Variability in parafoveal cone mosaic in normal trichromatic individuals

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Abstract: Parafoveal function is important for daily visual tasks such as reading. Here the variability in cone density along the four cardinal meridians in parafoveal regions of the retina was investigated in vivo using an adaptive optics fundus camera. Ten healthy normal trichromatic individuals were included in the study. There were significant differences in cone density between individuals at all four tested eccentricities (0.5, 1, 2 and 3°) and meridians. Cone density ranged from 34,900 to 63,000 cones/mm² at 1° horizontally, and from 31,600 to 60,700 at 1° vertically. The results were consistent with those of Curcio et al. (1990), although between-individual variability is greater than previously reported in the parafovea from 1 to 3.2°.

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1. Introduction

Important daily visual tasks such as reading rely on good optics and a densely packed cone mosaic in the foveal center, as well as signals from cones and associated neural circuitry in the parafoveal region [1]. Curcio and associates [2] were the first to publish histological data on between- and within-individual variability in cone density from the foveal center to the midperiphery of the human retina. Cone density peaked in an area as large as 0.032 deg² with a large between-individual variability ranging from 98,200 to 324,100 cones/mm². The large variability near the foveal center disappeared at about 1° in their seven individuals. Curcio and Sloan [3] followed up with an analysis of cone mosaic regularity in one individual, revealing greater variation in the regularity of the cone mosaic in the foveal center than in the parafovea. Several laboratories where the cone mosaic has been imaged in vivo with different adaptive optics (AO) retinal imaging systems have reported cone density measures [1,4–7] and cone mosaic regularity calculations [8] near the foveal center that are mostly in keeping with Curcio and associates. Cone density or photoreceptor mosaic regularity has been shown to be reduced in some genetic disorders that cause red-green [9–12] and tritan color-vision deficiencies [13], but this reduction is not always significant because parafoveal cone density may vary more in a larger population than what is evident from Curcio’s data.

To be able to make appropriate inference at a cellular level of the retina with regards to changes that may be pathological it is necessary to increase the knowledge about variation in the normal population. Here we report the first set of cone density measures from a Scandinavian population of ten normal trichromats imaged in vivo with the Kongsberg adaptive optics ophthalmoscope (KAO). The results reveal statistically significant between-individual variation in parafoveal cone density and cone mosaic regularity.

2. Methods

2.1. Subjects

Ten normal trichromatic subjects, (8 females, 2 males) aged 20–30 yrs with axial lengths in the 21.43–25.75 mm range and best-corrected logMAR acuity 0.1–(−0.08) were included in the study. The study followed the principles embodied in the Declaration of Helsinki (Code of Ethics of the World Medical Association) and was approved by the Regional Committee for Medical Research Ethics for the Southern Norway Regional Health Authority. Informed consent was obtained from each subject after explanation of the nature and possible risks of participating in the study.

2.2. Clinical measures

Each subject was refracted to best monocular logMAR letter acuity with natural pupils at 6 meters. Each subject’s color vision was confirmed to be normal after testing with a battery of color vision tests including the Cambridge Colour Test (Cambridge Research Systems Ltd,

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Cambridge, UK), and Rayleigh and Moreland anomaloscopy and luminance matching (HMC Oculus Anomaloscope MR, Typ 47700, Oculus Optikgeräte GmbH, Germany).

Fundus photos of the central 45° (Topcon TRC-NW6S), and spectral domain optical coherence tomography (SD-OCT) 30° scan-width with 2 and 49 B-scans (100 and 19 frames respectively), 512 A-scans/B-scan, (Spectralis SD-OCT system, Heidelberg Engineering, Heidelberg, Germany) were performed on each subject and found to be normal and free of eye disease. Axial lengths were measured on each individual with an IOLMaster (Carl Zeiss, Germany).

2.3. Flood-illuminated adaptive optics retinal imaging

High-resolution images of the cone mosaic were obtained with the KAO. Figure 1 shows a schematic diagram of the system. The KAO is similar in design to that by Rha et al. [14]. The KAO employs 780 nm and 840 nm super luminescent diodes (SLD; Superlum, Ltd., Ireland) as light sources for the wavefront sensing and imaging channels respectively. The imaging source is used in conjunction with a step-index multimode fiber (Fiberguide Industries, NJ, USA) to reduce speckle noise. AO correction was performed over a 6.8 mm diameter pupil with a Mirao52 (Image Eyes, France) deformable mirror and a custom-built Shack-Hartmann wavefront sensor (SHWS: lenslet array 0300-7.6-S: Adaptive Optics Associates, Inc., MA, USA; camera Rolera-XR Fast 1394: QImaging, British Columbia, Canada). The magnification of the subject’s pupil to the Mirao52 and to the SHWS was 2.2 and 0.73, respectively. The adaptive optics control algorithm calculates the control signals to send to the deformable mirror by subtracting the product of the control matrix by the SHWS spot displacement vector and a scalar factor (the loop gain) to the previous control signal vector reduced by a small bleed factor to remove the potential build up of un-sensed mirror modes. The control matrix is the pseudo-inverse of the system response matrix, with some modes filtered using a singular value threshold. When closing the loop, monochromatic aberrations were measured with the SHWS and corrections were applied at 20 Hz until an acceptable mean SHWS spot displacement was reached. Then a sequence of retinal images was acquired by illuminating a retinal area of diameter 2.0° with a flash. The sequences of photoreceptor mosaic images were collected with a 12-bit high-speed CCD camera with a light sensitive area of 1024 x 512 pixels (Cam1M100-SFT, Sarnoff Corporation, NJ, USA) over a period of 180 ms at 167 Hz (6 ms exposure time resulting in 30 frames per sequence). Less than 250 image sequence exposures were acquired per observer. The pixel spacing in the resulting images is 0.587 \( \mu \)m/pixel in an eye with axial length 24.0 mm [15].

The subject’s head was stabilized with a dental impression on a bite bar. The dominant eye was dilated and accommodation suspended with Cyclopentolate 1%. Subjects were instructed to fixate on particular intersections of a black grid on a white background. Multiple locations across the fovea and parafovea, from 0.5° to 3° eccentricity along the four cardinal meridians, of the dominant eye were imaged.

The 780 nm AO SLD was continuous wave (CW) and the power at the cornea was up to 1.1 \( \mu \)W. The 840 nm imaging SLD was pulsed with a mechanical shutter so that each image sequence of 30 frames corresponded to a 180 ms exposure. If the mechanical shutter was to be left open, the CW power measured at the cornea was less than 1.6 mW. A maximum of 10 image sequences were collected at each retinal location, in bursts of 3 sequences at a time, with gaps of over 22 seconds in between bursts. The light exposure between bursts can be ignored, as the shutter of the imaging source was closed and the subjects were either asked to close their eyes and/or move away from the bite bar. The CW and pulsed maximum permissible exposures (MPEs) are performed using the ANSI guidelines [16] yield 0.56 and 14.4 mW respectively, for the AO and imaging sources. The sum of the fractional exposures as indicated by the ANSI indicates that the light exposure of both light sources combined was about 9 times below the ANSI MPE [17].

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2.4. Image analysis and statistics

Between 4 and 20 frames with good resolution when viewed with the naked eye were selected from the sampled images for each position and used in the analysis. Individual cones were identified via automated and manual methods in images produced from averaged co-registered frames [8,12,13] after removing artifacts with flat-field correction [14]. Retinal cone density [cones/mm²] was estimated over two 0.16° × 0.1° windows after correction of image scaling for individual axial lengths [15]. Mosaic regularity was assessed by constructing Voronoi domains for each cone by defining points in the plane that were closer to it than any other cone in the mosaic [8,13]. The number of neighbors of each cone was calculated over two windows containing 126–175 cones each, and subsequently the number of cones with six-sided Voronoi domains was determined; calculations were done with an automatic method [8]. Control calculations with windows with varying number of cones (76–125, 126–175, 176–225, 276–325 and 376–425) showed negligible differences: the standard deviation in percentage of cones with six-sided Voronoi domains was less than 3.5%. Each of the mosaic regularity windows was centered on each of the 0.16° × 0.1° windows used for cone density calculations.

Statistical analyses were done with StatPlus:mac 2009 (AnalystSoft Inc., USA). Pearson correlation coefficients were calculated and paired t-tests were performed when comparing two data sets. A one-way repeated measure ANOVA was performed to test within-individual difference, and one-way between-groups ANOVA with post-hoc tests were performed to test between-individual differences. Differences were considered significant when $p \leq 0.05$. Bonferroni adjustments were made to compensate for multiple comparisons where necessary.
3. Results

3.1. In-vivo images of the cone mosaic

Figure 2 shows images of the cone mosaic for two individuals at three different eccentricities. The darker regions surrounding the bright cone photoreceptors correspond to rod photoreceptors. The increase in cone size with eccentricity is evident for both individuals.

![Fig. 2. Cone mosaic images at three different eccentricities. Shown are images for two females 4008 and 4010 at 1° (a) and (d), 2° (b) and (e), 3° (c) and (f), from temporal and nasal parafovea respectively. Scale bars are 50 μm. Retinal magnification estimates are 268.4 μm° for (4008) and 288.0 μm° (4010).](image)

3.2. Parafoveal cone density

Cone density declined on average 6600 ± 3200 cones/mm² per degree, as shown in Fig. 3, where cone density values along all four cardinal meridians have been pooled together. The filled circles represent the median, and the vertical extent of each box represents the interquartile range. There was a significant difference in cone density between individuals at all four eccentricities and meridians (p ≤ 0.001). This difference was still significant at 0.5°, 2° and 3°; but not at 1° after Bonferroni adjustments were made. The cone density [cones/mm²] ranges estimated at 1° are 34,933–63,009 nasally; 36,706–59,305 temporally; 37,930–52,160 superiorly and 31,579–60,730 inferiorly, which translates to a cone spacing range of 6.05–4.28 μm. A tendency towards lower cone density with increasing axial length between 0.5 and 2° was observed. This tendency however, was only significant in the temporal meridian at 0.5° and 1° (R = –0.67 and –0.69 respectively; p < 0.05, data not shown). Analysis of within-individual variability revealed no difference in cone density at 0.5° in the four cardinal meridians (nasal, temporal, inferior, superior) analyzed. Within-individual cone density was significantly lower in superior parafovea when compared with nasal, temporal and inferior parafovea at 1° and 2° (p ≤ 0.05), whereas it was significantly lower in both superior and temporal parafovea at 3° (p < 0.05).

3.3. Parafoveal photoreceptor mosaic regularity

Figure 4 shows the range of percentages of cones with six-sided Voronoi domains represented by the extent of the vertical lines at each eccentricity in nasal and temporal parafovea. The largest between-individual variation in mosaic regularity was at 1° temporal; the individual with the lowest percentage had a mosaic where only 34% of the cones had six neighbors,
whereas the individual with the highest percentage had a mosaic where 64% of cones had six neighbors.

![Fig. 3. Spread in cone photoreceptor density between individuals at four different retinal eccentricities. The filled circles are the median values; the vertical lines encompass the full range between the minimum and maximum values, and each box shows how the middle half of all cone density values distributed at each eccentricity.](image1)

![Fig. 4. Cone photoreceptor mosaic regularity in terms of cones with six-sided Voronoi neighbors between individuals at four different eccentricities. Other details as for Fig. 3.](image2)

Comparison of the percentage of cones with six-sided Voronoi domains revealed a difference in cone mosaic regularity between-individuals at all four eccentricities, but the difference was not significant after Bonferroni adjustments had been made. Analysis of within-individual variability revealed no difference in mosaic regularity in the four eccentricities and meridians analyzed.
4. Discussion

4.1. Parafoveal cone density

This is the first report of between-individual variability in cone density and mosaic regularity within the 0.5–3° of the parafovea in a young Scandinavian population. Table 1 shows mean cone densities and standard deviations at 1° and 3° temporal in comparison with densities from other studies that have reported values along the temporal meridian. Between-individual differences in cone density at 1° were consistent with previous in-vivo studies [10,12], but almost twice as large as reported in histological studies [18]. The individuals with the highest cone density had between 46 and 56% more cones than those with the lowest cone density (Fig. 3).

Table 1. Mean cone density and standard deviations (SD) along the temporal meridian

|                         | 1°   | 1°   | 2.5–3° |
|-------------------------|------|------|--------|
| Axial lengths           | Mean | SD   | Mean   | SD   |
| This study              | 21.43–25.75 | 20–30 | 30,963 | 5419 |
| Curcio et al. 1990      | 21.165 | 2425 |
| Chui et al. 2008        | 22.52–27.41 | 21–31 | 16,497 | 3170 |
| Carroll et al. 2009     | 22.52–27.41 | 18–31 | 25,721 | 3506 |
| Wagner-Schuman et al. 2010 | 22.51–27.41 | 18–32 | 48,903 | -    |

- Data not available.
- Male/females.
- Mean ± SD.
- 2.5°.
- 3.2°.
- 2.8°.
- Data not available.

The mean cone density and variation were 70–80% larger at 3° (0.8–0.97 mm) than in Curcio’s data set at 2.8° (0.8 mm) and 3.2° (0.9 mm). In another histological study that included 27 individuals aged 27–90 yrs it was concluded that mean cone density within the central 4 mm was stable throughout adulthood, but the youngest individuals did have higher mean cone density than the older ones [19]. The one with the highest mean cone density was the only one below 30 years of age and it was suggested that there might be an early decline in extrafoveal cone density [19]. This may explain the higher mean cone density observed at 3° here, and it may also explain the larger between-individual variation as it is likely that the range of cone densities within a young and narrow age group (20–30 yrs) is larger than the range across individuals from wider age groups (Table 1). The observed differences between histology and in-vivo studies is probably related to the fact that Curcio et al. [19] did not know individual axial lengths and based their calculations on the same retinal magnification factor for all.

One of the in-vivo studies reports much lower mean cone densities at about 3° (0.9-1.0 mm) [6]. Some of the variation between the in-vivo studies may be related to localization of the fovea and subsequently the accuracy of the position where the measurements of cone density was taken. There has also been some discussion regarding whether cone density decreases with increasing axial length [6,7], and there is a tendency for this in our data set between 0.5 and 2°, but not at 3°. Nevertheless, if the three data sets are considered as one population then what emerges is a clear indication that the variation in cone density is greater in the region around 3° than can be observed from just Curcio’s data set.
The finding that cone densities were lower along the superior meridian as compared with the other meridians confirms results from histology [18], but is at odds with one of the AO studies where they reported that cone densities were lowest along the inferior meridian [6].

4.2. Parafoveal photoreceptor mosaic regularity

The calculation of the percentage of cones with six-sided Voronoi domains is a metric commonly employed to assess the regularity of cellular mosaics in the retina (e.g. [3,8,20,21]). Between 34 and 64% of the cones assessed in this study were hexagonally packed, with little variation in median percentages at each retinal eccentricity, but larger variations in the range of percentages across the four eccentricities (Fig. 4). The total range of percentages is in agreement with histological- as well as in-vivo AO-studies [e.g. 3,8,10,13]. Curcio and Sloan [3] assessed the regularity of the mosaic of one individual and reported that it was more regular at 1.25° (0.36 mm), on the edge of the rod-free zone, than closer to fovea, and that regularity decreased towards 3° (0.8 mm), with little change beyond this eccentricity. The mosaic of the ten individuals assessed here was on average more hexagonally packed at 1° than at 0.5°, but the greatest variation was around 1° (Fig. 4). Analogous variation has been reported at 1.25° in normal trichromats, red-green and tritan color-deficient observers (40–70% Ref [8], 44–57% Ref [10], 47–75% Ref [12], 55–70% Ref [13]). The large variation observed in vivo around 1–1.25° may be because this is beyond the edge of the rod-free zone in some individuals, hence a more irregular mosaic. There is just one AO-study with reports on mosaic regularity around 3°, although they did not calculate regularity from Voronoi domains, but Fourier spectra. Their results suggest that it is first at 5° that the mosaic is considerably more irregular than at 1° [22].

Although a regular mosaic is expected to be important in terms of even sampling of high spatial frequencies [3,23], it is of great interest to begin to investigate what the implications of the large between-individual variation in cone density and the subsequent neural circuitry might be regarding daily visual tasks like reading.

5. Conclusions

The large between-individual variation in cone density calls for following individuals over time, which is of importance if one is to be able to differentiate emerging pathological changes from normal aging. It is understandable that the variation in mean cone density in the parafoveal region is considered small when compared with the much larger variation observed in the foveal center [1,18]. But, there seems to be a great redundancy of cones in the foveal center, and the question is if the statistically significant difference in mean cone density in the parafovea observed between individuals also reflects redundancy or whether it actually has a practical significance.

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