject, nine measures were taken for each of 12 targets and the 7-deg normalizing target in a single session, lasting ~1 h. The order of test loci was pseudorandomized, but corresponding discs and annuli were always paired, i.e., tested consecutively. The order of each target pair was also pseudorandomized (disc versus annulus). The subject adjusted the energy of the blue target component until a percept of no flicker was achieved.

2.3.2 *Experiment 1 results*

Figure 2 shows the MPOD retinal distributions averaged for all eight subjects in experiment 1. The OD values for ring stimuli (open circles) and disc stimuli (filled circles) are plotted together for comparison. An exponential was fit to the ring data. This was done to check the measured distributions for consistency with an exponential retinal distribution, which MP is known to typically follow. Also, the exponential fit to the disc data facilitates comparison to the ring data. The exponential was fit to the ring data because those data represent the reference to which the corresponding disc data are compared. The error bars represent ±1 standard error of the mean. Notice that the correspondence between the ring and disc stimuli is quite good. In fact, results for the two types of targets are nearly identical up to a radius of 0.75 deg. The two largest stimuli, 1.5 and 2.0 deg, exhibit a slight (~0.04 OD) departure from a perfect edge effect. Only the 1.5 deg data reach statistical significance (t test, $p = 0.02$).

Figure 3 shows the results for each subject. The range of MPOD at the 0 deg locus, estimated from the fitted exponential curve, is large ranging from 0.18 (subject JS) to 1.0 (subjects BW and MB). Notice that, as for the averaged data, the individual subjects show no systematic departure between the discs and rings up to 0.75-deg radius. Inspection of the results of subjects BW and MB suggests that they contribute most to the higher disc values at 1.0, 1.5, and 2 deg exhibited in the averaged data of Fig. 2. To a much lesser extent, subjects RB and WS show the same departure.

In general, the edge effect, i.e., the correspondence between the data for the disc and ring stimuli, holds well for each subject up to a radius of between 0.75 and 1.0 deg and for six of the eight subjects for all of the stimuli, up to the 2-deg radius. These

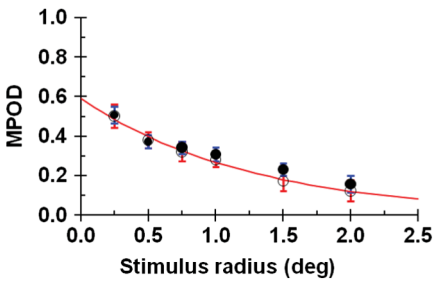

Fig. 2 Macular pigment optical density (MPOD) data averaged across all eight subjects are represented for the disc (closed circles) and annular (open circles) stimuli as a function of the stimulus radius. An exponential function is fit to the annular data. Error bars represent ± one standard error of the mean.

generalizations can, perhaps, be seen more clearly in Fig. 4 where we have plotted the individual MPOD data as scatter plots for the discs versus rings; each panel represents a given stimulus radius. If the edge effect held perfectly, the data points for each panel would be clustered around the diagonal line, which has a slope of 1.0 and an intercept of 0.0. As can be seen, the 0.25-, 0.5-, and 0.75-deg stimuli show R's of well over 0.9, slopes of nearly 1.0, and intercepts of essentially 0.0. The corresponding features for the 1.0- and 1.5-deg stimuli are almost as well described by the line of slope 1.0 and intercept 0.0. The data for the 2-deg stimuli, however, depart from supporting the edge effect. While the slope and intercept (0.82 and 0.05) are not so different from the smaller stimuli, the R of 0.51 is clearly inferior. Note that the poor R is not due to the subjects making more variable null-point settings for the 2-deg stimulus than for the others. In fact, the average standard deviations for subjects in each of the disc and ring conditions were essentially identical, i.e., 0.04 and 0.05 MPOD, respectively. Rather, the poor R for the 2-deg condition reflects primarily the slightly higher disc-target values of subjects BW and MB, as discussed above with respect to Figs. 2 and 3. The same can be said for the 1.5-deg data, but the differences are smaller.

2.4 *Experiment 2*

2.4.1 *Procedure*

In experiment 2, we explore a possible explanation of the edge effect based upon the variation in the spatial-temporal properties across the central retina. Experiment 2 was motivated by the awareness that in experiment 1, optimal flicker rates for the disc stimuli increased as the radius increased. This casual observation reminded us that the quantitative aspects of most visual tasks vary dramatically as a function of retinal locus, e.g., flicker thresholds, motion detection, color perception, spatial summation, or acuity (to name a few). Most relevant to our HFP task is the vast psychophysical and physiological literature related to critical flicker fusion (CFF), dating from around the beginning of the 19th century, e.g., Ref. 14, to more recent times, e.g., Ref. 15. We wondered if an explanation for the edge effect might emerge from considering the spatial and temporal properties across the central retina.

Our first task was to carefully determine the relation between the center-fixated discs from experiment 1 and the optimal flicker that gave a narrow null zone (as defined above). The results for our three subjects are displayed in Fig. 5 along with the averaged data. Notice that the data are fit well with a linear regression model with $r = 0.973$. The different slopes illustrate that there is significant individual variation between subjects for the same size targets. Having established ideal flicker frequencies for each subject, we then chose three stimulus sizes (radii = 0.5, 1.0, and 2.0 deg), defined as the reference discs, for the next task (see Table 1 for the values). The null zones for all discs smaller than each reference disc were tested by using the optimal flicker rates shown in Table 1. For example, BW's optimal (narrow null zone) flicker rate of 19.7 Hz for the 2-deg disc was used in the determination of the extent of the fusion zone for all smaller discs, i.e., 1.5, 1.0, 0.75, 0.5, and 0.25 deg. A bracketing procedure was used to define the limits of the fusional zone for each disc. For each target, the blue radiance was set by the experimenter to a low value that provided the subject with a distinct flicker percept. The subject then used an

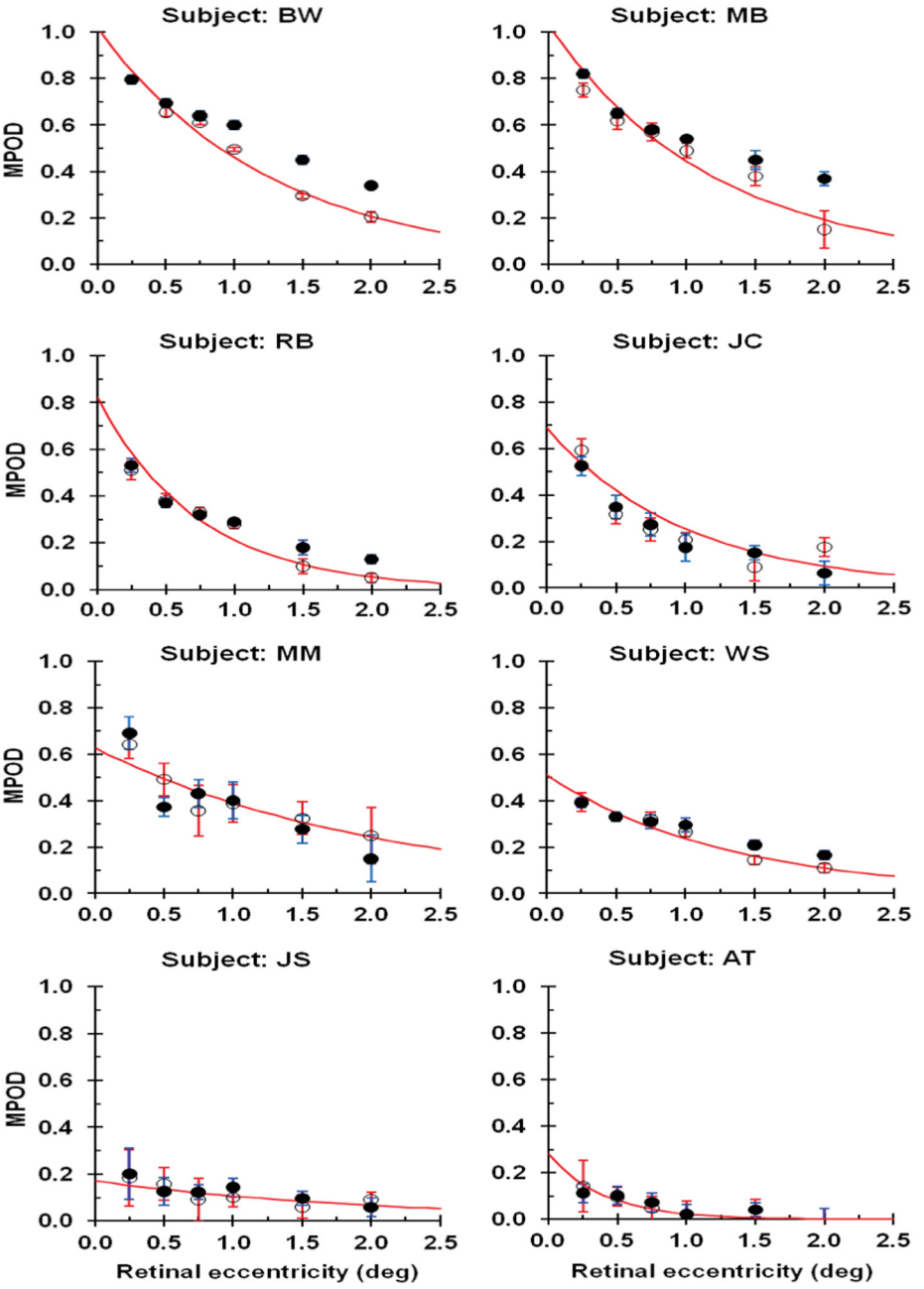

Fig. 3 The MPOD distribution for the eight individual subjects is plotted as a function of stimulus radius. The subjects were tested with disc (closed circles) stimuli with radii ranging from 0.25 to 2.0 deg and with corresponding annular (open circles) stimuli. An exponential function was fit to the annular data. Error bars represent ± one standard deviation.

adjustment knob to increase the radiance until the flicker ceased. The blue radiance was then set to a value high enough to again provide the subject with distinct flicker. The subject adjusted the radiance down until the flicker ceased. This was repeated three times for each target. The average log relative energy of the three high and low blue radiance settings were taken to be the upper and lower limit of the null zone respectively. The same procedure was used for the 1.0- and 0.5-deg-radius reference discs.

2.4.2 *Experiment 2 results*

Results for the three subjects and the averaged data are shown in Figs. 6 (BW and WS) and 7 (AT and averaged). A specific case will help understand the general pattern of the results. For BW, for example, compare the 2.0-deg-radius reference disc with the 0.25-deg-radius disc. Recall that the frequency of the 2.0-deg reference was adjusted to the optimal rate of 19.7 Hz so that the blue radiance value for no-flicker shows an extremely narrow null zone—almost a point. When the disc is switched to the 0.25-deg-radius disc (keeping the same frequency and blue radiance setting), the smaller disc exhibits no flicker, i.e., it is contained within a now large null zone. The reason for this is simple: the optimal frequency for a 0.25-deg-radius disc (see Fig. 5) is 11 Hz, far from the 19.7 Hz set for the 2.0-deg-radius disc. An important point is that no flicker is apparent for the 0.25-deg-disc despite the fact that the blue radiance setting, which reflects the amount

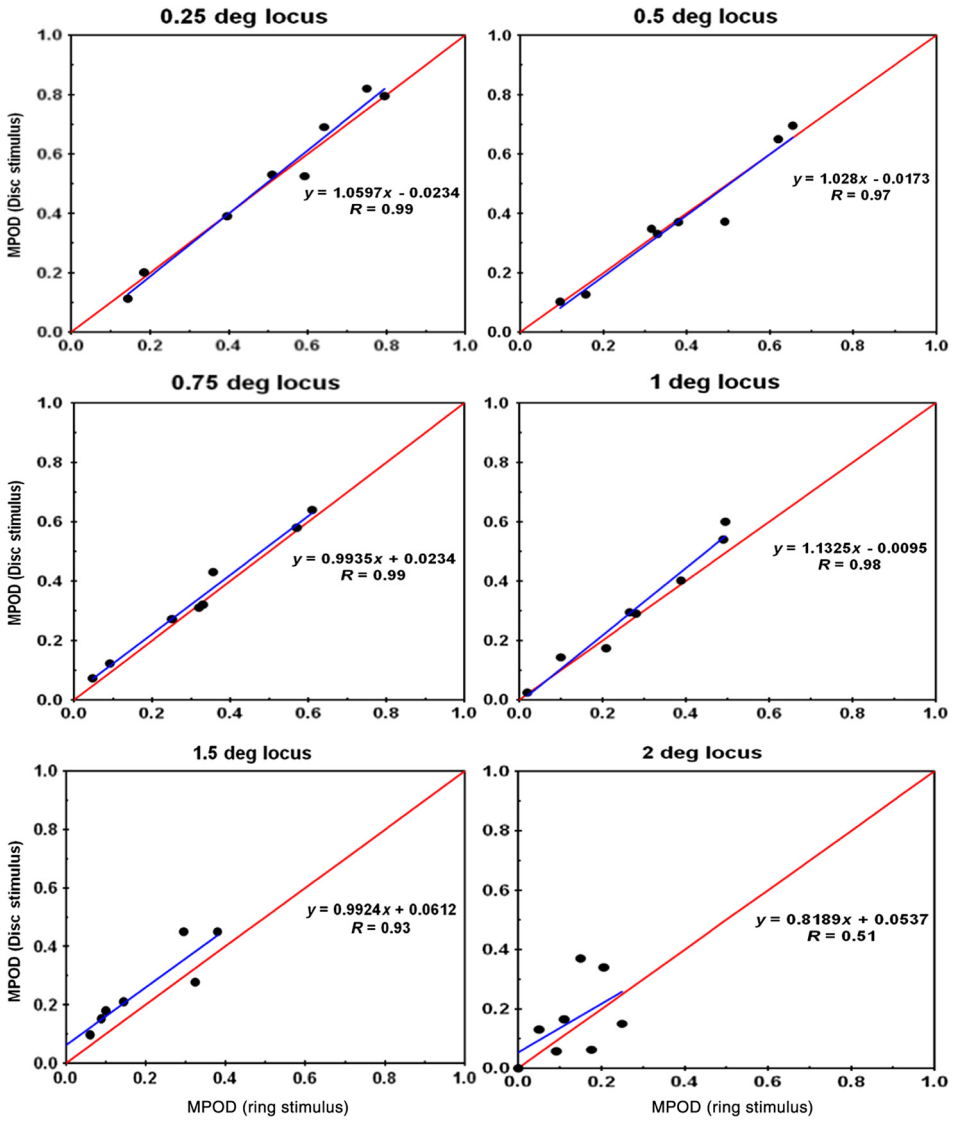

Fig. 4 The two stimulus types, ring (abscissa) and disc (ordinate), are plotted against each other for all measuring loci. Least squares regression lines were fit to the data; corresponding linear equations and Pearson correlation coefficient values are noted on each graph. The diagonal lines represent a hypothetical perfect match, i.e., slope of 1.0, intercept of 0.0.

of MP at 2.0 deg, is not a no-flicker match, due to the much greater MP at 0.25 deg (see BW's MP spatial profile in Fig. 3). The limits of the null zone for the 0.25-deg disc are determined by varying the blue energy up and down until flicker appears. These limits are shown by the horizontal tick marks above and below the dotted line defined by the blue radiance setting for the 2.0-deg-radius disc. The tick marks show a large null zone of no flicker that captures within it the appropriate blue setting for the 0.25-deg-radius disc. The discs between the 0.25- and 2.0-deg-radius discs show the same result but with smaller null zones as the size is increased from small to large. The same pattern is displayed by the other two subjects (WS, Fig. 6 and AT, Fig. 7) and is summarized by the averaged data (Fig. 7). In summary, small discs exhibit large null zones when tested using optimal frequencies appropriate for larger discs.

3 General Discussion

Several reports have concluded that when using centrally fixated disc targets and HFP to measure the MPOD in the central fovea, the resulting values are determined by the edge of the stimulus. Two published studies tested the edge hypothesis for 0.5 (Ref. 8) and 1.0 (Ref. 9) deg radius discs and found substantial confirmation. Bone,[6] however, examined the issue using annular stimuli in conjunction with a disc of 0.75-deg radius and concluded that the edge hypothesis fails: the average of 10 subjects corresponded not with the edge of the disc, but with a point approximately halfway between the edge and the center. Because their subjects showed unusually large individual differences and in view of the importance of the issue to the meaning of HFP determinations of MPOD, we felt that more data are needed to resolve the issue.

Our results do not agree with Bone within the range where the two studies' test targets overlap in size, i.e., 0.5- to 0.75-deg radius. This conclusion is reached by inspecting the averaged data (Fig. 2) and the individual data (Fig. 3). It is seen most clearly from the scatter plots of the individual data (Fig. 4): the three panels showing the 0.25-, 0.50-, and 0.75-deg data exhibit near-perfect agreement between MPOD estimates

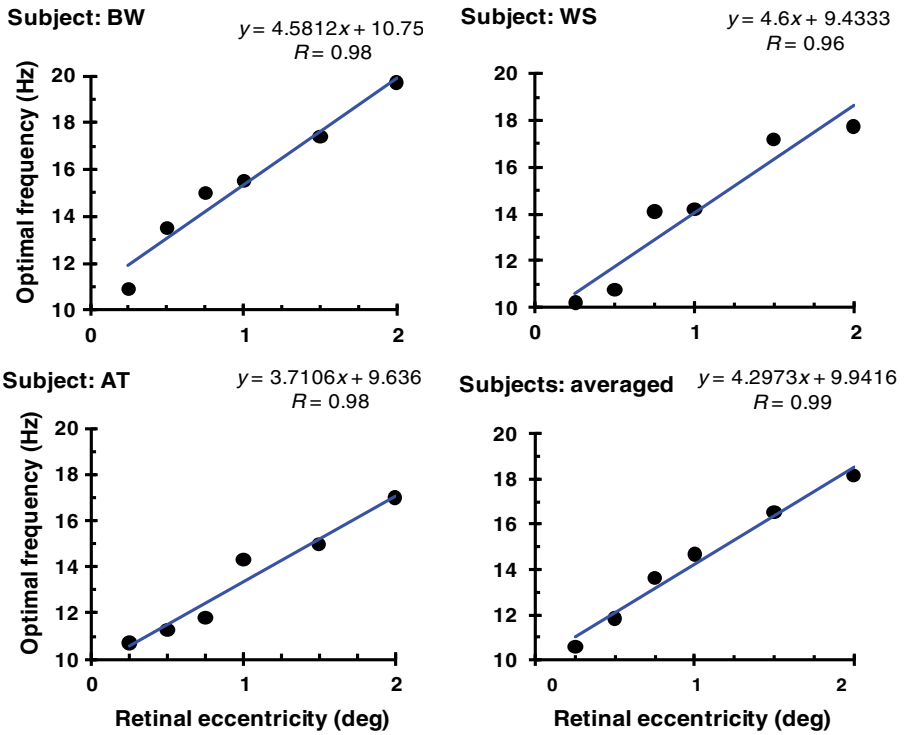

Fig. 5 Optimal flicker frequency for the disc targets is plotted as a function of retinal eccentricity. Three subjects, a subset of the original subject pool, were tested for optimal flicker frequency at each of the six retinal loci. In the lower right panel, the averaged frequencies for all three subjects are plotted.

from the ring and disc targets: slopes ranging from 0.99 to 1.05, *y*-intercepts of essentially 0.02, and Pearson *R*'s between 0.97 and 0.99. An interpolation procedure of our averaged data (Fig. 2) also illustrates the stark discrepancy with respect to the data of Bone. Figure 8 plots our estimate of the radii at which our disc and ring MPOD values correspond. Notice that within the range of Bone's stimuli (shown to the left of the dashed line), the three values are at essentially 100% of the radius, i.e., the edge effect is exact within experimental error.

The asterisk indicates Bone's analogous data: the intersection point fell at an eccentricity of 0.38 deg, which is 51% of the 0.75-deg radius. Another comparison between the results of the two studies is shown in Fig. 9 with scatter plots (details are shown in Table 2). Our data (filled circles) are the same as in Fig. 4 for the 0.5- and 0.75-deg panels. Bone's data are replotted from their Fig. 6 for the 0.5-deg condition and from their Fig. 3 for the 0.75-deg condition.[6] The solid and dashed lines are regression fits through our data and Bone's, respectively (see Table 2 for details of the fits for each condition). The lines describing our data are nearly identical to the expected slope of +1.0 for a perfect edge effect. Bone's data, on the other hand, exhibit slopes of 0.82 and 0.87 for the 0.5- and 0.75-deg conditions, respectively. All of the data points significantly above the slope of 1.0 line represent failures of a strict edge effect. Here again, the two experiments yield significantly different results and, therefore, conclusions.

Table 1 The table displays flicker rates for obtaining an optimal null zone for the displayed disc sizes for three subjects and the averaged rate for each disc. The optimal null zone was defined as a range of log blue energy no more than 10% of the arithmetic mean of the upper and lower blue radiance limits of the null zone.

	Flicker alternation rates for optimal null zone (Hz)			
Disc radius (deg)	Subject BW	Subject WS	Subject AT	Averaged data
0.5	13.5	10.7	11.3	11.8
1.0	15.5	14.2	14.3	14.7
2.0	19.7	17.7	17.0	18.1

Note: BW, second author; WS, first author.

Our experiment extends beyond 0.75 deg out to 2.0 deg. Inspection of Figs. 2–4 and 8 shows that beyond a 0.75-deg radius, the edge effect is less perfectly realized. The averaged data show that for stimuli of 1.0 deg and greater, the discs give a slightly greater MPOD than the rings, but only by ~0.05 OD. The individual curves show that this discrepancy was mainly contributed to by BW and MB and to a lesser degree by RB, the subjects with the highest MPOD. The effect on the estimate for the radius at which the ring and disc have equal values is ~83% of the disc radius (Fig. 8). This is less than a perfect edge effect (100%), but still far from Bone's 51% value.

We conclude that our data support a virtually perfect edge effect out to a radius of 0.75 deg, which is in stark distinction to Bone's results supporting their conclusion that the edge effect fails. Our conclusion is also contradictory to his model that HFP yields MPOD values that reflect an average over the area of the retina stimulated. Our conclusion is that MPOD as determined by our HFP method refers to the edge of a centrally fixated target for radii up to 0.75 deg. Beyond that, some subjects (two or three out of eight) in our study exhibited small but significant departures. Therefore, in using our method, it would be prudent

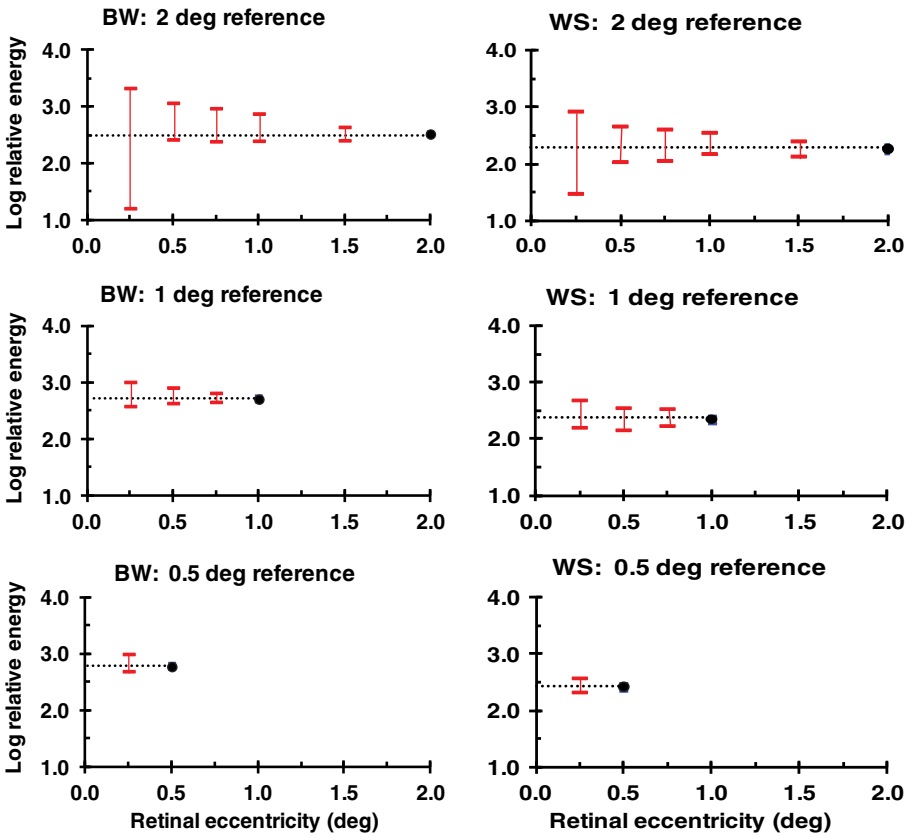

Fig. 6 The filled circles represent the 2-, 1-, and 0.5-deg reference disc stimuli (top, middle, and bottom panels, respectively). Each target was set by each subject by adjusting the blue energy and the frequency for a near-perfect null point. The frequency is shown in Table 1. The log relative blue energy is plotted on the ordinate and the radius of the stimulus is plotted on the abscissa. The tick marks represent the upper and lower limits of the no-flicker zones for the stimuli smaller than the reference stimuli.

to only use discs up to a 0.75-deg radius (as has been the practice). For stimuli >0.75 deg, an appropriate annulus or spot should be utilized and has been the practice.

An explanation for the contradiction between our results and those of Bone is not obvious. The answer, or answers, must be in the differences in apparatus, stimuli, and/or procedure used in the two studies.

The apparatuses were quite different in design. Bone used a Maxwellian-view optical system with a quartz-halogen light source and a neutral density wedge to control the radiance of the blue light. We used a Newtonian (free) view optical system with LED light sources and electronic control of radiance. Our blue-green interchange was accomplished with electronic switching, where Bone used a sectional, white-surfaced, rotating disc. Aside from the difficulty of alignment in Maxwellian view, we can see no reason to believe that difference in apparatuses could explain the disparate results.

The stimuli provided by the optical system of Bone and ours were different in several respects. Bone used a higher luminance and a larger stimulus array. Neither of these factors, however, would be expected to affect the results of MPOD determination. One difference, however, between their stimulus array and ours could potentially account for all or part of the conflicting results. Our target was a pedestal located at the center of a 6 deg, homogeneous, background and 1 log unit above the detection threshold. Thus, the edges of the target were always in strong contrast with respect to the background. Bone used a center-surround configuration, i.e., their target was adjacent to a white surround of the same luminance as the green reference field. Thus, as the blue radiance was adjusted up and down with reference to the green standard, the brightness of the combined target would vary accordingly. So, at the null point, the luminance of the combined green and blue components would match that of the surround. This luminance relation between the center and the surround, however, creates a potential for understanding why their results differ from ours.

When two adjacent fields of different colors are placed side-by-side, with a sharp boundary between them, a clear edge is generally perceived. It is possible, however, to adjust the radiance of one of the fields until the edge separating them becomes indistinct or even disappears altogether. Boynton and Kaiser[16] called this the "minimally distinct border" (MDB). Further work demonstrated that when spectral lights are matched to a white standard, the resulting spectral sensitivity is an exact match to the spectral sensitivity derived from heterochromatic flicker photometry.[17] Recordings from the retina of the rhesus monkey have conclusively shown that both criterion, i.e., MDB[18] and HFP,[11] are precisely accounted for by the response of phasic retinal ganglion cells. Furthermore, Lee et al.[11] showed that chromatic channels do not contribute to the HFP response criterion. In fact, MDB and HFP are simply two different response criteria (one spatial and the other temporal) that are mediated by the same mechanism, both defined by phasic retinal ganglion cells.

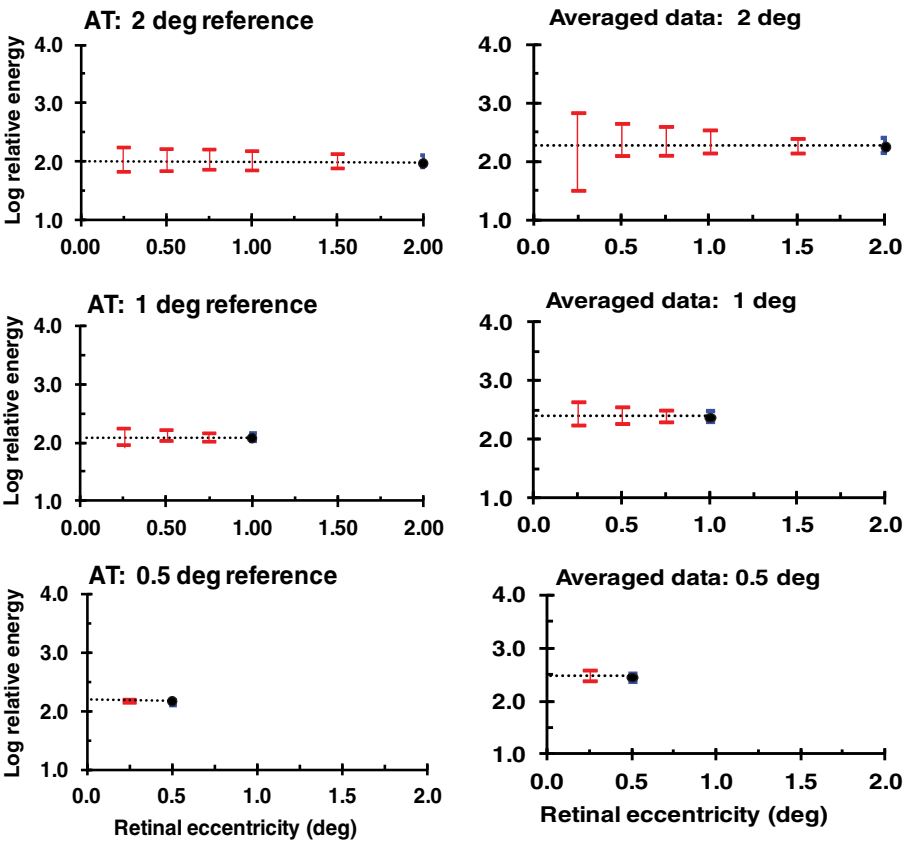

Fig. 7 Same as for Fig. 6 but for subject AT (left panels) and for the averaged data of the three subjects (right panels).

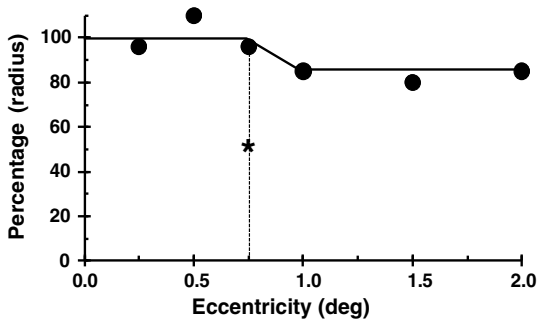

Fig. 8 The filled circles represent interpolated values from Fig. 2 of the percent of the radii of the annular stimuli corresponding to the radii at the paired disc stimuli. The asterisk indicates Bone's datum point for this 0.75-deg stimulus.

In current models of color perception, this underlying mechanism is called the achromatic channel and carries the percept of "white." Its spectral sensitivity is taken to be that of the luminosity curve and is frequently (but incorrectly) called the luminance channel. When two lights are equated by MDB or HFP, they have the same luminance, except for small differences between subjects. Thus, when Bone et al. equate their target and surround for luminance, they are also effectively creating a minimally distinct border between the center and the adjacent surround. When the border is minimally distinct, there is essentially no luminance discontinuity. There remains a chromatic difference between the blue-green mixture of the target and the white surround.

In sum, the center-surround configuration of Bone could create a situation where at the blue-green null point, there is no effective edge, or at the very least, a weak one. If this is true, then the very border needed to define the edge effect would simply not exist, or be very weak. Under these conditions, the effective position of correspondence between a disc and appropriate ring might well shift toward the center of the target.

Another aspect of the data reported by Bone that contrasts with our results is the variability. As the authors point out, "... there is wide variability among individual subjects." For example, the average standard deviation of their MPOD values for the ring stimuli was 0.22 (taken from their Fig. 4); the value for our comparable ring stimuli was 0.05. A related aspect of their data was the extreme range of their cross-points (where the ring and disc values were equal based upon interpolation): as shown in our Fig. 8, their average was at 51% of the radius, but the range was from 0 to 100%. In some subjects, there were striking differences in the MPOD distributions comparing left and right eyes (this comparison is possible because Bone tested both eyes in their 10 subjects). This would be surprising, if verified, because several studies have concluded that the MPOD distributions (and the absolute values) in primates (including humans) of the two eyes are normally highly similar. This has been demonstrated *in vivo*[19] and *ex vivo*.[20] The rather large variability reported by Bone may be understood, at least partially, by considering further an issue associated with the equating of the test and surround luminances.

The CIE photopic luminosity function (V_λ) was determined by averaging a large number of subjects mainly using HFP.

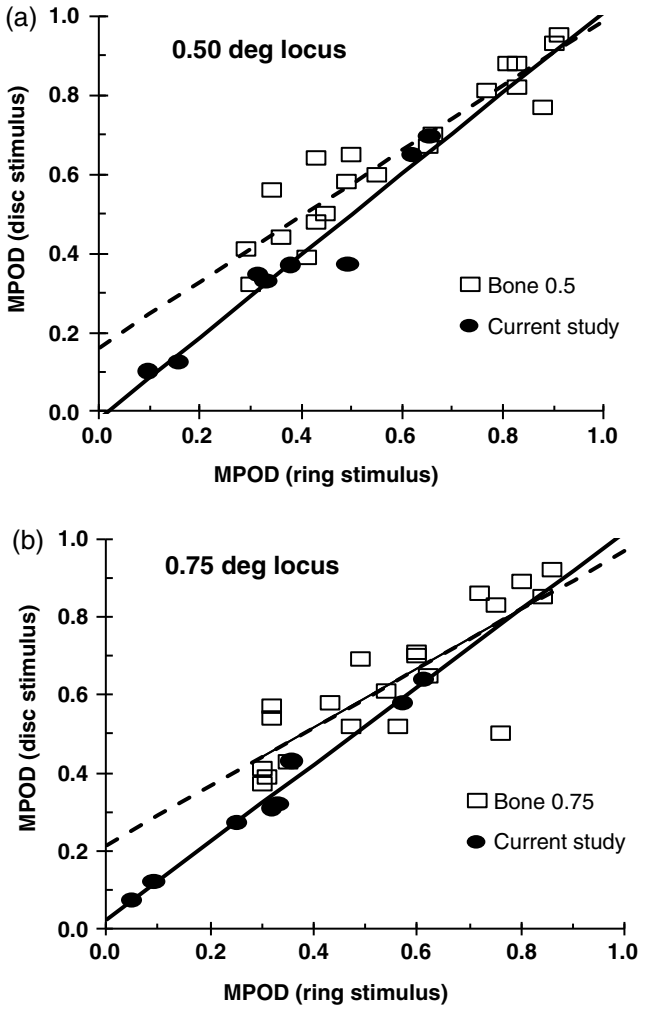

Fig. 9 Scatter plot of MPOD values or disc and ring stimuli using the (a) 0.5 and (b) 0.75-deg stimuli, respectively. Bone's data are shown by open squares; our data are shown by filled circles. The dashed and solid lines are determined by least squares regression. Details are shown in Table 2.

However, V_λ was created as a basis for photometry, and it serves that function well. It will not precisely match a given individual's luminance function, termed sensation luminance by Kaiser,[21] especially in the short-wave region, which is strongly affected by individual differences in lens and MP absorption. The implication for the 10 subjects in the Bone study is clear: each subject's sensation luminance will likely be different from the CIE V_λ function and to different degrees across individuals, especially in the short-wave region of the spectrum. Thus, there will be different degrees of border distinctness in proportion to differences between each person's sensation luminance function and the V_λ function. The variability of border distinctness could cause different cross points of the MPOD data for disc and ring stimuli and, therefore, contribute to the variability found in the Bone study.

Table 2 A comparison of the relationship between macular pigment optical density measured using a solid disc and an annulus. The regression lines are based on data collected in the current study and by Bone (2004). The data are plotted in Fig. 9.

Stimuli radii (deg)	Line equation–Bone	Line equation–current study
0.5	$Y = 0.82X + 0.16$, $r2 = 0.89$	$Y = 1.03X - 0.17$, $r2 = 0.94$
0.75	$Y = 0.75X + 0.21$, $r2 = 0.71$	$Y = 0.99X + 0.02$, $r2 = 0.98$

In addition to spatial factors, there are important differences in the time domain between Bone's study and ours that contribute to the disparate results with respect to the variability and the qualitative aspect of the data. Of particular interest are the procedural differences between the two studies whereby flicker matches were made.

The basic HFP procedure, common to all specific procedures, requires the manipulation of two physical variables:

1. the frequency (f) at which the test light (heavily absorbed by MP) is alternated with the comparison light (not absorbed by MP)
2. the radiance ratio (r) of the test (blue) and standard (green) lights.

Although every HFP method requires that f and r be manipulated in order to reach the goal of equating the test and comparison lights in visual effectiveness, methods differ with respect to the response criterion that defines visual effectiveness. Our method and Bone's differ importantly in this regard, and these differences are crucial to understanding the disparate data and conclusions. Bone's procedure for determining MPOD for the two disc stimuli (0.75 and 0.5 deg radii) was to fix the frequencies at 30 and 15 Hz for the fovea and parafovea, respectively. For the ring stimuli, he allowed the subjects to set the frequency for optimum location of the null point.[22] Fixing the frequency, however, promotes variability between subjects because a disc with a fixed frequency generally looks different to different subjects. Consider the 30-Hz foveal target. To an individual with average flicker sensitivity, such a rate might be optimal, i.e., at the proper r, the null zone would be very narrow, maybe almost a point. On the other hand, a person with superior flicker sensitivity would never see the flicker eliminated; instead the best r would yield only minimal flicker. A subject with below-average flicker sensitivity would see the disc in yet another way: as r is varied, there would be no null point or even a minimum flicker; rather, they would see a zone of fusion over a range of r's. Furthermore, flicker sensitivity is influenced by many factors, such as health and age. In fact, it varies considerably even within a healthy age cohort. The point is that if the physically constant stimulus looks different to the subjects, then it is not having the same physiological or perceptual effect, so the subjects are not uniformly using the same response criterion. In a sense, the subjects are enrolled in three different experiments. For this reason, we have taken a different approach toward setting f and r.

The ideal no-flicker match criterion is that the zone of fusion be a point, requiring that the corresponding r be an exact value with 0.0 standard deviation. To achieve an exact r requires a corresponding f with a standard deviation also of 0.00. In practice, such precision is impossible. What can be achieved, however, is an acceptable approximation to the ideal by iterating between adjusting f and adjusting r until a very narrow null zone is reached. We start by selecting a plausible f and then

adjusting r. If no r can be found that eliminates flicker, then f is increased. If no r can be found that results in a narrow null zone, then f is reduced. We go back and forth until a null zone is reached with a range within ~10% of the blue radiance. We find that this null zone criterion results in acceptable variation of the MPOD values, giving typical standard deviations of ~0.06 with five repetitions. We follow this procedure for each stimulus and for each subject, thus allowing the same response criterion for every subject in every condition: a narrow null zone (almost a point) at a specific (f, r). In addition to reducing variability, this procedure yields a surprising, and perhaps even more important dividend.

When a subject makes a setting using a disc stimulus following the response criterion of a narrow null zone, i.e., optimizing (f, r) by iteration, perceived flicker disappears over the entire area of the disc. This is true from the smallest disc that we employed (0.25 deg) to the largest (2.0 deg). We want to emphasize that the discs are totally devoid of perceived flicker from the center to the edge. Why is this surprising? Because it does not necessarily follow logically from our selecting (f, r) pairs defining narrow null zones, particularly in view of the exponential distribution of MPOD (Fig. 2) and the validity of the edge effect. That is to say, if a narrow null zone is made for a person with 0.30 MPOD at the edge of a disc stimulus, and they have, say, 0.60 MPOD at the center, the receptors in the center are being stimulated twice as much (an OD difference of 0.30) compared to the receptors at the edge. Yet, no flicker is seen at either site (or anywhere in between). These observations raise two related questions:

1. Why does the match point settle on the edge of the stimulus?
2. And then, why is no flicker seen within the disc stimulus, even in the center where the receptors are being strongly modulated?

Both of these questions can be answered by considering the spatial-temporal interactions revealed in our experiment 2 using disc stimuli. Our HFP results are strikingly similar, at least qualitatively, to the much earlier studies examining the related phenomenon of CFF.

Granit and Harper[23] found for centrally fixated discs, ranging from ~0.5- to 2.5-deg radius, a linear relation between CFF and the logarithm of area. This relation, known as the Granit–Harper law, was confirmed by many subsequent investigators. Roehrig[24] showed that it held up to at least a 25-deg radius and that, using annuli, the CFF for discs was determined near the edge. He concluded that "CFF is determined not by the total area of the retina illuminated, but by the portion(s) of the retina which is capable of the best temporal resolution."

The Granit–Harper Law and Roehrig's results are, of course, similar to our findings using the HFP criterion response.

The first half of experiment 2 basically replicates Granit and Harper, but with HFP, one detail is different. Whereas they found a linear relation between CFF and log disc area, we found a linear relation between optimal HFP rate and disc radius (Fig. 5). (When replotting our data using log disc area, the relation was a slightly positively accelerating function.) This difference should not be surprising in view of the very different stimulus conditions, although the criterion response (fusion) was identical. The important point, however, is that for both sets of data, the criterion of fusion is directly proportional to the radius of the edge. Furthermore, the results from experiment 1 show that it is the edge of a disc that largely (or entirely for stimuli < ~0.75- deg radius) determines the optimal HFP flicker rate. This is in accordance with Roehrig's conclusion quoted above for CFF. The remaining question to be answered is why the areas central to the edge do not contribute to the response, even though both in our stimulus conditions and in Roehrig's, the central receptors are being modulated far more than those near the edge.

Why is there complete fusion over the entire disc with our procedure of optimizing the flicker rate and the blue-to-green ratio? Consider a hypothetical typical subject A, as represented in Fig. 5 by the averaged data of our three subjects. A's optimal flicker frequency for the 2-deg-radius disc was 18.1 Hz; for the 0.25-deg disc, it was 10.3 Hz. Now consider two potential observations. First, when viewing the 2-deg disc at 18.1 Hz, no flicker is seen. If now the 2-deg target is switched for the 0.25-deg one (while keeping the f and r for the larger disc), the smaller target will be seen as fused over its entire area. Flicker can be restored by altering the blue-to-green ratio up or down, thus breaking out of the large fusional zone. In other words, the optimal (f, r) for 2 deg is not optimal for 0.25 deg. A narrow fusional zone for the smaller disc can only be realized by setting a lower f (appropriate for the poorer temporal sensitivity at the 0.25 deg retinal locus) and a higher r (appropriate for the larger amount of MP at the 0.25 deg retinal locus). In summary, when a disc of a given size is set at its optimal (f, r), all smaller discs with the same (f, r) will appear fused because of their lower flicker sensitivity, despite significant receptoral modulation. Figures 6 and 7 show that this generalization held for all three subjects. If we assume that the behavior with the smaller discs reflects the characteristics of the corresponding retinal areas contained within any given disc, then we have an explanation of why flicker is abolished over the entire area of the disc. These spatiotemporal factors that provide an explanation for our results may also, in part, explain Bone's very different results and conclusions.

Recall that Bone, for discs, used fixed frequencies (f) for the fovea and parafovea, 30 and 15 Hz, respectively. As noted earlier, this means that at the best match point (r), the stimulus would either be flickering at a minimum, show a relatively wide fusional zone, or exhibit a near null point. These three possible different response criteria could lead to profoundly different MPOD estimates. For example, for the subjects who can only minimize (but not eliminate the flicker by adjusting r), it is not clear where the minimum would settle with respect to the radius. Flicker would most likely be seen everywhere over the disc's surface, but because of unequal receptor activation as a function of position along the radius and a fixed f with variable manifestations in each subject, it is simply not clear where the cross-point between discs and rings would occur. For those observers who would see a relatively wide null zone, the interpretation might be different, but with the same result: the match could be anywhere within the null zone, resulting in considerable variability. Conceivably, this outcome could be made unambiguous by carefully determining the null zone endpoints and taking the midpoint as a match. In the absence of any explicit instruction to the observer, we cannot know what response criteria individuals used. For the rare case where the fixed flicker values resulted in narrow null zones upon adjusting r, the results should be similar to ours. In summary, without an explicit iterative procedure, systematically adjusting f and r until a near null point is achieved, it would

be the rare case where a fixed frequency would correspond to the optimal frequency for that of the edge radius. If our interpretation is correct, this lack of an optimal (f, r) would account for the highly variable data and a lack of an edge effect for most subjects.

We believe that our results strongly support the existence of the edge effect associated with the HFP criterion response. We also feel that we have provided a convincing explanation of the phenomenon based upon the psychophysical finding that for center-fixated discs, the optimal frequency is at or near the edge, resulting in flicker fusion over the entire surface of the stimulus. We now consider the underlying physiological substrates that could account for our explanation.

Lee et al.[11] have examined the physiological basis of HFP by recording from the retinal ganglion cells of macaques (*M. fascicularis*). Using HFP stimuli, they found that phasic cells show minimization of activity when the radiance of the chromatic components of the stimuli were near equal luminance. Furthermore, the HFP spectral sensitivities of the cells were virtually identical to those of human observers minimizing flicker in the same optical system. These cells also obeyed the laws of additivity, transitivity, and proportionality (as does HFP in humans). Tonic, or color opponent, cells showed no such results. They concluded that phasic cells are the substrate underlying the HFP task. Further physiological data relevant to our psychophysical results come from a study, also of macaque ganglion cells, by Solomon et al.[25] These authors determined complete temporal contrast sensitivity functions for phasic cells as a function of retinal eccentricity. They found that the cells were more sensitive to the higher frequencies (including CFF estimates) as retinal eccentricity increased. These findings are in agreement with an earlier study by Seiple and Holopigian[26] comparing flicker sensitivity derived from the focal electroretinogram (ERG) and psychophysics in the same subjects and with the same apparatus. They found that for both the ERG and psychophysics, sensitivity to higher frequencies (including CFF) increased as the stimulus was moved away from the fovea. These papers taken together offer a physiological basis for our interpretation of the edge effect. Assuming that the phasic ganglion cells are the basis for the HFP response criterion, and given that their sensitivity to higher frequencies increases with eccentricity, using our optimal (f, r) procedure, we would expect that f would occur at or near the disc edge (highest sensitivity) and that no flicker would be seen anywhere within the disc (despite high receptoral modulation near the center). This interpretation implies, of course, that although the receptors are responding when an (f, r) match is made, the center-surround organization of the phasic cell's receptive field renders the ganglion cells silent or nearly so. Thus, the purely temporal properties of the phasic retinal ganglion cells, across the retina, constrain the input to the more central mechanisms of flicker perception. But another factor, beyond just the presence of more sensitive flicker detecting phasic cells near the edge of a center-fixated disc, is the effect of small fixational eye movements on those cells.

As discussed earlier, Kaiser et al.[18] showed that the phasic retinal ganglion cells are the physiological substrate not only of HFP, but also the MDB response criterion. They recorded vigorous single-cell responses by moving borders across their receptive fields. These cells then are exquisitely sensitive to flicker and to luminance borders. While the HFP and MDB response criteria are quite distinct, they both rely upon the same spatial-temporal properties of the retina. After all, the flickering disc used in HFP studies has a distinct edge (at least in our paradigm) and the border used in MDB experiments is swept back and forth by fixational eye movements. Thus, phasic cells near the edge of a flickering disc are temporally modulated by the alternating components of the stimulus and by the moving luminance step at the edge. Cells at some distance from the edge toward the center are modulated only by the temporally alternating stimulus components. We conclude that given an optimal match fusion point (f, r), the phasic cells are silent across the entire image of the disc, except near the edge. Why are they active near the edge in view of the fusion over the rest of the disc? The answer lies in the spatial organization of the receptive fields, i.e., a center-surround, antagonistic configuration. Such cells respond poorly, or not at all, to a continuously presented homogeneous stimulus, i.e., as for the interior of our disc stimuli. Eye movements have no effect on them since the edge is well outside their receptive fields. And, even though the receptors are modulated, the phasic cells are silent due to the fusion conditions created by the (f, r) match. For the same reason, cells near the edge would also be silent were it not for the heavy modulation driven by the edge as it sweeps back and forth across the center-surround receptive fields. Two perceptual questions immediately arise. One, why do we not see any flicker at the edge if corresponding phasic cells are strongly modulated? Two, why do we see the disc as homogeneous given that only the cells near the edge are active? The answer to both of these questions involves fixational eye movements and the mechanisms of border perception.

The answer to the first question is that the (f, r) can be explicitly chosen to abolish any perceived flicker over the surface of the disc including the edge. In the absence of eye movements, this would result in the phasic cells being silent, even at the edge. With the high frequency (70 to 80 Hz) eye movements, however, the phasic cells are driven to a sustained, steady, level of activity, i.e., they are active, but well above fusion frequency. This pattern of activity is exactly the same as that resulting from viewing a disc that is not physically flickering, i.e., sustained phasic cell activity at the edge, driven by fixational eye movements. In both cases, the discs are seen as homogeneous and not flickering. These phenomena underscore the necessity of fixational eye movements in the perception of a contour. This was dramatically shown in the classic experiments of Riggs et al.[27] and Ditchburn and Ginsborg,[28] who found that the perception of form fades away when the image is optically stabilized on the retina. Emphasizing the importance of fixational eye movements even more is the finding that activity of phasic cells along a luminance border is actually synchronized, thus defining the edge even more distinctly.[29] These mechanisms provide a convincing explanation of the importance of fixational eye movements in defining contours and edge effects at the retinal levels. They do not, of course, explain how the vigorous activity at the edge of a defined form determines the homogeneous percept across the interior. This "filling in" process is beyond the scope of the present discussion.

4 Summary and Conclusions

In summary, we find that with our apparatus and procedures, the edge effect holds nearly perfectly for eight subjects out to a radius of ~0.75 deg. We conclude that for stimuli in this range, HFP provides MPOD values that can be referenced to the retinal loci corresponding to the edge of a centrally fixated

disc. This conclusion is consistent with results from van der Veen et al.,[30] who found that when comparing MPOD sampled using HFP and fundus reflectance, the edge effect holds. The contradiction with respect to Bone's results can be understood by considering the differences in stimuli and psychophysical procedures used in the two studies. We also find support for the edge effect out to a radius of 2 deg, although there were slight discrepancies for two or three of our subjects starting at a radius of ∼1 deg. The explanation of these slight failures is not clear, although parafoveal myopia and increased chromatic aberration (compared to the fovea) are issues that could be explored. We show that the edge effect can be explained on the basis of the differential spatial-temporal characteristics of the center regions of the visual field. We also consider the spatial-temporal properties of phasic retinal ganglion cells and fixational eye movements as the physiological basis of the edge effect.

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