Vision Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

# Distribution differences of macular cones measured by AOSLO: Variation in slope from fovea to periphery more pronounced than differences in total cones

Ann E. Elsner *, Toco Y.P. Chui, Lei Feng [1], Hong Xin Song, Joel A. Papay, Stephen A. Burns

*Indiana University School of Optometry, 800 E. Atwater Ave, Bloomington, IN 47405, United States*

ARTICLE INFO

*Article history:*
Received 8 April 2016
Received in revised form 22 June 2016
Accepted 28 June 2016
Available online xxxx

*Keywords:*
Adaptive optics scanning laser ophthalmoscopy
Cones
Cone density
Cone distribution
Fovea
Foveal specialization

ABSTRACT

Large individual differences in cone densities occur even in healthy, young adults with low refractive error. We investigated whether cone density follows a simple model that some individuals have more cones, or whether individuals differ in both number and distribution of cones. We quantified cones in the eyes of 36 healthy young adults with low refractive error using a custom adaptive optics scanning laser ophthalmoscope. The average cone density in the temporal meridian was, for the mean ± SD, 43,216 ± 6039, 27,466 ± 3496, 14,996 ± 1563, and 12,207 ± 1278 cones/mm$^2$ for 270, 630, 1480, and 2070 μm from the foveal center. Cone densities at 630 μm retinal eccentricity were uncorrelated to those at 2070 μm, ruling out models with a constant or proportional relation of cone density to eccentricity. Subjects with high central macula cone densities had low peripheral cone densities. The cone density ratio (2070:630 μm) was negatively correlated with cone density at 630 μm, consistent with variations in the proportion of peripheral cones migrating towards the center. We modelled the total cones within a central radius of 7 deg, using the temporal data and our published cone densities for temporal, nasal, superior, and inferior meridians. We computed an average of 221,000 cones. The coefficient of variation was 0.0767 for total cones, but higher for samples near the fovea. Individual differences occur both in total cones and other developmental factors related to cone distribution.



## 1. Introduction

A full understanding of the mechanisms of damage and the potential to rescue cones requires normative data with sufficiently narrow confidence limits that the effects of disease or treatment can be assessed. Cone density measured *in vivo* by using adaptive optics to correct for the ocular aberrations of the human eye, along with highly magnified retinal images, has large inter-individual variations not due to methodological considerations, particularly varying with aging (Chui, Song, & Burns, 2008a; Chui et al., 2012; Liu et al., 2014; Obata & Yanagi, 2014; Song, Chui, Zhong, Elsner, & Burns, 2011). At the fovea, individual differences in young, healthy eyes are reported to have a range of 1.81:1 (Zhang et al., 2015). The decrease in cone density with aging is found across both the central and more peripheral macula, but is particularly striking in the central fovea where the cones are densely packed in young adults. Nevertheless, in the healthy older eye there is still a general decrease of cone density with increasing retinal eccentricity. The marked central decrease with aging in foveal cone density is predicted from previous imaging studies of cone photopigment density, in which the central fovea has both decreased cone photopigment and decreased macular pigment (Elsner, Burns, Beausencourt, & Weiter, 1998). Similarly, there is a decrease with increasing age in foveal phase retardation, well-modelled by the near radial symmetry in the fovea of the birefringent Henle fiber layer (VanNasdale, Elsner, Hobbs, & Burns, 2011).

Another important factor in the measurement of cone density is the axial length of the eye, particularly noted for myopic eyes even when disease is not present (Chui, Song, & Burns, 2008b; Li, Tiruveedhula, & Roorda, 2010; Obata & Yanagi, 2014; Park, Chung, Greenstein, Tsang, & Chang, 2013). The increase in axial length alone is sufficient to lead to large individual differences, and the eye shape does not necessarily change in an identical manner for all subjects (Chen et al., 1992; Clark, Elsner, & Konynenbelt, 2015). Further, the retina does not stretch in an identical manner across the retina (Chui et al., 2008b).

* Corresponding author.
*E-mail addresses:* aeelsner@indiana.edu (A.E. Elsner), ypchui@indiana.edu (T.Y.P. Chui), fengleisx@126.com (L. Feng), hongxin_song@urmc.rochester.edu (H.X. Song), japapay@indiana.edu (J.A. Papay), staburns@indiana.edu (S.A. Burns).
[1] Dr. Lei Feng was on leave from the Eye Center, The Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, PR China.

http://dx.doi.org/10.1016/j.visres.2016.06.015
0042-6989/

The numbers of specific types of cells in the retina as measured by histology, is partly determined by cell fate, which is strongly related to genetics and species differences (Jelcick et al., 2011; Whitney et al., 2011). Other developmental factors may influence retinal neural cell numbers, such as the prenatal environment including exposure to light (Rao et al., 2013).

It has long been known that foveal specialization continues long after birth in humans, requiring 4–6 yr to develop a deep foveal pit free of inner retinal cells and having densely packed cones (Provis & Hendrickson, 2008; Yuodelis & Hendrickson, 1986). Cone densities in the foveal region, as measured from human fetal tissue, were different for two eyes from the same donor at 24 weeks of age, 28,300 and 37,900 cones/mm$^2$. The migration of cones from the more peripheral portions of the macula leads to a fovea containing densely packed cones, and this migration is not complete until after birth. With development, cone density increases greatly in the foveal region, with estimates varying among donors but reaching 100,000–324,000 cones/mm$^2$ in the foveas of adults (Ahnelt, 1998; Curcio, Sloan, Kalina, & Hendrickson, 1990). The re-distribution via migration of cones can be disrupted by diabetes, even if the overall numbers of cones may not be yet decreased by disease, as compared with healthy adolescents of similar age (Tan et al., 2015).

While the total number of cones is established before birth, the distribution is not. Retinopathy of prematurity can interrupt the process of photoreceptor re-distribution, and the fovea does not assume the normal pit shape with high densities of cones (Hammer et al., 2008). We examined to what extent the large variation in cone density is also found in the total numbers of cones in the macula. Although it is technically possible to image and count all the cones within the macula (Chui et al., 2008a), this requires considerable time for both the subject and the post-processing. For many subjects, particularly older ones, the time to collect data must be limited due to the tear film stability decreasing over time during a measurement session, as well as discomfort from maintaining stable posture and steady gaze for long periods of time. In contrast, using a smaller sample of data from which the cone densities across the macula and the total cones within the central circle of 7 deg radius, i.e. 14 deg diameter, can be modelled provides the opportunity to incorporate a wider range of subjects. Further, this type of model allows using arbitrary regions of interest to provide control data for cone density, so that studies with retinal disease can be compared to normative data with narrow confidence limits of for regions of interest not tested in controls. Without a model, the pronounced decrease of cone density with eccentricity, particularly in young subjects, leads to wide confidence intervals and a lack of statistical power if too large a range of eccentricities is averaged in the normative data. Thus, we modelled the total number of cones from samples of 36 subjects in two datasets, constraining our sample to be young healthy eyes with axial lengths within a narrow range.

## 2. Materials and methods

### 2.1. General

Cone density was measured as a function of retinal eccentricity, using the Indiana Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO) previously described (Burns et al., 2014; Chui et al., 2012; Song et al., 2011). Cone density data were obtained from the two groups of subjects described below: Group 1 subjects provided cone density data along the temporal, nasal, superior, and inferior meridians.

The Group 1 data were used to model the total cone count for each subject, by computing the relation between measurements of cone density along the temporal meridian to measurements of cone densities as a function of eccentricity in the temporal, nasal, superior, and inferior meridians. We modelled the relation of cone density, eccentricity, and meridian so that a total cone count could be obtained. Group 1 data were taken from our previously published data on aging (Chui et al., 2012; Song et al., 2011).

Group 2 subjects were newly recruited and data were analyzed only along the temporal meridian, where the larger retinal vessels are fewer than in the other meridians, to avoid errors in cone density due to sampling near large blood vessels (Fig. 1).

#### 2.1.1. Participants

All subjects had a full eye examination, including a subjective refraction and fundus examination. Axial length was measured with an IOLMaster (Carl Zeiss Meditec, Dublin, CA). The length measurements were used to exclude subjects with long eyes from the study, and to correct measurements of cone density for individual differences in axial length as described previously (Chui et al., 2008a, 2008b, 2012; Song et al., 2011). These criteria avoid the alteration in cone density due to retinal disease, aging, myopia or other factors leading to an abnormal eye length and therefore inaccurate cone density.

The data from Group 1 included cone density measurements along temporal, nasal, superior, and inferior meridians, from just outside the fovea to 7 deg eccentric (Song et al., 2011). These 10 normal subjects (6 females, 4 males, 21–28 yr old, 24.4 ± 2.17) were selected from this previously analyzed younger subjects group. Some of the data were re-sampled to use the right eye or left eye to achieve the least interference of blood vessel or other artifacts and to minimize interpolation and avoid extrapolation. The eyes that contributed data had the following criteria: refractive error from −2.50 to +0.25 D (−0.60 D ± 1.13), and axial length from 23.2 to 24.7 mm (24.2 mm ± 0.497).

Group 2 included 26 additional subjects (7 females, 19 males, 18–34 yr old (24.4 ± 3.83) with healthy eyes on ophthalmic exam and who had an axial length < 26 mm. Axial length for these eyes ranged from 23.2 to 25.8 mm (24.3 ± 0.463 mm). The mean age for the total of 36 subjects was 24.4 3.42 yr. Axial length was 24.2 ± 0.61 mm.

The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from all subjects, and we used consent forms and a protocol approved by the Indiana University Institutional Review Board for Human Subjects.

#### 2.1.2. Apparatus

Cone densities were measured using a second generation AOSLO, with the methods described previously (Burns et al., 2014; Chui et al., 2012; Song et al., 2011). Retinal images covering 530 × 550 μm of the retina were acquired at 820–840 nm ± 20 nm at 185 microwatts. Sequential groups of samples, with at least 50 video frames per fixation of were collected. For all Group 1 subjects, the samples formed a "+" shape around the fovea to include temporal, nasal, superior, and inferior meridians to at least 7 deg eccentricity. For Group 2 subjects, an abbreviated protocol was used for some of the subjects to ensure the highest quality data for the temporal meridian. Data collected in overlapping samples at eccentricities from fixation up to at least 7 deg, and the shorter session avoided a decrease in contrast found for some subjects due to tear film stability decreasing over time.

### 2.2. Image processing and data analysis for cone densities

To obtain a sample of cones with all pixels having the same size on the retina, a custom dewarping program adjusted each image to counteract the image stretching due to the sinusoidal scanning of

Please cite this article in press as: Elsner, A. E., et al. Distribution differences of macular cones measured by AOSLO: Variation in slope from fovea to periphery more pronounced than differences in total cones. *Vision Research* (2016), http://dx.doi.org/10.1016/j.visres.2016.06.015