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Effect of cone spectral topography on chromatic detection sensitivity

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Abstract

The spatial and spectral topography of the cone mosaic set the limits for detection and discrimination of chromatic sinewave gratings. Here, we sought to compare the spatial characteristics of mechanisms mediating hue perception against those mediating chromatic detection in individuals with known spectral topography and with optical aberrations removed with adaptive optics. Chromatic detection sensitivity in general exceeded previous measurements and decreased monotonically for increasingly skewed cone spectral compositions. The spatial grain of hue perception was significantly coarser than chromatic detection, consistent with separate neural mechanisms for color vision operating at different spatial scales.

1. INTRODUCTION

Human color vision is based on three classes of photopigments, labeled as long (L), middle (M), or short (S), differentiated by their peak spectral absorptions. A comparison between cones of different types is essential for color to be retrieved in an image independent of intensity. One posited consequence of these necessary comparisons is spatial information loss and consequently lower spatial resolution for hue perception compared to intensity modulation. In the extreme case at higher spatial frequencies, patterns of dark and light can fool the visual system into seeing color—a phenomenon also known as chromatic aliasing. That such aliases are rarely observed in natural vision [1] is evidence for the yet unknown, but clever processing scheme that the visual system uses to achieve a trade-off between wavelength and intensity. Defining the rules that govern this trade-off, including its spatial frequency characteristics, and dependence on cone spectral topography, are essential to unravel the associated mechanisms.

Visual performance for sinusoidally varying isochromatic and isoluminant red–green grating stimuli have been measured routinely to characterize human color vision capacities and in

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attempts to understand the underlying mechanisms [2-10]. However, there are limited studies where performance was assessed unhindered by chromatic and monochromatic optical aberrations, especially in subjects for whom the underlying cone spectral topography is known. Considering broadly the retinal basis for color perception allows appreciating the importance of accounting for the eye's optics and cone composition on such measurements, particularly in the fovea. Postreceptoral pathways encode intensity and wavelength variations in the retinal image via center-surround antagonism. Midget retinal ganglion cells (RGCs) account for ~90% of all RGCs in the central retina, possess both spectral and spatial antagonism, and have spatial density ideally suited to sustain high acuity achromatic and red-green vision across the parafovea [11]. In peripheral midgets, nonselective wiring predicts that, depending on the spectral balance between the center and surround, a cell may contribute to signaling a continuum between achromatic and chromatic contrast [12]. Foveal midgets, unlike those in the periphery, draw inputs from just one cone in the center of their receptive field, leading to two testable hypotheses. First, by virtue of possessing single-cone centers, foveal midgets are posited to have high resolution for detecting both pure intensity and red-green modulations. It is well-known that these cells subserve achromatic or intensity modulation at high resolution [11], but their spatial response properties in detecting high spatial frequency red-green gratings remain less clear. Second, given how the center receives excitatory input from just one cone, the composition of the surround may weigh heavily in deciding the fate of a foveal midget to signal chromatic contrast, achromatic contrast, or some continuum between the two [13], predicting that a skewed L:M cone ratio may be worse at red-green chromatic detection compared to a cone composition that is balanced [14]. To recover hue independent of intensity requires the activity of multiple cells across space to be combined given how any one cone or midget RGC alone cannot disambiguate hue from spatial variations in intensity. Such a comparison posits a coarser spatial grain for hue perception than achromatic detection. To test the hypotheses above necessitates knowledge of the cone spectral composition together with access to the retina at the resolution of individual cones by overcoming both monochromatic and chromatic aberration. Our goal in this study was to: (a) develop a method for efficient cone spectral classification and detail the spatial characteristics of (b) red-green detection, and (c) hue perception using adaptive optics (AO)-corrected visual stimuli in individuals with known cone spectral topography.

Cone spectral types have been identified using AO assisted retinal densitometry as described previously [15-17]. The basic principle involves using classical retinal densitometry to probe the differential spectral absorptions of LMS cone photopigments. The increased resolution offered by AO allows assigning these absorptions to individual cones. For identifying L and M cones, the densitometry protocol involves using two selective bleaching lights centered at 680 and 470 nm to preferentially bleach L or M cones, respectively. Following the selective bleach, L and M cones have different concentrations of remaining pigments—L cones have higher and lower remnant pigment concentration after 470 and 680 nm bleach, respectively. The converse holds for M cones. Following selective bleach, the retina is subjected to AO imaging with 543 nm illumination. This wavelength is chosen because the absorption of L and M cones is high and they have similar absorptance. The retinal imaging at 543 nm bleaches the remaining photopigment in L and M cones. Simultaneously, it offers a high-

resolution image of cones. The relative image intensity—immediately after the selective LM cone bleach and when the retina is fully bleached—contains information about differential absorptions in L and M cones. For instance, after selective bleaches at 680 and 470 nm, candidate L cones imaged with 543 nm ought to exhibit low absorption (high intensity) and high absorption (low intensity), respectively. The converse is true for M cones. Besides the longer time required for two separate bleaches, an a priori knowledge of L:M cone ratios was required to optimally titrate the selective bleaching doses. In this study, we sought to test the feasibility of cone spectral classification with single-wavelength imaging densitometry and obviating the need for selective bleaches. In contrast to the single snapshot AO fundus camera images used in Roorda and Williams [16] and Hofer *et al.* [17], more recent AO densitometry has used video-rate (30 Hz) imaging to probe bleaching dynamics [15]. The temporal features of video-rate densitometry depend critically on the spectral sensitivity of cones for the wavelength used for imaging. Given that L and M cones have similar sensitivity for the 543 nm imaging wavelength used thus far, sufficient segregation of cone types was not achievable based on the temporal aspects of densitometry. By using a wavelength where the L and M cones have different spectral sensitivity, we also sought to test whether cone types can be segregated via their time-dependent bleaching signatures.

In two previous reports, in addition to chromatic detection, subjects were asked to describe their color appearance for red–green gratings. A peculiar change in color appearance as a function of spatial frequency with “pure color” red–green gratings was reported [5,18]. At low spatial frequencies, intensity appeared to be relatively uniform across the colored stripes of a sinewave grating, and hue changed from red to green. However, at higher spatial frequencies (20 c/deg), even though the stripe pattern was clearly visible, it appeared to be a monochromatic grating where stripes were reported to vary in intensity rather than hue. Using the visibility of hue as the criterion, visual acuity for pure red–green colored patterns was about ~ three times worse than the acuity for monochromatic patterns that vary in intensity but not in wavelength. Granger and Heurtley [5] also measured “color pattern detection,” where thresholds for detecting red–green gratings were measured without regard for their hue appearance. Acuity in this task was high, approaching a spatial frequency cutoff of ~30 c/deg, similar to normal acuity with the eye’s natural optics for isochromatic or intensity modulations. A similar achromatic appearance was observed for gratings and checkerboards of complementary colors by Moulden *et al.* [19] who termed these “transchromatic” stimuli. In a motion detection task, Stromeyer *et al.* [20] observed that a chromatic stimulus provided a sensation of motion but not of hue. A simple explanation for these observations might be that subjects were actually responding to intensity modulation instead of hue, caused by optical artifacts produced by the chromatic aberrations of the eye or an inadequacy in matching the intensity of the red and green primaries. Depending on the red and green primary wavelengths, longitudinal chromatic aberration (LCA) can cause an optical defocus blur up to ~1 diopter (D) between red and green stripes of the grating [21]. Transverse chromatic aberration can cause a wavelength-dependent offset between the red and green stripes of a red–green grating [22], such that in the extreme case, they can be superimposed over one another yielding a pure isochromatic modulation. Either of these factors or a combination of them could convert the color vision task into an intensity discrimination yielding the observed high spatial frequency cutoff. An alternative hypothesis

may point towards the ability of the visual system to retrieve information efficiently based on wavelength differences at all spatial frequencies up to the cone sampling limit, such that at low spatial frequencies, the wavelength variations are coded as hue, whereas at high spatial frequencies, wavelength variations are used for detection but do not lead to conscious hue percepts.

We performed experiments here aimed at distinguishing these two hypotheses, i.e., whether the curious appearance of red–green gratings is the result of optical artifacts or if it reflects the presence of two neural mechanisms operating at different spatial scales to encode wavelength variations.

Isolating chromatic mechanisms is an important consideration in such experiments to confirm that stimuli are detected based on wavelength differences alone independent of intensity. We have taken the approach used to establish color vision capacities in animals where no assumptions are made about the underlying neural mechanisms of color and intensity [23]. Rather, intensity is made an irrelevant cue by varying the relative intensities of the red–green stimuli over a wide range. Consistent discrimination of the red–green stimuli over the entire relative intensity range is taken as evidence for a color vision capacity, and if discrimination fails at some relative intensity of the two wavelengths, it follows that discrimination is based on intensity, not color. To eliminate artifacts produced by the eye's optics, visual stimuli were generated in an AO vision simulator with a filter-based Badal LCA compensator. This instrument corrected for both monochromatic and chromatic aberrations of the eye [24]. The L:M cone ratios of subjects were estimated using flicker photometric ERGs and molecular genetics and were compared against their cone spectral topography obtained with the new method for AO densitometry.

2. METHODS

A. Subjects

Four color-normal subjects, three females and one male, were tested (age range of the subjects is 23–36 yo). Subjects S1–S3 were emmetropes, and S4 was a – 2.75 D myope. Subjects were cyclopleged with Tropicamide 1% eye drops. The research was approved by the University of Washington institutional review board, and all subjects signed an informed consent before their participation in this study.

B. Multiwavelength Adaptive Optics Vision Simulator with a Filter-Based Badal LCA Compensator

The psychophysical experiments were performed with subjects viewing stimuli generated in a multiwavelength AO vision simulator system equipped with a filter-based Badal LCA compensator, described in detail elsewhere [24] and shown in Fig. 1. Light from a superluminescent diode (906 ± 8 nm; Inphenix, California) was collimated, spatially filtered, and then introduced into the eye via a pellicle beam splitter (BS). The backscattered light was relayed to the deformable mirror (DM Mirao52d, Imagine Eyes, Orsay, France) and a Shack–Hartmann wavefront sensor by several afocal telescopes. An extra conjugate pupil plane allowed the insertion of trial lenses to compensate for sphere and cylinder. The

psychophysical channel had a multiwavelength digital light projector as a visual display in which two light-emitting diodes centered at 532 and 661 nm illuminated a digital micromirror device that generated the grating stimuli providing a 1° field of view. The psychophysical channel was coupled with the wavefront sensing/correction path through a cold mirror. An artificial pupil ensured an optimal pupil size for psychophysics. For all experiments described here, aberrations were corrected dynamically for a 6.5 mm eye's pupil, while psychophysics was performed over an artificial 6 mm pupil.

The experiments described here entail careful control of chromatic aberration. LCA was compensated with the filter-based Badal compensator whose operation is described in Jiang *et al.* [24]. Briefly, the relative defocus between the red and green wavelengths was altered by translating mirrors M2 and M3 a distance “z” in Fig. 1(a), such that LCA present natively in the eye was minimized. Subjects adjusted the relative focus subjectively until a 20/20 Snellen letter “E” appeared best focused *simultaneously* in both the red and green channels. In Jiang *et al.* [24], optimal visual performance and retinal image quality were demonstrated upon compensating LCA using the filter-based Badal system. Transverse chromatic aberration (TCA) was compensated using a red–green hyperacute alignment task [Fig. 2(a)]. Subjects were asked to position the red squares within the four green squares using arrow keys and the method of adjustment. Three measurements were averaged to yield relative TCA offsets that were compensated during stimulus generation. The same subjective paradigm was validated by Harmening *et al.* [25] against an objective TCA measurement in an AO-scanning laser ophthalmoscope (AOSLO). Once reliably estimated, pupil position during fixation is the dominant factor affecting TCA variability [26–28], and, therefore, pupil position was monitored continuously during the experiments. The pupil displacement measured during subjective alignment was set as baseline (y axis = 0 in Fig. 2) and deviations of pupil position from baseline were tracked with respect to time using a tracking system detailed in Domdei *et al.* [28]. Pupil positions estimated during six psychophysical runs of 250 s each are shown in Fig. 2(b). Blinks are filtered out from the pupil position traces. The average variability in pupil location was less than 100 μ m, corresponding to ~ 0.21 arc-min of TCA according to the relation established by Thibos *et al.* [27].

C. Cone-Resolved Densitometry

1. Optimizing the Bleaching and Imaging Wavelength—Given that all prior AO densitometry involved a two-step procedure—selective retinal bleaches followed by 543 nm imaging—we sought to test whether absorption imaging, with a single wavelength different than 543 nm, chosen preferentially to favor either L or M cones, can isolate their spectral classes. Ideally, the chosen wavelength to perform such selective wavelength densitometry ought to exhibit high absorption, and simultaneously, high differential L and M cone sensitivity. At $\lambda < 460$ nm and $\lambda > 660$ nm, the difference in sensitivity between L and M cones, is high, but absorption is too low to obtain reliable densitometry. To obtain a measure of absorption, double pass optical density (OD), defined as the log₁₀ ratio of image intensity after a complete bleach to the image intensity after dark adaptation, is used and was measured as ~ 0.3 – 0.4 log units for cones at 1.5 deg for 543 nm [15]. To determine the optimal wavelength and assess the extent of pigment absorption, we measured the OD in individual cones in the range from 500–600 nm using a multiwavelength AOSLO described

in Jiang *et al.* [24]. Briefly, the AOSLO consisted of multiple wavelengths originating from a super-continuum source (SuperK Extreme, NKT Photonics, Denmark), of which four illumination and detection channels could be simultaneously employed. Of these, two channels were reserved for wavefront sensing and near IR imaging, with illumination at 900 ± 16 nm, and 840 ± 15 nm, respectively. A superluminescent diode (Superlum Diodes Ltd., Ireland) provided the 840 nm illumination for near-IR imaging. The remaining two channels were used for densitometry with narrowband interference filters (25 nm FWHM) centered at 496, 520, 543, 578, and 598 nm. Besides a wavefront sensor, the detection arm consisted of confocal imaging channels dedicated to capturing near-IR (840 nm), green–red (520, 543, 578, 598 nm) and blue–green (496 nm) light.

Subjects were dark-adapted for 4 min, and 1 deg field of view AOSLO videos were recorded for the aforementioned wavelengths at 1.5 deg temporal eccentricity. The videos were registered [29], and individual cones were located in the high signal-to-noise ratio registered image. The mean intensity for each cone versus time was fit to an exponential of the form below and its derivative calculated:

$$I(t) = \gamma - \beta e^{-\left(\frac{t}{\tau}\right)}, \quad (1)$$

$$\frac{dI(t)}{dt} = \frac{\beta}{\tau} e^{-\left(\frac{t}{\tau}\right)}. \quad (2)$$

The change in image intensity (dark adapted to bleached) was averaged across individual cones and yielded the OD across the different wavelengths (Fig. 3).

2. Dynamic Densitometry to Determine Cone Spectral Types—In the bleaching model above, γ is related only to OD of individual cones, while β and τ are related to double pass OD and, in addition, the spectral sensitivity. For 543 nm bleach/imaging, L and M cones should have similar β and τ , provided they have similar OD. At other wavelengths where there is a sensitivity differential between L and M cones, the dependence of the rate of change of intensity on β and τ is posited to yield differences in the time-dependent bleaching signatures of L and M cones. Therefore, we sought to test whether the slope of image intensity is sufficient to separate L and M cones. The rate of change of cone image intensity (dI/dt) was subjected to Gaussian mixture model clustering analysis, similar to previous reports [15-17], and cones were assigned to L and M subtypes based on their assigned clusters. The L:M cone ratios thus obtained were compared against flicker-photometric electroretinograms (ERGs) and genetic testing [30]. S3 was not imaged in AO retinal densitometry, and her cone ratio was obtained only from ERG.

3. Flicker-Photometric Electroretinograms and Genetic Testing—Spectral sensitivity was determined by adjusting the intensity of a narrow band test light until the ERG signal it produced exactly matched that produced by a fixed-intensity reference light. The test and reference lights were alternately presented at 31.25 Hz. These “null points” were obtained for a range of wavelengths, and this was done twice for each subject. The final spectral sensitivity values were corrected for lens absorption with an age-dependent

lens correction. To obtain an estimate of the L:M cone ratio, a subject's spectral sensitivity data were best fit to a weighted sum of an L- and an M-photopigment template [31]. Individual differences in the spectral sensitivity of the L-cone photopigment have been shown to greatly influence estimates of the L:M cone ratio derived from flicker photometry [31]. We remove variability in the L-cone spectral sensitivity as a source of error by sequencing each subject's L gene and using an individualized L-cone photopigment template to estimate their L:M ratio [30]. The derived L:M cone ratios have been demonstrated to reflect cone ratios obtained from AO imaging densitometry [17].

D. Psychophysics to Assess Red–Green Color Detection and Appearance

Previously, we reported results on AO isochromatic contrast sensitivity functions (CSFs) in an orientation discrimination task using the same instrument in Jiang *et al.* [24]. This was performed to ensure that retinal image quality in red (660 nm) and green (532 nm) was similar after LCA correction and exhibited optimal visual performance. The isochromatic CSFs showed a high spatial frequency cutoff above 50 c/deg, indicating that performance with monochromatic and chromatic aberration correction was ultimately close to the limit imposed by the foveal cone photoreceptor mosaic. Two separate experiments were conducted here as below involving chromatic stimuli. Note that the visual stimuli in the multiwavelength AO vision simulator differed in critical ways in comparison to conventional methods, most importantly in the characteristics of the physical stimulus and the one impinging on the retina. The red and green light beam paths in the apparatus that constituted the *external* stimuli were adjusted in their relative position and vergence to precompensate for the eye's chromatic aberration. In effect, the external stimulus was preconditioned in a manner so that after traversing through the eye's optics, it created the desired *retinal* stimulus, whose red–green grating constituents had the optimal spatial phase and wavelength-dependent focus. Henceforth, to differentiate the two and compare against previous work, we will refer to the physical stimuli and that falling on the retina as the *external* and *retinal* stimuli, respectively.

1. Red–Green Color Detection Sensitivity—The retinal stimuli were either horizontal or vertical gratings with counter-phase red–green spatial modulations overlaid with a Gaussian envelope ($\sigma = 0.25^\circ$). The retinal stimulus contrast was converted to cone contrast space using the Stockman and Sharpe 2 deg cone fundamentals [32] and expressed as the vector sum of L and M-cone contrasts for computing threshold. The maximum M-cone and L-cone contrasts were 0.89 and 0.13, respectively. The subject's task was to respond to the grating orientation with a keypress, either vertical or horizontal, for retinal stimuli shown foveally, varying in contrast and spatial frequency. Stimulus duration was 500 ms. No feedback was provided.

CSFs for red–green gratings were measured in four subjects using the quickCSF paradigm [33]. This method entails an adaptive estimation of the logCSF in each stimulus trial. The logCSF is of a log-parabolic shape, defined by four parameters—the peak gain, peak spatial frequency ($f_{\max}$), the bandwidth, and truncation. The cutoff spatial frequency, f_c , where the sensitivity drops to zero, was calculated as a function of the gain, bandwidth, and $f_{\max}$ (Eq. (3) in Ref. [34]). The bandwidth was not analyzed in favor of f_c because the former becomes

redundant. Because low spatial frequencies were not tested, the truncation parameter was not relevant. The spatial frequencies were sampled evenly in log space across nine points in the range 7–50 c/deg. The spatial frequency of 63 c/deg was tested in addition for S2. The priors were set to a peak gain of 70, $f_{\max} = 3$ c/deg and $f_c = 28$ c/deg. These are similar to the priors used in Lesmes *et al.* [33], where they also showed the robustness of the chosen priors. Because Lesmes *et al.* [33] reported similar CSFs (RMS error of 0.86 dB on average) using the ψ method and the qCSF method after 100 trials per run, each qCSF run here was terminated after 100 trials. For each subject, three–five runs were completed. The number of trials per spatial frequency is variable and is noted in Table 1. qCSFs were also measured across a range of red–green intensities from 25%–75% $R/(R + G)$ similar to Mullen [7] to assess whether the minima in contrast sensitivity occurs at slightly different $R/(R + G)$ ratios across spatial frequencies under our specific conditions. This was performed experimentally by adjusting the drive signal and the resultant intensities of the red and green LEDs. The retinal stimulus contrast obtained for the range of red–green intensities was converted to cone contrast space in the same manner as above.

2. Hue Perception Sensitivity—The retinal stimuli were either: (a) chromatic red–green counter-phase gratings (as the above experiment) whose red and green intensities were perceptually set for each subject, such that the appearance of the mixture was at the equilibrium of the red–green axis, i.e., to appear yellow, or (b) a grating with the same proportion of red and green intensity, but in a summed-modulation as opposed to counterphase, to form an isochromatic grating. Color appearance was assessed in a two-interval, forced choice paradigm in an effort to remove ambiguity in subjective reports of appearance. In a given trial, two intervals were shown in succession (interinterval duration: 1 s), containing either: (a) the red–green counterphase grating or (b) red–green summed grating. The subject was instructed to pick the interval containing the chromatic grating with a keypress. No feedback was provided. Stimulus duration was 500 ms. The retinal stimuli were varied in spatial frequency and contrast and shown foveally. The orientation was different in both intervals, either horizontal or vertical, and assigned randomly. At each spatial frequency, the starting contrast was set to 100% and adjusted in a two-down, one-up staircase up until five contrast reversals were obtained. The mean of the last three reversals was taken as threshold. Two spatial frequencies were interleaved per run. Three to five runs per spatial frequency for each subject were completed. To avoid intensity cues within the two intervals, a subset of the experiments was run under conditions where the mean luminance of the stimulus was altered by ± 5 percent, pseudo-randomly for one of the two intervals.

3. RESULTS

A. Cone Classification with AO Densitometry

Figure 3 shows the mean $\pm$ standard deviation OD across all L and M cones versus the wavelength of bleach and imaging. The highest OD values are obtained at 543 nm with values decreasing on either side of this peak as expected from the average of L- and M-cone sensitivities. By virtue of confocal light detection, the AOSLO has advantages in measuring OD by reducing extraneous stray light introduced due to scatter from other retinal layers and

the anterior optics. However, high macular pigment absorption at 1.5 deg eccentricity leads to insufficient density at 496 nm preventing its use for further cone typing analysis. From the ODs, we concluded that at 578 and 598 nm, sufficient density may be available for probing differential L and M cone bleaching responses. We report cone classing with 578 nm wavelength densitometry alone for delineating L and M cones here.

The cone ratios of the three subjects (S1, S2, and S4) varied over a wide range, as illustrated in Fig. 4. There was good agreement between the results from imaging and those from the flicker photometric ERG. For the subject with lowest L:M ratio, the value obtained from imaging was 1.8:1 L:M, and the estimate from the ERG was 1.9:1 L:M. S2 was estimated to have an L:M ratio from imaging of 2.8:1 L:M compared to 2.7:1 L:M from the ERG. S4 is a carrier of a deutan color vision deficiency according to her genetic analysis and from imaging she was estimated to have a 13.3:1 L:M ratio from imaging and a 12.9:1 L:M ratio from ERG.

B. Red–Green Detection Sensitivity

Table 1 summarizes the spatial frequency range, the number of trials per spatial frequency (averaged over the number of qCSF runs), and the number of qCSF runs for each subject. The mean $\pm$ std (averaged across the separate qCSF runs) of the two parameters—the peak gain and the cutoff spatial frequency f_c —are noted for each subject. High sensitivity as denoted by the gain and f_c was obtained for red–green retinal stimuli detection once monochromatic and chromatic imperfections were removed (Fig. 5). Figure 5(a) shows the individual qCSF runs for each subject, and Fig. 5(b) shows the mean $\pm$ std qCSF for each subject together for comparison. The CSF reproducibility for each subject is noted in Fig. 5(a), Table 1 gain and f_c , and Fig. 5(b) shaded region. The subject with the most skewed L:M ratio exhibited the poorest chromatic discrimination and showed the lowest spatial frequency cutoff (f_c) of all four subjects. The cutoff spatial frequency (f_c) was monotonically related to L:M ratios, while the gain was similar. Table 2 shows the p -values obtained from unpaired, two-tailed, Student's t -test for a subject-wise comparison of these two parameters obtained from the qCSF. The p -values are not corrected for multiple comparisons.

Contrast sensitivity was also measured for a series of $R/(R + G)$ ratios. The qCSF curves for the different ratios are shown in Fig. 6(a). These measurements are from S1, where two qCSF runs of 100 trials were obtained at the relative intensity ratios shown. The qCSFs plotted in Fig. 6(a) are the means of both measurements. Overall, no systematic difference in the CSFs was observed by varying the relative intensities of the red and green primaries in the external stimulus. To summarize the effect of these intensity ratios on the CSF, the area under the log CSF (AULCSF) [33] is plotted in Fig. 6(b), where the lack of variation is also evident. Overall, in Figs. 5 and 6, higher sensitivities for red–green detection were measured here compared to previous studies. However, low spatial frequencies, where the lack of band-pass is typically observed in isoluminant detection, were not testable reliably here because the field of view and resolution constraints were preferentially chosen to favor higher spatial frequencies.

C. Red–Green Hue Perception Sensitivity

Figure 7 shows contrast sensitivity thresholds for hue perception for two subjects compared against their red–green detection sensitivity. Overall, in a two-interval forced choice paradigm, subjects were able to discriminate colored from isochromatic gratings with a high spatial frequency cutoff of 16–20 cycles/deg in the retinal stimuli. This is significantly lower than that for red–green detection. However, this result of contrast sensitivity of hue perception compares well against a previous report where isoluminant red–green detection thresholds were measured by counter-phase drifting red and green stripes imaged on the retina using laser interferometry upon bypassing the eye’s monochromatic and chromatic optical aberration [8].

4. DISCUSSION

There were three goals in this study. First, we investigated the feasibility of using single wavelength imaging densitometry to rapidly and efficiently classify cone types in human retina. Second, in subjects with known L/M ratios, and under conditions where chromatic and monochromatic aberrations are corrected, we sought to characterize thresholds for detecting red–green grating stimuli on the retina. Finally, we aimed to compare thresholds for detecting red–green retinal stimuli to thresholds for hue discrimination.

Cone classification has been performed with AO retinal densitometry, initially with an AO fundus camera and more recently using an AOSLO [15–17]. Besides the higher image contrast and resolution offered by the latter, the AOSLO provides a time-varying bleaching signature, that has the potential to provide key advantages over the single time point fundus camera snapshots of pigment absorptance. Thus far, the time varying aspect of the bleaching signatures was not used to classify L and M cone types, primarily because the temporal aspects of the bleaching model are dependent on pigment sensitivity to the imaging wavelength, and given how only ~543 nm was used for imaging, differential temporal L versus M cone characteristics were inaccessible. With the improved image signal-to-noise ratio available in an AOSLO, we measured sufficient OD at 528, 578, and 598 nm to potentially access differential pigment absorptance in L and M cones, given how at these wavelengths, L and M cones have sensitivity difference of 0.07, 0.17, and 0.37 log units, respectively. Here, we show feasibility of using densitometry at 578 nm and the rate of change of the bleaching signatures together to delineate L and M cones. However, cone image reflectivity variations [35] with stimulus and with time have the potential to reduce the accuracy of cone typing with single wavelength imaging alone. Therefore, moving forward, we suggest combining information from densitometry with at least two wavelengths to normalize these variations. Also, it will be interesting to compare the extent to which the amplitude, slope, or both characteristics of the bleaching signatures together are valuable in improving the accuracy of cone classification. Overall, it is encouraging that the cone ratios thus obtained with just single wavelength imaging densitometry have excellent correlation with ERG and genetic screening across three subjects with variable L:M ratios.

We observed significantly high sensitivity for red–green detection under optical correction. Moreover, contrast sensitivity for grating detection was correlated with the L/M cone ratio—the subject with the most skewed L:M ratio had the poorest chromatic discrimination with

the lowest spatial frequency cutoff of all four subjects. At the relative red:green external stimulus intensity ratio where red–green detection was measured, the M-cone contrast is 0.89, while for L cones it is only 0.13. Therefore, the M cones experience a contrast ~seven times higher than L cones. As the relative number of M cones decreases in our subjects, the L-cone dominated retina has access to 0.13 as the maximum quantal catch ratio in the red–green grating, while a progressively sparser mosaic of M cones has a far greater 0.89 as the maximum quantal catch ratio. Thus, it is reasonable to expect a higher L:M cone ratio to elicit a progressively lower high spatial frequency cutoff as observed in our experiments. Thresholds were only modestly affected over a range of relative intensities of the red and green primaries in the external stimulus, however, this was measured only in S1, whose L:M cone ratio is 1.8:1. More importantly, the search for an intensity match by varying the ratio of the two external stimulus lights failed to reveal any relative intensity where retinal stimulus discrimination failed completely; rather, the sensitivity remained relatively high across the entire range of ratios tested. That high spatial frequency red–green grating detection operates close to the receptor limits might be explained simply if subjects were using intensity artifacts caused by chromatic aberration for discrimination. Measurements of pupil displacement and its effect on TCA, together with the high performance obtained in isochromatic CSFs after LCA compensation, minimize this potential confound. Furthermore, given how detection of intensity modulations ought to remain immune to cone spectral composition, the monotonic variation of red–green color detection with L:M cone ratio we observed would be highly unlikely if the task relied on intensity discrimination caused by artifacts in transforming the external physical stimuli to retinal stimuli. Therefore, we conclude that at high spatial frequencies, red–green grating stimuli on the retina can be discriminated based predominantly on wavelength variations alone, and that its spatial characteristics approach the limits imposed by the spatial and spectral topography of the cone mosaic.

In comparison to thresholds for detecting red–green gratings, the thresholds for hue discrimination were significantly reduced. Subjects were able to discriminate colored from isochromatic gratings with a high spatial frequency cutoff of 16–20 c/deg compared to cutoff spatial frequencies of ~50 c/deg for red–green detection. The lower number of subjects in the hue perception task is a limitation, though the substantial difference between the color appearance and color detection tasks is similar between the two subjects tested here, suggesting that the result would be representative. Again, intensity artifacts in the transformation of external stimuli to retinal stimuli caused by the optics of the eye or an inadequacy in equating the red and green physical primaries for intensity might explain this behavior, however, the aforementioned reasons help exclude this. We sought to examine if other optical considerations in the conversion of the external stimuli could account for our observations. In a 15 c/deg red–green grating in the external stimulus, the spacing between the red and green maxima is 2 arc-min in visual angle. An equivalent amount of TCA in the eye would be required for the red and green bars of the external stimuli to be completely superimposed over one another in the retinal stimuli, to create an isochromatic grating with pure intensity modulations. Thibos *et al.* [27] described that TCA is proportional to LCA and pupil displacement. This relationship was later confirmed by Privitera *et al.* [26] and Domdei *et al.* [28] combining a pupil tracking system with objective TCA measurements in

an AOSLO. According to this relationship, a shift in TCA of 2.1 arcmin per mm pupil displacement is expected for our red and green primaries. Therefore, 1 mm pupil displacement will be needed to reduce a 15 c/deg red–green grating in the external stimulus to have pure intensity modulations in the retinal stimulus, a magnitude that is exceedingly high on the basis of our pupil position estimates in Fig. 2. The possibility that subjective TCA measurements may not fully capture the eye’s TCA and the objective measures can also be ruled out because both have been validated against one another previously [25]. The reliability of objective TCA measurements has been demonstrated in a few ways, in particular, Winter *et al.* [36] used it to quantify the human eye’s TCA and showed good agreement with previous theoretical estimates [37]. In addition, cone-targeted psychophysics undertaken in an AOSLO with eye-tracking employed the same objective TCA compensation and showed reliable percepts associated with single cone stimulation [38–41]. Given the reliability of TCA measurement and pupil monitoring, we can effectively eliminate it as a factor affecting our hue perception experiment.

Compared to previous work where typically lower sensitivity has been observed for the detection of red–green gratings, our results may at first seem surprising. Of most relevance is the work by Sekiguchi *et al.* [8] where all optical imperfections were bypassed via dual-laser interference fringes and red–green detection cutoff spatial frequencies of ~20 c/deg were observed—very similar to the hue discrimination performance measured here. In Sekiguchi *et al.*, subjects adjusted the contrast until no chromatic stripes were visible at any time during each stimulus drift period. Because the detection threshold criterion of isoluminant red–green grating was tied closely to the visibility of hue at all spatial frequencies, this previous experiment draws close parallels to our psychophysical experiment here aimed at measuring hue perception. The main difference is our use of a forced-choice method compared to a method of adjustment used previously. Therefore, it is reasonable to expect good similarity between them. The red–green detection measurements conducted here used an orientation discrimination task, and subjects did not receive instructions pertinent to perceiving hue. Subjects verbally reported that at times, both the high spatial frequency isochromatic and red–green gratings appear to have a superimposed indistinct splotchy pattern of desaturated colors, implicating chromatic aliasing [42]. Similar chromatic noise appearance was reported by observers in Sekiguchi *et al.* [8]. A key advantage of laser interference fringes over correcting aberrations with AO is that the effect of diffraction due to the finite pupil size of the eye can be eliminated in interferometry. The red and green primaries, 660 and 532 nm, respectively, undergo slightly different spatial frequency dependent contrast attenuation (~1–6% in the range between 5 and 30 c/deg) due to diffraction for a 6 mm pupil. This difference, albeit small, could potentially lead to a cue for detection. A larger red intensity than green can, in principle, be used to alleviate the higher contrast attenuation at longer wavelength due to diffraction. However, we did not observe variations in detection thresholds across a range of red–green intensity ratios in the external stimulus (Fig. 6).

Given how both tasks were performed without regard to the subjective appearance of the retinal stimuli, these experiments can be used to draw inferences that assess the human visual system’s capacity to use wavelength information for spatial vision versus hue discrimination. Two observations about the subjective appearance of the gratings are worth

noting: (1) Even at the highest spatial frequencies, subjects had the strong impression of seeing relatively distinct bars of a particular orientation, indicating that the visual system can use chromatic information for fine spatial vision tasks at or near the limits imposed by the foveal cone mosaic, even though the hue itself is not consciously or readily available. Hue, on the other hand, is restricted at a spatial scale considerably below the resolution of the cone mosaic. (2) At spatial frequencies above 16–20 c/deg, the appearance of a red–green grating closely resembles a high contrast intensity modulation, indicating that the same neural mechanism may mediate high spatial frequency red–green detection and isochromatic or intensity modulations.

The coarse resolution of hue discrimination is reminiscent of earlier findings where only a small fraction (~1/3rd) of cones were associated with strong hue sensations [39,41], while the majority signaled achromatic sensations. That the cone mosaic readily serves the dual role of both high acuity spatial vision and red–green hue sensations indicates that the patterns of cone activity certainly encode sufficient information to retrieve wavelength and intensity independently from the environment. However, how the signals from the cones are parsed to serve these diverse functions remains unknown [43]. Detailing the spatial characteristics of hue and achromatic detection as such allows probing postreceptoral pathways responsible for this segregation. The coarser spatial grain of hue perception observed here compared to chromatic/achromatic detection reveals a fundamental bottleneck of the parvocellular system separable from mechanisms limiting spatial resolution.

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Fig. 1.

LCA compensation and system layout—adapted from Jiang *et al.* [24] here for completeness. (a) A typical Badal optometer consists of two focusing elements (L1 and L2) and flat mirrors (M1–M4) that are together used to form an afocal telescope. M2 and M3 are placed on a translation stage. Moving the stage (z) can alter the optical distance between L1 and L2 introducing extra vergence at the exit pupil (the eye’s pupil). Without the long-pass filters (LPF) in place, the eye’s longitudinal chromatic aberration forces the two wavelengths—red and green in this case—to be defocused relative to each other. Once the two LPFs are inserted between the mirror pairs M1 and M2 and M3 and M4, the longer (red) and shorter (green) wavelength undergo different relative focus that can be tuned exactly to be equal and opposite the eye’s longitudinal chromatic aberration. (b) Optical layout of the multiwavelength adaptive optics vision simulation (AOSIM) system published in Jiang *et al.* [24]. L, lens; M, Mirror; LP, Long-pass filter; BS, Beamsplitter; DM, Deformable mirror; WFS, Wavefront sensor; SLD, Superluminescent diode; DMD, Digital micromirror device; P, Pupil plane; R, Retinal plane; AP, Artificial pupil. Details in text.

Fig. 2.

TCA measurement and pupil tracking example in one subject. (a) A red–green hyperacute alignment task based on method of adjustment was performed to assess and compensate TCA once LCA was compensated. (b) Pupil position (x : blue, y : orange) traces during the course of six psychophysical trials lasting about 250 s. The standard deviation in pupil position was in the range of 50–90 μm . Average variability defined as the mean of standard deviation across these trials was 66.6 μm . The range of pupil displacements did not exceed $\pm 300 \mu\text{m}$.

Fig. 3.

Double pass optical density of individual cones. AO densitometry was used to assess optical density in single cones for wavelengths centered at 496, 520, 543, 578, and 598 nm. The data points and accompanying labels represent the mean $\pm$ standard deviation OD across all L and M cones.

Fig. 4.

LMS cone mosaics in the retinae of the three subjects. Each histogram and the image below it represent an individual subject's retina at 1.5 deg temporal eccentricity. L and M cones appear as two distinct clusters of cones in the histograms on the basis of their relative change in intensity under selective wavelength densitometry. Based on where a cone appears in the S versus L/M and L versus M clustering analysis, it is shaded as "blue," "green," and "red" to represent S, M, and L cones, respectively, in the retinal images. The scale bar is 2 arcmin.

Fig. 5.

Cone contrast sensitivity for red–green gratings obtained from the qCSF model fit. CSFs for individual subjects with different L:M cone ratios (a) show reproducible measurement across different runs. In (b), the mean $\pm$ std of the qCSF runs for each subject denotes decreasing spatial frequency cutoff for increasingly skewed L:M ratio. These CSFs were obtained for a red:green intensity ratio of 50:50. In (a), the open circles denote spatial frequencies where greater than 10 trials were obtained in each qCSF run. In (b), the open circles denote spatial frequencies where greater than 10 trials were obtained on average across runs.

Fig. 6.

Cone contrast sensitivity with variable red and green intensity ratios in the external stimulus for S1 obtained from the qCSF model. The retinal stimulus contrast obtained for the range of red–green intensities were converted to cone contrast space using the Stockman and Sharpe 2 deg cone fundamentals as described in Methods. In (a), the mean of 2 qCSF runs at each ratio and is plotted in different colors. Additionally, the open circles denote spatial frequencies where greater than 10 trials were obtained in each qCSF run. In (b), the area under the logCSF [in $\log(\text{cone contrast sensitivity})$ units] is plotted for the 2 qCSF runs as a function of the variable intensity ratios to summarize the cone CSF curves obtained via the qCSF method.

Fig. 7.

Cone contrast sensitivity for hue perception. Cone CSFs for red–green grating detection and hue perception are shown for two subjects (solid and dashed, respectively) whose L:M ratio is indicated. In the detection CSF (solid line), the open circles denote spatial frequencies where greater than 10 trials were obtained in each qCSF run. High spatial frequency cutoffs of 16–20 cycles/deg were observed for a discriminating hue, and overall this task exhibited significantly lower performance compared to the red–green detection task. In the latter, subjects were asked to discriminate the orientation of red–green gratings without regard for whether the grating appeared to be modulated in hue or isochromatic intensity.

Table 1.

Subjects' Characteristics, Psychophysics Variables and qCSF Result Parameters

	L:M Ratio ERG	L:M Ratio AO	SF Range (c/deg)	# of qCSF Runs	Mean # of Trials per Run across SF	Peak Gain	Cutoff sf (fc)
S1	1.9:1	1.8:1	[7,9,12,15,19,25,31,40,50]	3 (100 trials per run)	[6,13,8,8,9,13,11,11,22]	194.2 ± 25.0	61.6 ± 4.3
S2	2.7:1	2.8:1	[7,9,12,15,19,25,31,40,50,63]	5 (100 trials per run)	[9,8,11,11,13,12,7,12,15]	168.4 ± 18.2	63.6 ± 5.8
S3	5.0:1	–	[7,9,12,15,19,25,31,40,50]	5 (100 trials per run)	[10,7,13,11,12,11,13,13,17]	223.1 ± 38.9	53.2 ± 6.5
S4	12.9:1	13.3:1	[7,9,12,15,19,25,31,40,50]	3 (100 trials per run)	[8,14,9,8,6,8,14,16,19]	211.2 ± 26.9	45.1 ± 4.7

Table 2.Unpaired, Two-Tailed, Student's t -Test p -Values for a Subject-Wise Comparison of qCSF Result Parameters

Peak Gain					Cutoff SF				
	S1	S2	S3	S4		S1	S2	S3	S4
S1	–	0.15	0.29	0.44	S1	–	0.63	0.10	0.01
S2	0.15	–	0.02	0.03	S2	0.63	–	0.03	0.003
S3	0.29	0.02	–	0.62	S3	0.10	0.03	–	0.11
S4	0.44	0.03	0.62	–	S4	0.01	0.003	0.11	–