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The organization of the cone photoreceptor mosaic measured in the living human retina

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ABSTRACT

The cone photoreceptors represent the initial fundamental sampling step in the acquisition of visual information. While recent advances in adaptive optics have provided increasingly precise estimates of the packing density and spacing of the cone photoreceptors in the living human retina, little is known about the local cone geometric arrangement beyond a tendency towards hexagonal packing. We analyzed the cone mosaic in data from 10 normal subjects. A technique was applied to calculate the local average cone mosaic structure which allowed us to determine the hexagonality, spacing and orientation of local regions. Using cone spacing estimates, we find the expected decrease in cone density with retinal eccentricity and higher densities along the horizontal as opposed to the vertical meridians. Orientation analysis reveals an asymmetry in the local cone spacing of the hexagonal packing, with cones having a larger local spacing along the horizontal direction. This horizontal/vertical asymmetry is altered at eccentricities larger than 2 degrees in the superior meridian and 2.5 degrees in the inferior meridian. Analysis of hexagon orientations in the central 1.4° of the retina shows a tendency for orientation to be locally coherent, with orientation patches consisting of between 35 and 240 cones.

1. Introduction

Studying the structural properties of the normal cone photoreceptor mosaic is important both to evaluate how the human visual system samples the world as well as to provide comparison date for understanding how aging and retinal diseases impact the sampling properties of the cone photoreceptors. The seminal paper by Curcio and colleagues (1990) expanded on earlier studies (Ahnelt, Kolb, & Pflug, 1987; Osterberg, 1935) to provide quantitative measures of the distribution and organization of the cone photoreceptors in post-mortem human retinae. It is now well accepted that for a given retinal eccentricity the cone density is higher along the horizontal (nasal and temporal) meridians than along the vertical (superior and inferior) meridians. Curcio and colleagues also computed local anisotropies in one eye (Curcio & Sloan, 1992) where they found that human cones are 10–15% farther apart along radii extending from the fovea than along isoeccentricity lines, (except at the edge of the rod-free zone, around 1° of retinal eccentricity).

Since that time, a number of approaches have been developed to make some of these measurements *in vivo*, including psychophysical experiments, based on interferometry (Coletta & Williams, 1987; Williams, 1988; Williams & Coletta, 1987) or speckle ocular interferometry (Marcos, Navarro, & Artal, 1996; Marcos, Tornow, Elsner, & Navarro, 1997) and scattering theory (Marcos & Burns, 1999). Most notable was the development of Adaptive Optics (AO) retinal imaging (Liang, Williams, & Miller, 1997), which allowed direct imaging of the cone mosaic in the living human retina. Using adaptive optics, it has been possible to individually identify cone photoreceptors and to quantify cone spatial organization (Chiu et al., 2013; Chui, Song, & Burns, 2008a, 2008b; Garrioch et al., 2012; Li & Roorda, 2007; Lombardo, Lombardo et al., 2013; Lombardo, Serrao, Ducoli, & Lombardo, 2013; Loquin et al., 2012; Merino, Duncan, Tiruveedhula, & Roorda, 2011; Roorda et al., 2002; Rossi & Roorda, 2010; Song, Chui, Zhong, Elsner, & Burns, 2011; Xue, Choi, Doble, & Werner, 2007; Zhang et al., 2015). A striking feature of quantitative photoreceptor data is the variability between and within individuals with a specific example being the variation in density of cones at the fovea. However, even at other fixed retinal locations, individuals vary widely in their photoreceptor packing density. While some studies reported the individual variability in cone packing between subjects (Li, Tiruveedhula, & Roorda, 2010; Song et al., 2011) other have described the variability in cone density within a subject at different retinal locations and

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eccentricities as well as global and local anisotropies in the cone photoreceptor packing within subjects (Chui et al., 2008a, 2008b).

The presence of anisotropies in the cone mosaic has been primarily studied using Voronoi diagrams (Shapiro, Schein, & De Monasterio, 1985). The Voronoi diagram, by connecting surrounding cones and characterizing the number of sides, allows assessment of the degree of hexagonality and how disease and aging can affect this aspect of packing geometry (Baraas et al., 2007; Carroll et al., 2009; Choi et al., 2006; Dees, Dubra, & Baraas, 2011; Lombardo, Serrao et al., 2013; Park, Chung, Greenstein, Tsang, & Chang, 2013). For Voronoi analysis to work well requires identifying every cone photoreceptor and positioning the center of each individual cone. Other methods, based on spatial frequency content, such as autocorrelograms (Rodieck, 1991) and the power spectrum of Fourier transform (Yellott, 1982), do not require the identification of each individual cone to quantify cone spacing and cones density. These studies have provided information on the hexagonality and spacing, but little information on local anisotropies of hexagonal packing. The presence of local anisotropies has been demonstrated in both human and non-human primate post-mortem tissue and the results suggest that cones tend to be clustered into relatively small regions of similar orientation (Ahnelt, 1998; Pum, Ahnelt, & Grasl, 1990).

In the current paper we introduce a technique, similar to the autocorrelation technique, which allows us to evaluate the cone mosaic on both a local and global basis. The new technique is based on cone-averaging to: (1) rapidly estimate cone spacing properties of the normal cone photoreceptor mosaic within relatively small areas without the need to identify every single cone (Burns, Zou, Qi, Zhong, & Huang, 2011); (2) evaluate the local anisotropy of the in-vivo cone photoreceptors mosaic, in the fovea as well as in the parafovea (up to 5° retinal eccentricity); and (3) provide estimates of the spatial organization and orientation mapping of the living human retina, similar to the analysis of Pum et al. (1990).

2. Materials and methods

2.1. Subjects

The right eye of each of 10 normal healthy subjects (ages of 24 to 36 years, mean 29.1 ± 3.6 yo) was imaged in the study. The average refractive error for the measured eyes was −1.55 ± 1.43 D (range 0 to −3.5D). Each subject's pupil was dilated with one drop of 0.5% tropicamide. The axial eye length for each subject was measured with a biometer (IOL Master; Carl Zeiss Meditec, Dublin, CA). Consent forms were obtained after a full explanation of the procedures and consequences of this study. The study protocol was approved by Indiana University Institutional Review Board and complied with the requirements of the Declaration of Helsinki.

2.2. High resolution adaptive optics scanning laser ophthalmoscope

We used the Indiana high resolution Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO) (Burns et al., 2014; Ferguson et al., 2010). In brief, the system uses a supercontinuum laser source (Fianium Ltd., Southampton, UK) to provide both the wavefront sensing (856 nm; 50 μW at the cornea) and the infrared imaging source (810 nm; 200 μW at the cornea), a Shack-Hartmann wavefront sensor and a woofer-tweeter wavefront control system (Zou, Qi, & Burns, 2008) to provide en-face high-resolution images of retinal structures, with the capability of focusing on superficial or deeper retinal layers. Images were obtained at a 28 Hz frame rate and a 15.1 kHz line rate. Light returning from the retina passes through a confocal aperture optically conjugated to the retinal plane. In the current study, the system was focused on the cone photoreceptor layer. Depending on the retinal location of the cone photoreceptors imaged, we used three different computer controlled field sizes to measure cone spacing at different eccentricities: 1° × 0.9° imaging field (size 1: 0.5 μm/pixel sampling) for eccentricities up to 0.86°, a 1.3° × 1.2° imaging field (size 2: 0.67 μm/pixel sampling) for eccentricities from 0.90° to 1.28°and a 2° × 1.8° imaging field (size 3: 1 μm/pixel sampling) for eccentricities from 1.38° to 5.15°. We used a 25 μm confocal aperture (0.5 Airy disk confocal aperture) when imaging with size 1 and a 75 μm confocal aperture (1.5 Airy disk confocal aperture) when imaging with sizes 2 and 3. The subject's head movements were stabilized using a chin and forehead rest.

2.3. Imaging the cone photoreceptors in the fovea and parafovea

Measurements of foveal cones in the center of the fovea were recorded while the subjects fixated at 9 locations of the 1° × 0.9° (size1) imaging field (4 corners, 4 middle edges and the center). Thus, nine retinal images were obtained comprising a ~2 × 2° montage of foveal cones with a 0.5 μm/pixel sampling size. A strip along the superior or inferior meridian – until finding a blood vessel (approximately 1° × 3° strip) – was also imaged by steering the imaging beam while the subject maintained fixation on a fixed central target provided by an auxiliary fixation system. This additional strip of images was used to improve alignment of montages derived from the same retinal location but with different sampling.

To image the parafoveal cones, four strips of cones along the four primary meridians (Temporal (T), Nasal (N), Superior (S), Inferior (I)) were recorded by steering the 2° × 1.8° (size 3) imaging field in a 1° step from the fovea to the parafovea up to 5° retinal eccentricity while maintaining fixation on the central fixation target. To ensure alignment between field sizes we repeated the measurement of foveal cones across the center of the fovea with the size 3 imaging field (subjects pointing their eyes at 9 locations of the imaging field as described for the small field size).

Additionally, 4 subjects (S2, S5, S7 and S8) were imaged by steering the 1.3° × 1.2° (size 2) imaging beam around the fovea in order to compute accurately the cone spacing and cone density at eccentricities from 0.90° to 1.28° along the four meridians. The measurements on S2, S5 and S8 were performed without dilation of the pupil as they had a pupil size larger than 6 mm. These measurements were performed in a different session on a different day – one to two months after the initial measurements.

The whole procedure for AOSLO imaging on each subject took less than 30 min.

2.4. Image processing and montaging with automated custom software

Images of cones were recorded as short videos (100 frames at 580 × 520 pixel/frame) digitized at each retinal location for later processing which involves the correction of scan distortion, an automatic selection of a template image from each video segment, and the alignment of the remaining frames at that location to the template frame. The result was a series of short video sequences with eye movement removed. We then generated averages based on the local best contrast (Huang, Zhong, Zou, & Burns, 2011) for each retinal location.

Images from different retinal locations were next automatically aligned to create continuous montages with a custom MATLAB routine that combined MATLAB (Mathworks, Natick MA), i2 k Retina (DualAlign, LLC) (using the command line executable of i2k Retina) and Photoshop (Adobe Photoshop CS6 extended) using the Photoshop MATLAB toolbox.

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