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***In-vivo* imaging of the photoreceptor mosaic in retinal dystrophies and correlations with visual function**

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PURPOSE. To relate *in-vivo* microscopic retinal changes to visual function assessed with clinical tests in patients with various forms of retinal dystrophies.

METHODS. The UC Davis Adaptive Optics (AO) Fundus Camera was used to acquire *in-vivo* retinal images at the cellular level. Visual function tests, consisting of visual field analysis, multifocal electroretinography (mfERG), contrast sensitivity and color vision measures, were performed on all subjects. Five patients with different forms of retinal dystrophies and three control subjects were recruited. Cone densities were quantified for all retinal images.

RESULTS. In all images of diseased retinas, there were extensive areas of dark space between groups of photoreceptors, where no cone photoreceptors were evident. These irregular features were not seen in healthy retinas, but were characteristic features in fundi with retinal dystrophies. There was a correlation between functional vision loss and the extent to which the irregularities occurred in retinal images. Cone densities were found to decrease with an associated decrease in retinal function.

CONCLUSIONS. AO fundus photography is a reliable technique for assessing and quantifying the changes in the photoreceptor layer as disease progresses. Furthermore, this technique can be useful in cases where visual function tests give borderline or ambiguous results, as it allows visualization of individual photoreceptors.

Retinal dystrophies are debilitating and currently incurable diseases that cause severe visual impairment from a very young age. Patients with cone and cone-rod dystrophies typically exhibit visual acuity loss, visual field impairment, color vision deficiency, photophobia and sometimes nystagmus,¹⁻⁷ whereas patients with retinitis pigmentosa (RP), rod or rod-cone dystrophies show night blindness, peripheral visual field depressions or scotomas and in some patients, residual central vision⁸.

Electroretinography (ERG) has been the most important objective tool in monitoring the progression of retinal dystrophies. To assess central retinal function, the multifocal electroretinogram (mfERG) has been used⁹⁻¹². It has been demonstrated that small remaining responses in the macular region can be reliably measured with mfERG in patients with RP¹²⁻¹⁴.

Color vision tests have also been used in monitoring the progression of retinal dystrophies as cones are affected in most cases. Fishman *et al.*¹⁵ examined 67 patients with various types of RP for color vision defects. They used the Farnsworth-Munsell 100-hue test and found that when an atrophic macular lesion was present or if visual acuity was less than 20/30, none of the patients had normal color vision. RP patients with cystoid macular edema tested better than patients with foveal lesions. Heckenlively *et al.*¹⁶ evaluated color vision abnormalities in all forms of inherited cone-rod dystrophy. Of 40 eyes, 37 had abnormal error scores on the Farnsworth-Munsell 100-hue test, even though the average visual acuity was 20/30. Tritanomalous defects were the most common finding in these patients. More recently, in a large psychophysical study, similar results were found: the majority of patients with cone and cone-rod dystrophies exhibited marked color vision defects, and the magnitude of defects correlated with the loss of visual acuity¹⁷.

Foveal cone functions in patients with retinal dystrophy can be inferred from visual

acuity and contrast sensitivity¹⁸⁻²⁵. In comparison to visual acuity, however, contrast sensitivity is a more sensitive indicator of abnormal central visual function in diseased retinas¹⁸⁻²². Patients with better visual acuity (20/30 or better, e.g., with RP) showed substantially reduced contrast sensitivity especially at high spatial frequencies, whereas patients with poor visual acuity (e.g. with cone dystrophy) showed normal sensitivity to lower spatial frequency gratings.

Numerous histological studies have demonstrated both rod and cone photoreceptor degeneration with progression of retinal dystrophies²⁶⁻³¹. Photoreceptors are either missing or have shortened and severely distorted outer segments. A common biochemical defect has been identified in early onset retinal dystrophies in three different species of animals: rats, mice and dogs³²⁻³⁴. In each of these animals, there was evidence of an abnormality in the cyclic nucleotide metabolism, leading to the death of photoreceptor cells. Mullen and Lavail³⁵ demonstrated that a failure of the phagocytic relationship between the photoreceptor cells and the RPE leads to degenerative changes in the photoreceptors in chimeric rats. Chaitin and Hall³⁶ were able to show more specifically that the defect is not in the initial part of the phagocytic mechanism (i.e., binding and recognition), but occurs in the middle stages of ingestion or engulfment in RCS rats.

Even though a great deal of information has been learned through histological studies, it can be limited by artifacts during preparation of the retina, which further complicates the interpretation of disease processes at different stages. With the availability of AO, it is now possible to image individual photoreceptors in living human eyes³⁷. Roorda reported the first AO images on a cone dystrophy patient and showed the retina was patchy due to photoreceptor dropout³⁸. An overview of high-resolution retinal imaging using AO is provided by Doble³⁹.

Previous studies have concentrated on imaging diseased eyes but none has attempted to correlate disease-associated changes in these images (i.e., cone loss) with tests of visual function.

The aim of this study was to quantify and correlate the changes observed in high-resolution AO retinal images with various visual function tests used in clinical settings to explore the utility of this imaging modality for diagnosis and monitoring of retinal dystrophies.

METHODS

Subjects

Five patients with different forms of retinal dystrophies of unknown family history and three control subjects were tested. The patients included three cases of rod-cone dystrophy, one with cone dystrophy and one with cone-rod dystrophy. The age of all subjects ranged from 13 to 44 years with either no or minimal refractive error (i.e., less than -2 D for both spherical and astigmatic correction). Table 1(a) and (b) summarize the characteristics of all 8 subjects. Refractive error was corrected with trial lenses prior to AO retinal imaging and psychophysical testing; the auto-refractor unit was used for the mfERG. All subjects had bilateral changes, therefore the one eye with greater variation of visual field defects was chosen for the study. The pupil was dilated with one drop of 1% tropicamide followed by one drop of 2.5% phenylephrine for the AO retinal imaging and the mfERG measurements. For AO retinal imaging, the subject's head movement was minimized by using a bite-bar with a dental impression. For the contrast sensitivity measurements, a combination of a forehead and chin rest was used. The tenets of the Declaration of Helsinki were observed and written informed consent was obtained after all procedures were fully explained and prior to any experimental measurements.

Procedure

1. Photoreceptor Imaging

Figure 1 shows a schematic of flood illuminated AO fundus camera in operation at UC

Davis. The system consists of two parts: (i) the AO control loop which measures and corrects the ocular aberrations (shown by the dark gray shading) and (ii) the imaging camera / projector which provides for imaging and vision testing respectively (light gray).

a. The AO control loop

Trial lenses are used to correct sphere and cylinder prior to the AO implementation. The subject was then instructed to fixate on a target consisting of a series of concentric circles calibrated to different retinal eccentricities in degrees from the fovea. The fixation target subtended a visual angle of $\pm 8^\circ$. A superluminescent laser diode (SLD) (Hamamatsu Photonics, Model L8414-04, Japan) operating at 835 ± 20 nm is used to form a wavefront sensor (WFS) beacon on the retina. The corneal irradiance was $63.9 \mu\text{W}$, incident through a 1 mm entrance pupil. This is a factor of 3 below the recommended ANSI standard⁴⁰. The SLD is coupled into the eye via a high quality pellicle beamsplitter (National Photocolor, Rochester, NY) and is delivered slightly off the corneal pole to avoid the corneal reflex⁴¹. The pellicle is optically flat in both transmission and reflection, which allows for a reference laser to be coupled into the system from the opposite side for calibration and alignment purposes.

Upon reflection from the retina, the 7 mm diameter exit pupil is relayed to the deformable mirror (DM) via a 10x magnification telescope. The DM was manufactured by Litton “ITEK” (DM109, Lexington, MA) and has 109 actuators arranged in a hexagonal array below a continuous front-surface mirror. The DM has an approximate mirror stroke of $\pm 2 \mu\text{m}$. This conjugate pupil plane is then relayed to the wavefront sensor (WFS) via reflection from a high quality hot mirror. The WFS is Shack-Hartmann based, comprising a lenslet array (Adaptive Optics Associates, Inc. Cambridge, MA) and a Dalsa CA-D7 CCD camera (Dalsa, Waterloo, Ontario, Canada) located in the back focal plane of the lenslet array. The lenslets have a 24 mm

focal length with a 400 μm pitch. The 7 mm exit pupil is magnified onto the WFS by a factor of 1.14 and is sampled by 20 lenslets across the pupil diameter. The DM is operated using direct slope control⁴². Individual DM actuators are pushed and the resulting Shack-Hartmann WFS spot deflections are recorded. The resulting matrix is then inverted and used to calculate the required voltages to be sent to the actuators, directly from the measured slope offsets. This method removes the need for any wavefront reconstruction in the AO control loop. The aberrated wavefront is sampled at 20 Hz providing an experimentally measured closed-loop bandwidth of ~ 0.9 Hz. A gain factor of 30 % is used. Both the WFS and DM are controlled through a standard 2.66 GHz personal computer. Typical RMS correction for a 7 mm pupil is ~ 0.09 μm . The before and after wavefront map and the resulting PSF are shown in Figure 2. There was a decrease in RMS by a factor of 3 with an associated 3-fold increase in Strehl ratio. For imaging purposes, a smaller pupil of diameter 6.5 mm is used to avoid the edge artifacts due to DM correction at the pupil margins.

b. Imaging camera

Once the eye's aberrations have been corrected, a shutter is opened and a retinal image acquired on the imaging camera; the imaging path is shown with the lighter gray shading. The imaging pulse follows the corrected path of the DM and is transmitted through the hot mirror and is re-imaged onto a cooled CCD camera (Princeton Instruments, VersArray XP). The imaging source is a 300 W xenon arc lamp regulated by a dc power supply (Oriel, Stratford, CT). The imaging wavelength is broadly tunable over the visible and near infrared spectrum by choice of an appropriate interference filter. The exposure duration was set to 10 msec with 0.6 and 0.25 μJ of light being delivered through 40 nm FWHM interference filters centered at 550 and 650 nm respectively. This is a factor of 40 below the recommended ICNIRP guidelines⁴³. To correct for

the ocular longitudinal chromatic aberration between the WFS beacon at 835 nm and the visible wavelengths used for imaging, the imaging camera is mounted onto an axial translation stage to provide the chromatic aberration correction.

Five to seven best images were selected and then registered together via an autocorrelation process to optimize the contrast and improve the signal-to-noise ratio of the image⁴⁴.

c. Cone density measurement

Photoreceptor density was determined using a Matlab program (The Mathworks, Inc., Natick, MA) that automatically counted the cones once the images were registered. The initial, registered images, I_{orig} , were first convolved with a Gaussian filter and then subtracted from the initial profile to give the new image intensity profile, I . The process is described by equation (1)

$$I = I_{orig} - I_{orig} * e^{-(x^2 + y^2) / 2\sigma^2} \quad (1)$$

where the exponential describes a Gaussian filter of width σ (*i.e.*, the standard deviation) and x , y denote the coordinates of a pixel⁴⁵. For this work, a σ value of 30 was chosen. Figure 3(a) and (b) show the initial and filtered images respectively. To aid visualization of the filtered images, the values of I were linearly scaled to increase brightness.

By determining the intensity of the dimmest cone, a threshold intensity level could then be set. The intensity profile of the image was divided into smaller sections of 10 intensity levels to search for the pixels that are brighter than the threshold intensity in each section. When a ‘hot’ pixel lies within a locus defined by the smallest center-to-center cone spacing, the program

ignores that pixel value. This step ensures that cones are only counted once. Figure 3(c) shows the example image with all the cones highlighted. It is then a straightforward step to calculate the cone density.

2. Functional Testing

a. Visual field analysis

Prior to AO retinal imaging, visual fields were measured in each eye using a Humphrey Visual Field analyzer (HFA II – I series, Carl Zeiss Meditec, Inc., Dublin, CA) with SITA standard settings to determine the areas of reduced retinal sensitivity within the central 20-50 degrees of visual field. This helped us to decide which eye to test, and to target the areas of different severity for the AO retinal imaging. Depending on the severity of visual field loss, either a 24-2 or a 10-2 full threshold test was used with size 3 stimulus (i.e., 3 mm circular stimulus on white background, 10 cd/m², with exposure duration of 200 ms).

b. Multifocal electroretinography

The multifocal electroretinogram (mfERG) is routinely used to isolate retinal response at discrete locations and to detect retinal dysfunction that has not yet lead to visible changes in the fundus or global decreases in visual performance. This technique is based on a cross-correlation between hexagonal black-and-white stimulus changes and the local response of the retina, thus reflecting the first stage of retinal processing⁴⁶. The protocol used was similar to the ISCEV Guidelines for multifocal ERG and was described in detail elsewhere⁴⁷. Briefly, the recording was made under room light conditions with a dilated pupil ($d > 6$ mm). Correction of refractive error (between + 6 and - 6 D) was obtained using the refraction correction unit as part of the VERIS system (EDI, San Mateo, CA). The stimulus consisted of 103 hexagons that were not

scaled with retinal eccentricity in order to facilitate comparison with the 1° retinal areas used for AO retinal imaging and contrast sensitivity testing. A central cross (X) was used for fixation. The flash intensity was $2.67 \text{ cd}\cdot\text{sec}\cdot\text{m}^{-2}$ ($200 \text{ cd}\cdot\text{m}^{-2}$), the intensity of the dark areas was $<0.05 \text{ cd}\cdot\text{sec}\cdot\text{m}^{-2}$ ($<4 \text{ cd}\cdot\text{m}^{-2}$) and the frame rate was 75 Hz. Burian-Allen contact lens electrodes (Hansen Ophthalmic Development Laboratory, Iowa City, IA) were used as active electrodes. Standard m-sequence stimulation mode ($m = 14$) was used resulting in a total recording time of 3.38 min. The responses were then band-pass filtered between 10 Hz and 300 Hz. Eight segments were used for each recording, and any segment contaminated by eye movements or artifacts was rejected and re-recorded. The pupil was continuously monitored with a camera.

c. Contrast sensitivity function

The contrast sensitivity function (CSF) was measured with a 33 cm CRT video monitor (Sony Multiscan G220) driven by a Macintosh G4 computer with an ATI Radeon 7500 video card (10-bit per gun). Gabor patches (sinusoidally modulated gratings windowed by a Gaussian envelope) were presented at the same size (1°) and retinal position as the AO images. Contrast thresholds were measured at 5 spatial frequencies (1, 2, 4, 8 and 14 cycles per degree) using software written in Matlab 5.2.1 and with the Psychophysics Toolbox extensions^{48,49}. The monitor was characterized using a Minolta colorimeter (CS 100 Chroma Meter) and gamma corrected using the procedures set out in Brainard *et al.*⁵⁰. Observer position was stabilized by a forehead and chin rest so that the screen was viewed monocularly at a distance of 80 cm. All experiments were performed in a dark room. Before the CSF was measured, subjects were adapted to the luminance level of the background ($31.3 \text{ cd}/\text{m}^2$) onto which the stimulus patch was presented for 10 min, and then asked to maintain steady central fixation by looking at a fixation target throughout the experiment. At each retinal location, the contrast sensitivity

threshold for all five spatial frequencies, presented in random order, was measured by using a QUEST adaptive tracking procedure⁵¹. The threshold value for each spatial frequency was taken as the geometric mean of the threshold estimates from two interleaved adaptive staircase tracks. Then the same procedure was repeated at the next retinal eccentricity. The subject's task was to judge in which of two temporal intervals, the grating was presented and to record the response by pressing the corresponding button on the response pad.

d. Color vision tests

Three color vision tests were used: Farnsworth D-15, Neitz Anomaloscope and the Cambridge Color Test (CCT). All the tests were performed monocularly with corrective lenses. The CCT (Cambridge Research Systems) is particularly useful in examining the changes in color discrimination that occur as a result of congenital or acquired conditions. With this test, Landolt-C patterns (2 to 16 cd/m²) were presented on a calibrated computer monitor using a video board with 15-bit per color channel resolution. Following 5 min dark adaptation, the subject's task was to detect the location of the gap in a Landolt C using a four-alternative forced choice, based on the principles of pseudo-isochromatic plates⁵². Stimuli were displayed for 5 seconds in each trial. Discrimination thresholds were defined by the minimum chromatic contrast required for detection of stimuli varied in terms of the stimulation of short-wave (tritan), middle-wave (deutan) and long-wave (protan) sensitive cones.

RESULTS

Figure 4 shows the AO images of a normal retina (control 1) at six retinal locations. The scale bar represents 10 μ m on the retina. The photoreceptor mosaic is regular across the retina without any missing cones in the healthy eye. The cones and the gaps between the cones increase in size with increasing eccentricity, as expected⁵³.

Figure 5(a)-(c) show the AO retinal images taken from subjects with rod-cone dystrophy (RCD 1, 2 and 3) at different retinal loci. The retinal locations were determined based on the visual field results so that the areas with varying severity of functional loss could be imaged by the AO fundus camera. The consistent finding in all rod-cone dystrophy subjects was that the cone mosaic is not regular in these eyes (unlike that of normal eyes) at the retinal locations where the sensitivity is reduced, (i.e., there were areas of dark spaces between groups of cones, where no cones were visible). Figure 5(d) and (e) show the AO images from cone-rod dystrophy and cone dystrophy patients respectively at different retinal eccentricities. These subjects also showed photoreceptor irregularity at the locations where retinal sensitivity was reduced.

Cone density was calculated for each retinal image, and there was a clear positive correlation with retinal sensitivity threshold (from all the functional tests). The greater the reduction of retinal sensitivity was, the greater the loss of cones in the images. Table 2 summarizes the cone density measurements from the AO retinal images for each location of retina for all subjects, and they are compared to the extrapolated values from Curcio's histology data⁵³. The relative percentage values provided an indication of relative cone loss at each retinal location. Across all subjects, regardless of the type of retinal dystrophy, there was a decrease in number with an increase in retinal sensitivity loss. Depending on the extent of retinal damage, the range over which the percentage value decreased was different between subjects. The variation of cone density from the least to the most affected areas was 96%-49% for RCD 1, 59%-32% for RCD 2, 33%-22% for RCD 3 (3 rod-cone dystrophy cases), 41%-26% for CRD (cone-rod dystrophy) and 81%-9% for CD (cone dystrophy). These numbers provide a good quantitative index of cone loss in various forms of retinal dystrophy.

The mfERG was measured on all subjects. Figure 6(a)-(c) show the traces from rod-cone

dystrophy subjects (RCD 1, 2, and 3). The first figure, (i), in each section shows the recordings from all 103 locations in the central 45 degrees of the retina. The overall pattern of cone responses in mfERG matched closely to the visual field result, i.e., the areas in the visual field that showed depression in retinal sensitivity, were registered with flatter traces and longer latencies. The second figure, (ii), shows the averaged cone responses from hexagons that correspond to the retinal locations used for AO imaging. The resolution of the mfERG is not fine enough to make a 1:1 correspondence with the AO imaging location. The size of each hexagon was calculated to be 4.4 degrees on the retina. Therefore in some cases, a single hexagon represents the combined responses from more than one imaging location, e.g., in RCD 1, trace #1 represents averaged response from the area that includes both 2° NVF and 2° TVF and trace #3 includes 7° and 8° TVFs. All three subjects showed that as retinal sensitivity decreased, the mfERG trace became gradually flatter with longer latencies between peaks. Figure 6(a)(ii)-(c)(ii) demonstrate this trend. The top trace represents averaged response from the retinal location that corresponds to healthier part(s) of the retina used for AO imaging. The bottom trace represents that of the most affected part of the retina imaged and the middle trace(s) represent intermediate locations. At the retinal locations that were significantly affected by the disease, the amplitudes of mfERG evoked responses were reduced almost to the extent that there were no visible responses. Figure 6(d) and (e) show the mfERG recordings from subjects with cone-rod dystrophy (subject CRD) and cone dystrophy (subject CD) respectively. Both subjects had no visible responses across all retinal locations (refer to Figure 6(d)(ii)–(e)(ii)). For CRD, trace #1 represents averaged response from the area that includes 3°NVF and 4° NVF, whereas trace #2 represents averaged response that includes 1° and 2° NVFs.

The data displayed in the graphs in Figure 7(a)-(c) are the luminance contrast sensitivity

function (CSF) measurements from the three rod-cone dystrophy subjects (RCD 1, 2 and 3) at each retinal location, and the results were compared to those of control subjects. Contrast sensitivity decreases were similar to the other functional tests and AO images, and disease-related reductions in log contrast sensitivity were roughly uniform across all spatial frequencies. Figure 7(d) and (e) show the results from the cone-rod dystrophy and cone dystrophy subjects respectively. The cone-rod dystrophy subject had significantly reduced CSFs at all retinal locations compared to the control subject, and also the CSF decreased with a decrease in retinal sensitivity (as measured from other functional tests in this study and AO images), refer to Figure 7(d). The cone dystrophy subject did not have measurable contrast sensitivity at any of the retinal locations tested, therefore showing flat CSFs, see Figure 7(e).

Color vision test showed that those subjects with good central vision (i.e., rod-cone dystrophy subjects) had normal color vision, whereas those with poor central vision (i.e., cone-rod dystrophy and cone dystrophy subjects) had generalized color vision defects, showing highly elevated threshold on all three cone axes of the Cambridge Colour Test.

DISCUSSION

The data presented here show a clear correlation between functional visual loss and the extent to which cone density decreases as measured from AO retinal images of retinal dystrophies. It is generally recognized that, regardless of differences in the underlying cause, the final common pathway of retinal degeneration is the apoptotic death of the photoreceptors²⁶⁻³¹. Electron microscopic studies have revealed that cones of patients with retinal dystrophy have truncated outer segments that are composed of small groups of disoriented disk membranes and swollen inner segments. In the end, the course of the disease leads to death of both inner and outer segments leaving a residual photoreceptor cell of a spherical shape with little cytoplasm⁸.

Such cell degeneration could explain the dark spaces seen in the AO retinal images. In healthy retinas, the photoreceptors reflect incident light from the junction of the inner and outer segments, and this light is wave-guided back through the pupil. Each bright spot in the AO image represents a dominant reflection from an individual photoreceptor.

When the cone density measurements from AO retinal images were compared with the mfERG recordings from corresponding areas, the analysis indicated that there is a clear correlation between the strength of mfERG responses and the cone density measurements, i.e., lower amplitudes and longer latencies of mfERG responses were associated with lower cone density measurements. Given the effectiveness of AO fundus photography for identifying diseased retina at a very early stage of disease progression, AO imaging has the clear potential of becoming an integral part of ophthalmic and optometric diagnostic regimens.

A reduction in cone density due to cone photoreceptor cell death seems a likely explanation for the predominant contrast sensitivity loss. In contrast to previous findings, where the contrast sensitivity was found to be mainly affected at higher spatial frequencies in diseased retinas^{18,22}, the contrast sensitivity decreased across all spatial frequencies in all of our subjects with retinal dystrophies. The CSFs for the cone dystrophy subject were flat at all retinal locations indicating no residual functional vision at those locations.

This study confirms earlier histological data by showing apoptotic death of the photoreceptors in diseased retinas *in-vivo*. The ability to monitor retinal changes at a cellular level *in-vivo* opens the possibility of (1) visualizing cellular changes without introducing artifacts from retinal preparation (2) a more sensitive method for quantifying the retinal changes, and (3) much earlier detection of any changes, which makes it possible to intervene with appropriate treatments at much earlier stage of disease.

This is a clear demonstration of the applicability of the AO fundus camera in diagnosing as well as possibly monitoring the health of the retina at the sensitivity level that could not have been achieved by conventional clinical instruments. It will be of greater use, especially in cases where visual function tests lead to ambiguous or borderline results, by allowing one to visualize the photoreceptor layer *in-vivo*.

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Table 1. - Characteristics of (a) 5 subjects with various retinal dystrophies and (b) 3 control subjects.

(a)

Subject	Gender	Age	Diagnosis	Eye Tested	Visual Acuity	Visual Field
RCD 1	Female	33	Rod-cone dystrophy	Left	20/25	Temporal defect
RCD 2	Male	31	Rod-cone dystrophy	Left	20/20	Peripheral ring defect
RCD 3	Male	33	Rod-cone dystrophy	Right	20/20	Temporal arcuate defect
CRD	Male	44	Cone-rod dystrophy	Right	20/80	Central scotoma
CD	Female	13	Cone dystrophy	Left	20/100	Central scotoma

(b)

Subject	Gender	Age	Eye Tested	Visual Acuity
Control 1	Male	20	Left	20/15
Control 2	Male	33	Left	20/15
Control 3	Female	41	Right	20/15

Table 2. - Cone density measurement from AO retinal images for all subjects. The order in which retinal locations are listed is going from the least to the most affected areas. NVF = nasal visual field, TVF = Temporal Visual Field, TSVF = Temporal Superior Visual Field, TIVF = Temporal Inferior Visual Field, NSVF = Nasal Superior Visual Field, NIVF = Nasal inferior Visual Field.

Subject	Diagnosis	Retinal location (VF in degrees)	Cone density measurement (cells/mm ²)	Extrapolation from Curcio's data (cells/mm ²)	Relative %
RCD 1	Rod-cone dystrophy	2° NVF	41,450	43,000	96
		2° TVF	29,625	40,000	74
		4° TVF	15,075	25,000	61
		7° TVF	11,325	20,000	57
		8° TVF	7,825	16,000	49
RCD 2	Rod-cone dystrophy	2° TVF	23,425	40,000	59
		4° N 4° IVF	10,525	20,000	53
		2° N 7° SVF	5,625	18,000	32
RCD 3	Rod-cone dystrophy	4° T 4° IVF	6,500	20,000	33
		5° T 3° SVF	5,375	24,000	22
		5° T 7° SVF	4,550	18,000	25
		7° T 7° SVF	3,750	17,000	22
CRD	Cone-rod dystrophy	4° NVF	20,300	25,000	81
		3° NVF	22,125	33,000	67
		2° NVF	13,200	43,000	31
		1° NVF	7,175	76,000	9
CD	Cone dystrophy	4° N 4° SVF	10,257	25,000	41
		4° N 4° IVF	7,075	20,000	35
		4° T 4° IVF	5,125	20,000	26

FIGURE LEGENDS

- Figure 1 The Flood Illuminated Adaptive Optics Fundus Camera at UC Davis; the AO control loop is represented by dark gray shading while the imaging and vision testing paths are represented by light gray.
- Figure 2 The Wavefront (WF) and PSF plots before and after AO correction. The measurements were taken over 7mm pupil. The wavefront becomes flatter after AO correction. The RMS error decreases by a factor of 3. From the PSF plot, it can be observed that strehl ratio increases by a factor of 3.
- Figure 3 An example of the automated cone density measurement procedure. (a) Cropped original image, (b) Image after background subtraction, (c) Outcome of cone density measurement: all the cones are accurately picked.
- Figure 4 AO images of normal retina at six retinal locations on control subject 1. The scale bar corresponds to 10 μm on the retina. N = nasal, S = Superior, I = Inferior. VF = visual field.
- Figure 5 AO corrected retinal images for each subject at different locations of the retina are shown with the corresponding visual field results. Retinal location is denoted in terms of visual field in degrees from the fovea. Separate panels denote different subjects, as indicated. Other details as in Fig. 4.
- Figure 6 Multifocal ERG recordings showing the traces from all 103 hexagons as well as the ones that closely match with the retinal locations used for AO imaging. Changes in amplitude and latency were observed at different retinal eccentricities in each subject.

Figure 7 Contrast sensitivity functions for retinal dystrophy patients and control subjects at the retinal locations corresponding to the locations imaged with AO. Separate graphs denote different subjects, as indicated.

Figure 1.

(a) WF Map without AO correction.

(b) WF Map with AO correction.

(c) PSF without AO correction.

(d) PSF with AO correction.

Figure 2.

(a) Cropped original retinal image

(b) Retinal image after background subtraction

(c) Cone count result

Figure 3.

(a) 2° NVF

(b) 4° NVF

(c) 7° NVF

(d) 4° N 4° IVF

(e) 7° N 3° SVF

(f) 7° N 6° SVF

Figure 4.

(a) Subject RCD 1; Rod-cone dystrophy (OS)

(b) Subject RCD 2; Rod-cone dystrophy (OS)

(c) Subject RCD 3; Rod-cone dystrophy (OD)

(d) Subject CRD; Cone-rod dystrophy (OD)

(e) Subject CD; Cone dystrophy (OS)

Figure 5.

(i) (ii)

(a) Subject RCD 1; OS, Rod-cone dystrophy. The line plots (from top to bottom) correspond to (average response of 2° NVF and 2° TVF), 4° TVF and (average response of 7° TVF and 8° TVF) respectively.

(i) (ii)

(b) Subject RCD 2; OS, Rod-cone dystrophy. The line plots (from top to bottom) correspond to 2° TVF, 4° N 4° IVF and 2° N 7° SVF respectively.

(i) (ii)

(c) Subject RCD 3; OD, Rod-cone dystrophy. The line plots (from top to bottom) correspond to 4° T 4° IVF, 5° T 3° SVF, 5° T 7° SVF and 7° T 7° SVF respectively.

(d) Subject CRD; OD, Cone-rod dystrophy. The line plots (from top to bottom) correspond to (average response of 3° & 4° NVF) and (average response of 1° & 2° NVF) respectively.

(e) Subject CD; OS, Cone dystrophy. The line plots (from top to bottom) correspond to 4° N 4° SVF, 4° N 4° IVF and 4° T 4° IVF respectively.

Figure 6.

(a) Subject RCD 1; Rod-cone dystrophy (OS)

(b) Subject RCD 2; Rod-cone dystrophy (OS)

(c) Subject RCD 3; Rod-cone dystrophy (OD)

(d) Subject CRD; Cone-rod dystrophy (OD)

(e) Subject CD; Cone dystrophy (OS)

Figure 7.