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Variability in human cone topography assessed by adaptive optics scanning laser ophthalmoscopy

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Abstract

Purpose—To assess between- and within-individual variability of macular cone topography in the eyes of young adults.

Design—Observational case series.

Methods—Cone photoreceptors in 40 eyes of 20 subjects aged 19–29 years with normal maculae were imaged using a research adaptive optics scanning laser ophthalmoscope. Refractive errors ranged from –3.0 D to 0.63 D and differed by <0.50 D in fellow eyes. Cone density was assessed on a two-dimensional sampling grid over the central 2.4 mm × 2.4 mm. Between-individual variability was evaluated by coefficient of variation (CV). Within-individual variability was quantified by maximum difference and root-mean-square (RMS). Cones were cumulated over increasing eccentricity.

Results—Peak densities of foveal cones are $168,162 \pm 23,529$ cones/mm² (mean ± SD) (CV = 0.14). The number of cones within the cone-dominated foveola (0.8–0.9 mm diameter) is $38,311 \pm 2,319$ (CV = 0.06). The RMS cone density difference between fellow eyes is 6.78%, and the maximum difference is 23.6%. Mixed model statistical analysis found no difference in the

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DISCLOSURE:

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST. T Zhang, P Godara, E R Blanco, R L Griffin, X Wang, C A Curcio, and Y Zhang reported no potential interest.

Contributions of authors: conception and design (CAC and YZ); analysis and interpretation (TZ, PG, ERB, RLG, XW, CAC, and YZ); writing the manuscript (TZ); critical revision of the manuscript (TZ, CAC, and YZ); final approval of the article (TZ, PG, ERB, RLG, XW, CAC, and YZ); data collection (TZ, ERB, XW, and YZ); obtaining funding (CAC and YZ); and literature search (TZ, CAC, and YZ); technical support and clinical supervision (XW and YZ).

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association between eccentricity and cone density in the superior/nasal ($p=0.8503$), superior/temporal ($p=0.1551$), inferior/nasal ($p=0.8609$), and inferior/temporal ($p=0.6662$) quadrants of fellow eyes.

Conclusions—New instrumentation imaged the smallest foveal cones, thus allowing accurate assignment of foveal centers and assessment of variability in macular cone density in a large sample of eyes. Though cone densities vary significantly in the fovea, the total number of foveolar cones are very similar both between- and within-subjects. Thus, the total number of foveolar cones may be an important measure of cone degeneration and loss.

INTRODUCTION

Macular cone photoreceptors in human eye are responsible for high acuity vision and color vision.¹ The spatial density of cone photoreceptors (cells/mm²) is an essential metric for assessing the fundamental limit of human vision^{2–9} and the effect of aging and disease.^{10,11} To ensure the validity of spatial density, accurate knowledge of cone topography and variability in adults in normal chorioretinal health is of paramount importance.

In 1990, Curcio and co-workers presented a comprehensive 2-dimensional map of both cone and rod density in 7 short-post-mortem retinæ prepared as whole mounts.⁹ This technique enabled visualization, topography, and morphological detail of inner segments, while largely eliminating histological processing artifacts and counting errors due to cells split by sectioning.¹² This widely cited study quantified salient features of human cone topography hinted in previous studies examining small retinal areas.^{13–20} These features include a peak of cone density at the foveal center, a sharp decline from the center, a lesser decline with increasing eccentricity, isodensity contours elongated along the horizontal meridian, and higher density in nasal than temporal periphery. With accurately localized foveal centers, it was possible to compare within- and between-individual variability in cone density and find a striking (three-fold) range of peak cone density. A subsequent study by the same authors showed that despite this wide range in density, the total number of cones within the 0.8–0.9 mm-diameter cone-dominated foveola was much less variable.¹⁰ Photoreceptor distributions in one pair of fellow eyes were similar but not identical, with an 8% difference in cone number and density differences up to 30% in spots.

Recent advance in adaptive optics (AO) assisted retinal imaging^{21–28} has made it possible to assess in the living human eye cone density,^{29–35} which could be characterized previously only by histology. Although AO retinal imaging offers significant advantages in studying the impact of chorioretinal diseases on cones, the density and the variability of the foveal cones, especially cones in the very foveal center, have not been adequately assessed. Paucity of these data may impede use of this imaging modality for larger studies involving more individuals. Recent technical progress in using adaptive optics scanning laser ophthalmoscopy (AOSLO),^{22–24,28,31,36–38} including a new-generation AOSLO that is used herein,³⁷ has provided appropriate resolution for imaging foveal cones. The goal of this study is to better assess between- and within- individual variability of cone topography in a large number of young adult subjects and to determine the total number of foveolar cones.

Many of the same advances originally developed for accurate histology⁹ are now possible with *in vivo* imaging.

METHODS

The study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board at the University of Alabama at Birmingham. Written informed consent was obtained from participants after the nature and possible consequences of the study were explained. The study complied with the Health Insurance Portability and Accountability Act of 1996.

Subjects

Twenty (n=20) subjects between 19 and 29 years of age were recruited from the student and employee population at the University of Alabama at Birmingham (UAB). After obtaining written consent, a self-report questionnaire was administered to assess participant general health and ensure that only subjects in good chorioretinal health and free from systemic diseases were included. We excluded the subjects who had undergone any keratorefractive procedures such as LASIK, which could influence the accuracy of retinal magnification factor. Then, participants underwent assessment of best-corrected visual acuity (BCVA) using the Electronic Visual Acuity (EVA) Tester and measurement of refractive error using an automated refractor. Eye lengths were measured using an ocular biometer (IOL Master 500, Carl Zeiss Meditec, CA). To minimize the effect of refractive error,^{30,31} only participants with refractive error between -3 D to 0.625 D and fellow eye refractive error difference <0.50 D were included.

AOSLO imaging

Both eyes of subjects were imaged with the UAB AOSLO. Specifications have been reported elsewhere.³⁷ In brief, the light source is a superluminescent diode (SLD) (Broadlighter S840-HP, Superlum, Russia). The wavelength is centered at 840 nm with a spectral bandwidth of 50 nm. This low coherence light source produces high fidelity retinal images by reducing light interference. The pupil size of the instrument is set at 6 mm diameter for young subjects. The AO consists of a deformable mirror with 97 actuators (Hi-Speed DM97-15, ALPAO SAS, France) and a custom high-speed wavefront sensor. The robust performance of the AO ensured that all cone photoreceptors were resolved across the maculae in all eyes. The imaging light power measured at the cornea was 500 μ W, or 3.8% of the maximum exposure permitted by the ANSI standard.³⁹ The AOSLO imaging channel consists of a photomultiplier tube (PMT) (H7422-50, Hamamatsu, Japan) and custom signal processing electronics controlling the scanning system and forming the output of the PMT to a video signal. The AOSLO acquires retinal images with a frame rate of 15 Hz. The field of view subtends approximately $1^\circ \times 1^\circ$ and the image is recorded at 512×512 pixel/frame.

The subject's pupil was dilated with 1.0% tropicamide and 2.5% phenylephrine hydrochloride. The head was aligned and stabilized with a head-mount and chinrest. A fixation target consisting of a moving light dot on a calibrated grid was placed in front of the eye via a pellicle beam splitter. At each point on the grid, the light dot paused for 2–3

seconds so that at least 30 frames were acquired. The subject was asked to follow and focus on the light target, while the AOSLO continuously recording video across an area of $10^\circ \times 10^\circ$ in the central macula. The continuously recorded retinal video allows a large macular image to be montaged from a series of successive frames with precise cone-for-cone registration in the overlapping area. A typical imaging session for both eyes lasted approximated 40 minutes.

Image processing

Image distortion caused by nonlinear scanning by the resonant scanner and by eye movements was eliminated by custom software.⁴⁰ Registered images (typically 10–30 frames) were averaged to enhance the signal-to-noise ratio. Images from different retinal locations were manually aligned (Photoshop, Adobe Systems Inc., Mountain View, CA) to create a continuous montage. Alignment was done on a cone-for-cone basis in areas of overlap.

Retinal magnification factor (RMF)

The angular pixel size of AOSLO images (degree/pixel) was computed using a calibrated grid placed at the retinal plane of a model eye. To convert angular scale to linear on the retinal plane, we used the distance from the imaging space nodal point to the retina to calculate individual retinal magnification ratio (RMF).³¹ This distance was calculated with the Liou and Brennan eye model,⁴¹ which closely resembles in vivo biological eye.⁴² Model parameters, including anterior radius of curvature of the cornea (r_1), anterior chamber depth (ACD), and axial length, were measured from each subject's eye with the IOLMaster. Corneal thickness was subtracted from the measured ACD to obtain the distance from the posterior cornea to the anterior lens. The posterior radius of curvature of the cornea (r_2) was estimated by $0.8236 \cdot r_1$.⁴¹ The distance from the nodal point to the retina, N_L , was calculated with optical design software (Zemax EE, Radiant Zemax, Redmond, WA). RMF was obtained with the equation,

$$RMF = N_L \tan 1^\circ.$$

Cone density measurement

Accurate quantification of macular cone distribution and variability has several technical challenges. First, the singular point of high density, i.e. the foveal center, must be accurately localized to serve as the origin of a coordinate system. Second, the steep gradient of cone density dictates the use closely spaced sample points to avoid interpolation artifacts.⁴³ Third, it demands the use of appropriately sized sampling windows to avoid artificial dilution of cone density at the peak.^{9,43,44} Fourth, even with robust AO correction for the wave aberration of the eye, visibility of cones in the foveal center may still be affected by light interference.⁴⁵ To meet these demands, we sampled at closely spaced points in the fovea where the cone density gradient is steep, optimized window size to capture the maximum density within the foveal singularity, and pooled data from small windows enclosing well-visualized cones to utilize all possible data in the foveal center.

Retinal coordinate system

The location of peak cone density served as the origin of a coordinate system with abscissa along the temporal-nasal direction and ordinate along the inferior-superior direction.⁹ Based upon the individual RMF, we generated a fiducial grid to mark the locations of sampling windows (Figure 1). We put a $40\ \mu\text{m} \times 40\ \mu\text{m}$ window over the area with the highest apparent cone density and then put five small windows whose size are of $5\ \mu\text{m} \times 5\ \mu\text{m}$ within this large window. We pooled cones in the five small windows to obtain an average and adjusted the position of the large window to set the origin of the retina coordinate.

Sampling strategy

The sampling pattern was adapted from that used in the histological study,^{9,43} where it was determined empirically to produce smooth isodensity contour maps, even near the foveal singularity. As shown in Figure 1, cone density was assessed at each intersection on this grid. At eccentricities $< 150\ \mu\text{m}$, the sampling interval is $50\ \mu\text{m}$; between $300 - 1200\ \mu\text{m}$ eccentricities, the interval was $300\ \mu\text{m}$. In the fovea ($0 - 150\ \mu\text{m}$ eccentricity), sample windows were pooled, as described above. To avoid blood vessels and seams in the image montage, sampling window positions were slightly adjusted.

Optimal sampling window

We assessed cone densities with different size square sampling windows ($2.5\ \mu\text{m}$ to $40\ \mu\text{m}$ on a side) in the eyes of 4 subjects (Figure 4). The windows were selected based upon center-center spacing provided by histology,⁹ for example, at the foveal center, where cone spacing is $2.4\ \mu\text{m}$, we tested the cone density measurement using square windows of size from $2.5\ \mu\text{m}$ to $40\ \mu\text{m}$. The $2.5\ \mu\text{m}$ window encompassed a single cone thus it was used for ascertaining that no cone-like spot with size bigger than $2.5\ \mu\text{m}$ could be taken as cones. At $50\ \mu\text{m}$ eccentricity, where cone spacing is $> 2.7\ \mu\text{m}$, square windows of $5\ \mu\text{m}$ to $40\ \mu\text{m}$ were tested. Through this process, we determined a series of heuristically optimal windows that yielded both a monotonically decreasing cone density profile with increasing eccentricity and cone densities close to the histological reference data (Table 1).

Cone counting

Based upon the test in 4 subjects (Figure 4), cone densities in the eyes of the rest of subjects ($n=16$) were assessed with the optimal window at each eccentricity (Table 1). To count cones automatically, we used the “find Maxima” function of ImageJ (version 1.47g; <http://rsbweb.nih.gov/ij/>), with manual checking. A well-established practice to provide unbiased densities is count cells only on the top and left edges of sampling windows, excluding those on the bottom and right edges.⁴⁶ However, we adopted an alternative method. Using the ImageJ “find maxima” function, we counted cones including and excluding cells on the edges and then averaged the two measurements. Results obtained with these two methods in 4 subjects at the sampling points along the primary horizontal and vertical retinal medians differed by $< 3\%$.

Inter-rater reproducibility was assessed by the intra-class correlation coefficient (ICC) based upon cone densities of 10 subjects measured by authors TZ, PG, and ERB working independently. ICCs from the Shrout-Fleiss⁴⁷ reliability random set were assessed along

nasal-temporal and inferior-superior meridians. ICCs among three raters were greater than 0.99.

Quantitative analysis and statistical evaluation

We computed mean density, standard deviation (SD), coefficient of variation (COV), and the total number of cones cumulated within rings of increasing eccentricity. We compared fellow eyes as follows:

1. Maximum relative cone density difference. Relative cone density difference Δ_i at each sampling point is estimated by $(D_{OS} - D_{OD})/D_{OD}$, where D_{OS} and D_{OD} are cone densities in the left and right eye, respectively.
2. Root mean square (RMS) of the relative cone density difference at the individual sampling locations, which is calculated by $RMS_k = \sqrt{\sum_{i=1}^{i=N} \Delta_i^2 / N}$, N is the number of subjects.
3. Root mean square (RMS) of the overall relative cone density difference across the studied retinal area, which is calculated by $RMS = \sqrt{\sum_{k=1}^{k=M} RMS_k^2 / M}$, M is the number of sampling points.

Within-individual variability was evaluated statistically using a mixed-model approach.⁴⁸ The retina was divided into four quadrants: superior/temporal (S/T), superior/nasal (S/N), inferior/temporal (I/T), and inferior/nasal (I/N). The density within each quadrant was compared between fellow eyes, and a three-way interaction term was included to examine whether the association varied across quadrants. The null hypothesis was that the distributions of fellow eyes of the same individual did not differ significantly. Analyses were performed using SAS v9.3 (SAS Institute Inc., Cary, NC). A p-value < 0.05 was considered significant.

RESULTS

Subject characteristics

As summarized in Table 2, subjects were 23 ± 3.5 years old. All had BCVA of 20/20 or better in both eyes. There were no significant differences between the left and right eyes in refractive errors, axial lengths, or RMF.

AOSLO revealed a clear cone mosaic across the macula

In all subjects, cones could be counted unambiguously over the central $2.4 \text{ mm} \times 2.4 \text{ mm}$ of the macula, including the foveal center (Figures 2 and 3). Cone densities at eccentricity < $300 \mu\text{m}$ are affected by the size of the sampling window (Figure 4), with smaller windows yielding higher density, as expected.

Between-individual variability

As shown in Figures 5 and 6, in right eyes, the peak density is $168,890 \pm 21,348 \text{ cones/mm}^2$ (mean $\pm$ SD), ranging from 140,257 to 234,391 cones/mm². In left eyes, the peak density is

$167,434 \pm 26,068$ (cones/mm²), ranging from 136,132 to 247,061 cones/mm². Both right and left eyes exhibit sharp density gradients. At 150 μ m eccentricity, cone density is 55% of the peak. At 300 μ m, it is one-third of the peak, and at 1.2 mm, one-tenth of the peak.

Mean, SD, and COV of cone density are shown in Figure 6. The SD follows a similar distribution as that of density with a more compact peak and a faster decline than mean density. The COV is less than 0.5 and does not have a sharp peak at the fovea. At the foveal center, COV is 0.12 in right eyes and 0.15 in left eyes. With increasing eccentricity, the COV decreases to a minimum at 300 μ m and increases beyond 600 μ m.

We computed the total number of cones in circles of increasing radius centered on the fovea center in fellow eyes (Figure 7). The number of cones within 100 μ m of the foveal center ranged from 2,914 to 5,068 cones (mean = 3,591; SD = 453; COV = 0.13). The total number of cones within 1 mm eccentricity ranged from 91,561 to 95,524 (mean = 93,823; SD = 1,910; COV = 0.02). Within the cone-dominated foveola (eccentricity $\sim$ 450 μ m),⁹ the mean total number of cones was 38,311 (SD = 2,319, COV = 0.06). Thus, the density of cones is highly variable between eyes at the foveal center, yet all eyes have a similar number of cones within 1 mm of eccentricity.

Within-individual variability

Figure 8 presents contour maps of macular cone density in both eyes of all subjects, showing qualitatively similar cone topography in fellow eyes. Mean cone densities, foveal center peak density, and cumulative number of cones do not differ significantly between fellow eyes ($p > 0.99$, Kolmogorov-Smirnov 2-sample test). There was no difference in the association between eccentricity and cone density in fellow eyes for quadrants S/N ($p=0.8503$), S/T ($p=0.1551$), I/N ($p=0.8609$), and I/T ($p=0.6662$).

The maximum relative cone density difference at a single sampling point in all subjects is 23.6%. The overall RMS of differences across the macula is 6.78%. The RMS relative difference at individual sampling locations (Figure 9) is lowest in the central fovea, reaching a minimum of 3.04% at 100 μ m nasal and 50 μ m superior.

Comparison to histology

AO imaging-based estimates of cone density has been historically benchmarked against the histological description by Curcio and co-workers.^{9,10} Our results along two primary retinal meridians (Figure 6) agree well with histology ($p = 0.9983$ for both eyes, Kolmogorov-Smirnov 2-sample test), as do the total number of cumulated cones (Figure 7) ($p > 0.99$ for both eyes, Kolmogorov-Smirnov 2-sample test).

DISCUSSION

Accurate assessment of between- and within-individual variability in human cone topography is important for interpreting the effects of aging and disease on photoreceptors. Our study extends previous work in several ways: 1) we image the cone photoreceptors across the central macula, including the foveal center, in a larger number of subjects with tightly controlled age range and refraction status. 2) We created 2-dimensional maps of cone

topography. 3) We assess the variability not only between but also within individuals. 4) We estimate the total number of cones within the cone-dominated foveola. A major accomplishment of our study is foveal cone densities in a sample of 40 eyes, an order of magnitude more eyes than in previous studies and important for other aspects of accurate macular morphology.

Characteristics obtained by histology have been largely corroborated and extended by AO imaging assessment.^{29–35,49} Yet only 2 studies using AOSLO to date reported cone density or center-to-center spacing in the very foveal center, and both assessed fewer than 5 eyes.^{31,36} Large inter-subject variability of foveal cones was shown by Li and co-workers who analyzed foveal centers in 4 eyes.³¹ Recently Lombardo and co-workers used an AO flood illuminated fundus camera to quantify cones in fellow eyes (within-individual) along the parafoveal horizontal meridian only.⁵⁰ They reported a high intra-class correlation of density at each retinal eccentricity in fellow eyes. However, their data was limited by the resolution of the imaging system and did not include the fovea, which manifests the greatest variability.^{9,31}

Our study revealed that foveal cone density topography in the living human eye follows that provided by histology. Peak densities in our study ranged from 136,132 to 247,061 cones/mm², yielding a 1.81-fold difference, smaller than the 3.3-fold difference reported in histology. The variance and the COV in this study are qualitatively similar to those reported by Curcio and colleagues.⁹ The variance of cone densities is high within 300 μ m eccentricity (indicating a large inter-subject variability) and is low beyond 300 μ m. The COV has a high value at the foveal center and declines to a ring of local minima at about 300 μ m eccentricity, and then slightly increases beyond this ring. However, the variance in our study was lower than in histology. At the fovea center, the COV was 0.126 in right eyes and 0.156 in left eyes, compared to the histological COV of 0.46. The lower variance in this study could be attributed to two factors. First, our sample size was larger (40 eyes in 20 subjects as compared to 8 eyes in 7 subjects). Second, *in vivo* measurements are not affected by tissue volume change and distortion due to post-mortem edema and swelling, which are concerns in histological study of the delicate fovea.^{8,9}

The highest cone density we measured is 247,061 cones/mm², whereas histological measurements reported as high as 288,600 cones/mm² and 324,100 cones/mm².^{9,16} AOSLO uses the eye as an objective lens, and spatial resolution is ultimately determined by the useful pupil size and eye length after AO correction for ocular wave aberration. We wondered if the absence of very high densities from our sample is due to the resolution of our AOSLO. Resolution can be estimated with the Rayleigh⁵¹ and Sparrow criteria.⁵² With digital images, the Sparrow criterion is more appropriate, because it quantifies pixel intensity.⁵³ With the light of wavelength 840 nm, a 6 mm diameter pupil, and a focal length assumed to be 22.2 mm in an emmetropic eye, the lateral resolution of the UAB AOSLO is 2.18 μ m by the Sparrow criterion. From cone density d (cells/mm²), we calculate the center-to-center spacing S_{cc} (μ m) of foveal center cones, assuming a perfect hexagonal lattice,⁵⁴

$$s_{cc}=1000[2/(\sqrt{3}d)]^{0.5}$$

For right eyes, mean spacing in the foveal center is 2.61 μm (range, 2.22 to 2.87 μm). For left eyes, mean spacing is 2.62 μm (range, 2.16 to 2.91 μm). Thus, the smallest spacing measured in our study is comfortably within the resolving power of our AOSLO and comparable to published values.^{8,31,36} Although several other groups presented AOSLO images of the foveal center,^{28,31,36,38} none has yet reported cone density as high as those seen histologically. It is also possible that more densely packed cones may have been missed by our AOSLO due to the diffraction limit. This may be investigated by using light sources with a shorter wavelength and a larger pupil. Thus, we refrain from excluding the possibility of higher cone densities than those observed in this study. Despite this concern, the diffraction-limited performance of our AOSLO provides a good estimation of the biological center of the fovea.

Accurate foveal centration and dense retinal sampling enabled us to compute the cumulative number of cones at different eccentricities, and the number of foveolar cones is 38,311, in reasonable agreement with histological estimates.^{9,14} Cones cumulated from AOSLO images differ that from histological flatmounts at further eccentricities, where rod photoreceptors intrude. It has been hypothesized that variability in foveal cone density may be related to variability in rate, timing, or extent of retina involved in the distinctive developmental increase of cone density.^{6,9,18,19,55,56} Packing density of foveal cones is low at birth and increases through early childhood. Photoreceptors that are short and squat begin to thin, lengthen, and squeeze together, greatly increasing the packing density of inner segments. This process occurs concurrently with lengthening and centrifugal spreading of synaptic terminals that contact bipolar neurons displaced laterally by the foveal depression. We hypothesized that different eyes had a similar total number of foveolar cones yet variable peak densities due to genetically- or environmentally-induced variation in the developmental re-distribution sequence.^{6,9} The total number of cones within the foveola (COV=0.06) is relatively invariant relative to peak density (COV=0.14), both within and between subjects (Figure 7), consistent with this idea. Thus total foveolar cones can be considered as a robust metric for assessing the impact of age, disease, and treatments on the cone mosaic. Whether variation in foveolar cone density explains variation in visual performance is an interesting but heretofore unanswerable question. Our methods to visualize the finest cones can be combined with AO-assisted methods to assess visual acuity precisely,⁵⁷ in the same individual, making this question newly approachable.

Our results for fellow eyes may be compared and contrasted to those recently published by Lombardo and co-workers.⁵⁰ Relative to the Lombardo study, we enrolled subjects within a narrower range for both age (19–29 years vs 24–38 years) and refractive error (maximum error of –3 D vs –5.5 D).^{30,31} Relative to the instrument used by Lombardo et al, the UAB AOSLO has confocal imaging, a deformable mirror with more actuators to better correct the wave aberration and a low coherent light source with a broader spectrum to improve image fidelity.³⁷ These technical advantages enabled us to image foveal cones in all eyes (Figure 2) and produce a 2-dimensional map. In contrast, Lombardo and colleagues measured cone

density at only selected locations along the parafoveal horizontal meridian. While we agree with Lombardo and colleagues' overall conclusion that macular cone distributions in fellow eyes are bilaterally symmetric, we found a greater difference between fellow eyes (RMS=14% vs 8.3%) and closer to histology, especially in the parafovea (Figure 9).

In conclusion, our study provides a comprehensive assessment of macular cone distribution and variation between and within subjects. We confirmed that in living human eyes, though cone densities vary significantly in the fovea, the total number of cones within the cone-dominated foveola is less variable within and between subjects. This study was confined to subjects of age 19–29 and aging effect on foveal cone density remained to be examined.^{11,49} Further data are needed to assess disease-induced cone loss in subjects of any age. Our study establishes a baseline for assessing aging, disease, and treatment effects on the cone photoreceptors.

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Biography

Tianjiao Zhang is a senior undergraduate student at the University of Alabama at Birmingham, graduating in May of 2015 with a Bachelor Degree in Biomedical Engineering. Since 2012 he has been studying human photoreceptor topography using adaptive optics imaging under the supervision of Christine A Curcio, PhD, and Yuhua Zhang, PhD. He has been admitted to the PhD program in Bioengineering at University of California at Berkeley and will study neural engineering.

Figure 1.

The retinal coordinate system and sampling grid for assessing human cone photoreceptor density. Cone density was counted at the cross points of the sampling grid. Within eccentricity $\leq 150 \mu\text{m}$, cone density was assessed with an interval of $50 \mu\text{m}$; between $300 - 1200 \mu\text{m}$ eccentricities, the sampling interval was $300 \mu\text{m}$.

Figure 2. Foveal cone photoreceptors imaged by adaptive optics scanning laser ophthalmoscopy in fellow eyes of the same subject. OD: right eye. OS: left eye.

Figure 3.

Macular cone photoreceptor in the left and right eyes of the same individual imaged by adaptive optics scanning laser ophthalmoscopy. Each panel is of $75 \mu\text{m} \times 75 \mu\text{m}$, showing well-resolved cone photoreceptors (the bright dots). The corresponding cone densities in cones/ mm^2 are listed beneath each panel. OD: right eye. OS: left eye.

Figure 4.

Macular cone photoreceptor density estimated with different sizes of sampling windows at different eccentricities. Histological cone densities at each data points are drawn for reference. Cone densities were counted with a series of sampling windows with sizes of 2.5 μm, 5 μm, 10 μm, 20 μm, and 40 μm. All assessments were done in the right eyes of the subjects.

Figure 5.

Topography of macular cone photoreceptor density assessed by adaptive optics scanning laser ophthalmoscopy. The top, middle, and bottom rows show the contour maps of the average, the standard deviation, and the coefficient of variation of the cone density in the fellow eyes of the 20 subjects, respectively. OD: right eye. OS: Left eye.

Figure 6.

Cone photoreceptor density along primary retinal meridians in fellow eyes assessed by adaptive optics scanning laser ophthalmoscopy. The shaded region covers 1 standard deviation range of cone density values at corresponding eccentricity reported by Curcio and coworkers.⁹ To better show the comparison of the cone densities between our study and the histological study, the cone density plots are not drawn in linear scale along abscissa. Our results agree well with the histological results reported by Curcio and co-workers ($p = 0.9983$ for both eyes, Kolmogorov-Smirnov 2-sample test).

Figure 7.

Cumulative number of cone photoreceptor as a function of eccentricity estimated by adaptive optics scanning laser ophthalmoscopy. Our results agree well with the histological results reported by Curcio and co-workers ($p > 0.99$ Kolmogorov-Smirnov 2-sample test).

Figure 8.

Macular cone photoreceptor isodensity contour maps obtained by adaptive optics scanning laser ophthalmoscopy. The spatial density of cones (cones/mm^2) is plotted. Subjects' identification numbers are marked on the up-left corner of the maps. The bilateral symmetry of the cone density distribution in fellow eyes is apparent. OD: right eye. OS: Left eye.

Figure 9.

The root mean square of relative cone density difference assessed by adaptive optics scanning laser ophthalmoscopy. Large differences occur at the parafoveal retina.

Table 1

Optimal sampling window size for assessing cone photoreceptor density

Eccentricity	Optimal window size
0 – 100 μm	5 $\mu\text{m} \times 5 \mu\text{m}$
150 μm	10 $\mu\text{m} \times 10 \mu\text{m}$
300 – 1200 μm	40 $\mu\text{m} \times 40 \mu\text{m}$

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Table 2

Refractive error, axial length, and retinal magnification factor of the eyes used for assessing cone photoreceptor topography in this study

	Left eyes		Right eyes	
	Mean	Standard deviation	Mean	Standard deviation
Refractive error* (Diopter)	0.56	0.71	0.57	0.76
Axial length (mm)	24.47	0.82	24.42	0.78
Retinal magnification factor (μm/degree)	282.43	12.52	282.49	12.64

* Spherical equivalent