Variations in image optical quality of the eye and the sampling limit of resolution of the cone mosaic with axial length in young adults

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PURPOSE: To evaluate the variation in higher-order ocular wavefront aberrations and the Nyquist limit of resolution of the cone mosaic (Nc) in a population of young healthy subjects and the relation to axial length (AL).

SETTING: Fondazione G.B. Bietti IRCCS, Rome, Italy.

DESIGN: Case series.

METHODS: An adaptive optics retinal camera prototype (rtx1) was used to image the cone mosaic. Cone density and Nc were calculated at fixed eccentricity between 260 μm and 600 μm from the foveal center. Ocular higher-order wavefront aberrations were measured using the OPD Scan II device. The coefficient of variation (CoV) was used to analyze the variation in optical and retinal parameters. The correlation of optical and retinal parameters with AL was performed using Pearson analysis.

RESULTS: Twelve subjects (age 24 to 38 years; AL 22.61 to 26.63 mm) were evaluated. A high interindividual variation in the higher-order wavefront aberrations was found, ranging from 26% for corneal higher-order aberrations (HOAs) to 41% for intraocular HOAs. The CoV of cone density and Nc were 16% and 5%, respectively. The decline in cone density and Nc with AL was statistically significant at all retinal eccentricities (R² > 0.44, P < 0.001).

CONCLUSIONS: Although there appeared to be random variation in the eye’s optical wavefront aberration from subject to subject, the cone-packing density and Nc were highly correlated with AL. Although the eye’s overall image optical quality in the emmetropic group and the myopic group was comparable, the spatial sampling of the cone mosaic decreased with increasing AL.

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limits imposed by their optical aberrations because most visual tasks in our surrounding world are limited to seeing details in the range of low frequencies (between 5 cpd and 19 cpd). In general, the highest frequencies detectable by normal subjects viewing natural scenes are distributed between 20 cpd and 32 cpd.\textsuperscript{16}

With the advent of adaptive optics technology in vision science, it is now feasible to understand the relationship between optical quality and the sampling limit of resolution of the cone mosaic in the same subject. The in vivo analysis of the spatial distribution of cone photoreceptors can provide new information on the physical aspects of visual sampling. Researchers can now directly compare the optical and retinal performance in the same eye and further understand the basis of the variation in visual performance in the normal population.

Previous studies determined the photoreceptor cone density\textsuperscript{9–12} and the optical quality\textsuperscript{16–22} in healthy emmetropic and myopic eyes separately. In this study, we measured the interindividual and intrasubject variations in the optical quality of the eye and the sampling limit of resolution of the cone mosaic in a population of young adults. We sought to determine whether the optical and retinal parameters of the eye are correlated with the eye's axial length (AL).

**SUBJECTS AND METHODS**

Healthy volunteers participated in this study and signed an informed consent after receiving a full explanation of the procedure. All subjects were free of ocular or systemic disease, had 20/20 or better uncorrected or corrected distance visual acuity, and a scotopic pupil diameter of 6.0 mm or larger in both eyes. The study protocol had the approval of the local ethical committee and adhered to the tenets of the Declaration of Helsinki.

All subjects received a complete eye examination including noncontact ocular biometry (IOLMaster, Carl Zeiss Meditec AG), retinal imaging by scanning laser ophthalmoscopy–optical coherence tomography (Spectralis, Heidelberg Engineering GmbH), and ocular and corneal aberrometry (OPD-Scan II, Nidek Co., Ltd.; wavelength of light source centered at 808 nm).

Data Processing and Analysis

Image analysis of the photoreceptor mosaic was performed using Image J (version 1.45a, U.S. National Institutes of Health). For each eye, the acquired images were stitched together to create a larger montage (approximately 9 degrees x 9 degrees) of the photoreceptor mosaic using commercial software (Photoshop version CS3, Adobe Systems, Inc.). Each montage was verified by comparing it with the subject’s fundus image taken by the scanning laser ophthalmoscopy–optical coherence tomography device. The foveal center reference point for retinal coordinates was determined by finding the center of the image taken when the subject fixated on a yellow cross at a 0-degree angle compared with overlapping area of the 4 images when the subject fixated on a 1.5-degree upper nasal and temporal angle and on a 1.5-degree lower nasal and temporal angle. Cone density (cells/mm\(^2\)) was estimated at fixed locations, along the temporal and nasal meridians, at specific eccentricities from the foveal center (+260 \(\mu m\), +400 \(\mu m\), and +600 \(\mu m\)) throughout the montages. The program automatically identified the cone within two 50 \(\mu m\) x 50 \(\mu m\) windows (Figure 1). If required, the user performed manual editing to displace the window position in areas devoid of blood vessels and where cones were well resolved. Center-to-center cone spacing (intercone distance [ICD], minutes per arc [arcmin]) was calculated from the identified cone center considering that the photoreceptors are hexagonally arranged, as previously described.\textsuperscript{23} Accordingly, the Nyquist sampling limit of the resolution of the cone mosaic, \(N_y\) (cpd), was calculated as

\[
\sqrt{\frac{3}{2}} \times \text{ICD}
\]

Details of the model used in this study are given in the Appendix (available at http://jcrsjournal.org).

The retinal magnification factor was estimated using the nonlinear formula of Drasdo and Fowler\textsuperscript{24} taking into account the AL and the corresponding posterior nodal point distance in each eye. The spectacle-corrected magnification factor was then estimated for each eye by considering the

An adaptive optics retinal camera prototype (rtx1, Imagine Eyes) was used to evaluate the cone mosaic. The apparatus’ core components include a wavefront sensor (HASO 32-eye, Imagine Eyes), a correcting element (MIRAO 52-e, Imagine Eyes), and a low-noise, high-resolution, charge-coupled device camera (Rope Scientific). The adaptive optics system uses a low-coherence superluminescent diode centered at 750 nm to measure and correct optical distortions and simultaneously control the focus at the retinal layers. The second source (a light-emitting diode with wavelength centered at 850 nm) provides uniform illumination of the retinal field to be imaged (4 degree x 4 degree field).

Optimative retinal imaging sessions were performed after the pupils were dilated with 1 drop of tropicamide 0.5%. Both eyes of each subject were imaged; a video (40 frames) was recorded at various locations surrounding fixation. During adaptive optics imaging, fixation was maintained by having the patient fixate on a yellow cross that the investigator moved at fixed retinal locations.

After image acquisition, a manufacturer-provided program was used to correlate and average the captured image frames to reduce noise artifacts and produce a final image with an enhanced signal-to-noise ratio.

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spherical equivalent (SE) refraction (corresponding to the trial lens added to the system). The spectacle vertex distance was set at 14.0 mm for all eyes. The radial and area magnification factors were computed for each eye.

The ocular, corneal, and internal aberration data output for each eye were exported from the aberrometry system and processed using purpose-written software in Matlab software (version 7.0, The Mathworks, Inc.) for analysis. Excluding LOAs, 3rd- to 6th-order aberration data were computed by averaging 3 consecutive measurements over a simulated 6.0 mm pupil. The root mean square (RMS) values of the higher-order ocular, corneal, and internal wavefront aberrations were computed from the Zernike coefficients, and the more recent recommended notation was used. The modulation transfer function (MTF) and the point-spread function (PSF) in the eye, the anterior cornea and the internal optics were computed from the higher-order wavefront aberration (wavelength Z808 nm).

The Stiles-Crawford effect was incorporated into the calculation, using \( r = 0.05 \text{ mm} / C_0 \) as the shape factor value.

**Statistical Analysis**

The cone density was calculated by dividing the number of cones by the area of sampling window at each eccentricity. Representative cone-density measurements at different eccentricities were computed by averaging the density estimates at the temporal and nasal meridians. Two-way analysis of variance (ANOVA) was used to analyze the differences in cone density and \( N_c \) at different eccentricities.

The interindividual variance in the optical and retinal parameters was calculated using the coefficient of variation (CoV). The intraregional variance was calculated using the intereye ratio (expressed in %); that is, the ratio between the cone density or \( N_c \) differences in fellow eyes and the bilateral mean:

\[
\frac{R_{eye} - L_{eye}}{Mean R_{eye} - Mean L_{eye}}
\]

where \( R_{eye} \) and \( L_{eye} \) are the right and left data, respectively. The bilateral differences in refractive data, cone density, and \( N_c \) were evaluated using the paired Student t test.

The differences between emmetropic eyes and myopic eyes were evaluated using the unpaired Student t test. The correlation of optical and retinal parameters with AL was performed using the Pearson analysis. Statistical analysis was performed using SPSS software (version 17.0, SPSS, Inc.). Differences with a \( P \) value less than 0.05 were considered statistically significant for all the tests.

**RESULTS**

The study evaluated 12 subjects (3 men, 9 women). The patients’ age ranged from 24 to 38 years. The mean SE refractive error was \(-1.77 \pm 2.26 \) (SD) (range \(-5.50 \) to \(-0.25 \) D) with astigmatism less than \(-1.50 \) D when referenced to the spectacle plane. The AL was between 22.61 mm and 26.63 mm. In the emmetropic group, the mean age was 30.57 \pm 5.00 years, the mean SE refractive error was \(-0.14 \pm 0.16 \) D (range \(-0.25 \) to \(-0.25 \) D), and the mean AL was 23.37 \pm 0.46 mm. In the myopic group, the mean age was 30.00 \pm 4.43 years, the mean SE refractive error was \(-4.41 \pm 0.88 \) D (range \(-5.50 \) to \(-3.00 \) D), and the mean AL was 25.02 \pm 0.85 mm. No differences in SE refractive error or AL were found between right eyes and left eyes (\( P > 0.05 \), t test).

In general, the total amount of the whole-eye higher-order RMS was less than values of the anterior cornea or the internal optics except in 6 eyes (6/24 eyes, 25%). The ocular higher-order RMS values ranged from 0.20 to 0.53 \( \mu \text{m} \) (CoV, 29%). Corneal HOAs ranged from 0.21 to 0.59 \( \mu \text{m} \) (CoV, 26%). Intraocular HOAs ranged from 0.23 and 0.87 \( \mu \text{m} \) (CoV, 41%). Table 1 shows the mean values of the higher-order wavefront aberration...
data. Figure 2 shows the mean higher-order wavefront aberration maps. The corresponding mean PSFs and MTFs are plotted in Figure 3 and Figure 4, respectively.

There were no statistically significant differences in the mean total HOA values (mean difference 0.01 ± 0.08 μm), mean corneal HOA values (mean difference 0.02 ± 0.10 μm), or mean intraocular HOA values (mean difference 0.01 ± 0.14 μm) between right eyes and left eyes ($P > .05$, $t$ test).

There were no significant differences in total HOAs (mean difference 0.01 ± 0.01 μm) or corneal HOAs (mean difference 0.05 ± 0.02 μm) between emmetropic eyes and myopic eyes ($P > .05$, $t$ test); the amount of intraocular HOAs was, however, statistically significantly higher in myopic eyes than in emmetropic eyes (mean difference 0.13 ± 0.01 μm) ($P < .05$, $t$ test). There was no statistically significant correlation between the amount of HOAs and the AL ($r^2 = 0.005$ for total HOAs; $r^2 = 0.11$ for corneal

**Table 1. Mean AL, refractive error and RMS of ocular HOAs.**

<table>
<thead>
<tr>
<th>Group</th>
<th>AL (mm)</th>
<th>SE (D)</th>
<th>Total HOAs (μm)</th>
<th>Corneal HOAs (μm)</th>
<th>Intraocular HOAs* (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All eyes (n = 24)</td>
<td>24.13 ± 1.06</td>
<td>-2.15 ± 2.30</td>
<td>0.33 ± 0.10</td>
<td>0.38 ± 0.10</td>
<td>0.43 ± 0.16</td>
</tr>
<tr>
<td>Emmetropic RE (n = 7)</td>
<td>23.35 ± 0.47</td>
<td>-0.11 ± 0.20</td>
<td>0.32 ± 0.09</td>
<td>0.40 ± 0.12</td>
<td>0.35 ± 0.08</td>
</tr>
<tr>
<td>Emmetropic LE (n = 7)</td>
<td>23.29 ± 0.49</td>
<td>-0.18 ± 0.12</td>
<td>0.32 ± 0.13</td>
<td>0.40 ± 0.10</td>
<td>0.38 ± 0.08</td>
</tr>
<tr>
<td>Myopic RE (n = 5)</td>
<td>25.06 ± 0.76</td>
<td>-4.63 ± 0.86</td>
<td>0.33 ± 0.05</td>
<td>0.37 ± 0.09</td>
<td>0.51 ± 0.21</td>
</tr>
<tr>
<td>Myopic LE (n = 5)</td>
<td>24.97 ± 0.99</td>
<td>-4.29 ± 0.87</td>
<td>0.33 ± 0.11</td>
<td>0.34 ± 0.08</td>
<td>0.49 ± 0.18</td>
</tr>
</tbody>
</table>

AL = axial length; HOAs = higher-order aberrations; SE = spherical equivalent

* $P < .05$ between emmetropic eyes and myopic eyes ($t$ test)

**Figure 2.** Higher-order corneal wavefront aberration maps for average right eyes and left eyes in the emmetropic group and myopic group. The software overlaps the wavefront data for averaging with respect to the line of sight. The fixed color scale bar is in micrometers. The mean differences in RMS values between fellow eyes were lower than 0.03 μm both in the emmetropic and myopic groups. There is evidence of mirror symmetry in higher-order wavefront aberration between fellow eyes (HOA = higher-order aberrations).
HOAs, and $r^2 = 0.06$ for intraocular HOAs) (all $P > 0.05$).

**Cone Density**

Figure 5 shows the mean cone density and the mean $N_c$ as a function of retinal eccentricity. The mean cone density dropped from 49,393 ± 7,941 cells/mm² (peak 65,220 cells/mm²) at 260 μm eccentricity to 30,049 ± 4,954 cells/mm² at 600 μm eccentricity. The mean $N_c$ was 33 ± 2 cpd at 260 μm (peak 38 cpd) and 26 ± 2 cpd at 600 μm eccentricity. The CoV of the cone density and $N_c$ was 16% and 5%, respectively, at each retinal eccentricity, with higher values in myopic eyes (18% and 6%, respectively) than in emmetropic eyes (12% and 4%, respectively).

The intereye ratio of cone density was 6% at each eccentricity ($\leq 370$ cells/mm²; $P > 0.05$). The intereye ratio of $N_c$ was 3% ($\leq 0.2$ cpd; $P > 0.05$) at each eccentricity. There were statistically significant differences in cone density and $N_c$ between emmetropic eyes and myopic eyes at the specified eccentricities ($P < 0.01$, $t$ test) (Figure 5). Cone density and $N_c$ were statistically significantly correlated with AL (Figure 6). No correlation was found between the amount of HOAs and $N_c$ at any eccentricity ($P > 0.05$).

**DISCUSSION**

It is still under debate whether an improvement in the optical quality of the eye can optimize the visual resolution of the individual. Previous studies have shown that the main constraint to high-resolution visual acuity is the optics of the eye, mainly in cases of large pupils. However, there is a lack of knowledge about the variation in the theoretical maximum retinal resolution in the population. Cone density varies significantly among individuals; therefore, a direct correlation between optical quality and the retinal sampling in the same subject/eye would overcome the uncertainty caused by intersubject variability. With the advent of adaptive optics technology in clinical ophthalmology, it is feasible to estimate the
Nyquist limit of resolution of the cone mosaic \((N_c)\) by imaging the photoreceptor layer in vivo.

In this study, we evaluated the variation in the eye’s optical image quality and the Nyquist limit of the cone mosaic in a population of healthy young adults with SE refractive errors ranging between \(-0.25\) D and \(+5.50\) D and ALs ranging between 22.61 mm and 26.63 mm. We aimed to correlate the optical and retinal parameters of the eye to the AL. We believe that we therefore provide for the first time a detailed comparison of the optical and retinal performance between emmetropic eyes and myopic eyes.

Previous studies\(^1\),\(^\text{17},\)\(^\text{27},\)\(^\text{28}\) found high variation in higher-order wavefront aberrations. The interindividual variation in ocular aberrations, here expressed by CoV, ranged from 26% for corneal HOAs to 29% for total HOAs and 41% for intraocular HOAs. He et al.\(^\text{18}\) found a variation between 22% and 38% in the mean RMS values of corneal, intraocular, and total HOAs in 45 young subjects (age range 9 to 29 years; SE refractive error between +0.50 D and –7.75 D), which is in agreement with our data. The mean intraindividual differences in the higher-order RMS values were less than 0.02 \(\mu\)m. We can therefore confirm that although there appears to be random variation in the eye’s aberration from subject to subject, the variation in HOAs between fellow eyes is relatively low.\(^1\),\(^\text{17},\)\(^\text{21},\)\(^\text{22}\)

In general, the optical quality of the whole eye, here expressed by pupil and image-quality metrics, was better than that provided by corneal and intraocular optics alone, as expected\(^1\),\(^\text{18},\)\(^\text{19}\). In the young healthy eye, the result of the compensatory processing between the cornea and the lens has been shown to improve image optical quality at the fovea.\(^1\),\(^\text{20},\)\(^\text{21}\) No differences between emmetropic eyes and myopic eyes were found except for a higher amount of intraocular HOAs in myopic eyes. Data in the literature surrounding this issue are controversial. Some studies\(^\text{21}\)
found that the optical quality was worse in myopic eyes than in emmetropic eyes (expressed by increased HOAs), whereas others found no correlation between refractive error and monochromatic aberrations. No clear evidence supports the relationship between emmetropization and ocular aberrations. We cannot draw conclusions from our results because the intraocular HOAs showed the largest variance in this study. The influence of the Stiles-Crawford effect was incorporated into the calculation of the PSF and MTF to properly model the optical effect of HOAs. This phenomenon has a retinal basis in the optical behavior of cone photoreceptors because the photoreceptors act as optical fibers and have typical waveguide properties. From the data shown, the eye’s optics play a primary role in filtering out high-frequencies and therefore limiting the resolution in the normal eye, even when the correction for defocus and astigmatism is optimum (as modeled in our study). The overall image optical quality in the eye in the emmetropic group and the myopic group was comparable.

Along the horizontal meridian, cone density dropped from 49,393 ± 7941 cells/mm² at 260 μm from the foveal center to 30,049 ± 4954 cells/mm² at 600 μm from the foveal center. The interindividual variation in cone density and Nc was 16% and 5%, respectively, between 260 μm and 600 μm eccentricity from the foveal center. The mean differences in cone density or Nc between fellow eyes was 5% and 3%, respectively, and did not achieve statistical significance. A statistically significant difference in cone density between emmetropic eyes and myopic eyes at each specified eccentricity was found. The cone density estimates in the present study are in agreement with those in previous studies. In a population of 18 eyes of 18 healthy subjects (age range 23 to 43 years; AL range 22.86 to 28.31 mm), Li et al. found that cone density decreased significantly with increasing
AL at eccentricities between 100 μm and 300 μm from the foveal center ($r^2 = 0.56$, $r^2 = 0.65$, and $r^2 = 0.75$ at 0.3 degrees, 0.7 degrees, and 1.0 degree eccentricity, respectively). In 11 eyes of 11 healthy subjects (age range 21 to 31 years), cone density in moderately myopic eyes (up to $-7.50$ D) was significantly lower than in emmetropic eyes within 2.0 mm from the fovea. The interindividual variation in cone density was, on average, 18% between 0.3 mm and 2.0 mm from the fovea in previous adaptive optics studies.

In the current study, the mean Nyquist limit sampling of resolution of the cone mosaic was estimated to be $33 \pm 2$ cpd at 260 μm eccentricity, declining to $26 \pm 2$ cpd at 600 μm eccentricity. A statistically significant difference in $N_c$ between emmetropic eyes and myopic eyes was found, as previously reported. The $N_c$ was estimated to be 34 cpd at 1-degree eccentricity (approximately 270 μm) by Chui et al. Coletta and Watson estimated the $N_c$ to range between 50 cpd and 42 cpd at the fovea and between 24 cpd and 22 cpd at 4 degrees eccentricity (approximately 1.1 mm) in a population with SE refractive errors ranging between 0.0 D and $-14.0$ D. The lower cone density estimated in myopic eyes has been postulated to be caused by retinal stretching resulting from the increased AL in the eye.

A limitation of our study is that we were not able to resolve cones at the foveal center. Variability in cone density at close to the fovea (<0.2 mm) could be even greater between emmetropic eyes and myopic eyes based on histologic data. Beyond optical aberrations, scatter and dispersion of light, which were not measured in this study, can degrade the optical image quality in the individual eye at the retinal plane. We therefore were not able to measure the overall optical quality of the eye and model the Nyquist limit of resolution of the foveal cones.

Individual visual performance is the result of how the image is focused on the retina and how the visual system processes and integrates the information. The resolution depends on the eye’s optical factors, the structural properties of the cone mosaic, and the functional properties of the post-receptorial structures. The cone-packing density within 600 μm eccentricity from the fovea showed less variation than the eye’s optical aberrations in a population of young adults and was statistically significantly correlated with the AL. The spatial vision of the cone mosaic was slightly reduced with increasing AL from 22.60 to 26.60 mm. Our results indicate that optical improvement in the eye will likely benefit young emmetropic patients more than patients with moderate myopia. Previous studies support the hypothesis that retinal stretching at the posterior pole in myopic eyes reduces retinal sampling density. Although a difference less than 2 cpd might not be considered clinically meaningful, researchers have found that myopic eyes perform slightly worse than emmetropic eyes when acuity limits were tested with grating stimuli generated with high-contrast laser interference fringes and the effect of optical degradation was compensated for. The visual acuity in myopic eyes has been found to be even worse when measured with letter targets rather than with grating stimuli. A greater difference between myopic eyes and emmetropic eyes also occurred when the acuity limits were expressed in retinal units (c/mm) rather than in angular units (cpd). The discord between resolution and $N_c$ expressed in retinal acuity was primarily considered to be the result of the psychophysical and neural factors, including the neural sampling rate; that is, the receptive field density of the retinal ganglion cells. When modeled, the Nyquist limit of the retinal ganglion cells mosaic was estimated to be lower than the Nyquist limit of the cone mosaic and to be better correlated to visual acuity up to 5 degrees eccentricity from the fovea. Theoretically, the differences in acuity tasks between emmetropic eyes and myopic eyes might be even higher considering retinal stretching, which may ultimately decrease the neural sampling density of the myopic retina. The contribution of post-receptor neural factors to the individual visual function should be studied to determine in detail the spatial filtering properties of the human neuroretinal system.

**WHAT WAS KNOWN**
- The higher-order wavefront aberrations in the eye vary largely between individuals. The correction of HOAs is believed to benefit emmetropic eyes and myopic eyes to the same extent.

**WHAT THIS PAPER ADDS**
- Although the optical image quality in emmetropic eyes and myopic eyes was comparable, the Nyquist limit of resolution of the retinal cone mosaic was reduced with increasing AL.
- The theoretical full correction of the wavefront aberrations should therefore benefit emmetropic eyes more than myopic eyes.

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