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Rapid Measurement of Individual Cone Photoreceptor Pointing using Focus Diversity

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Abstract

A novel method is presented to rapidly measure the pointing direction of individual human cone photoreceptors using adaptive optics (AO) retinal imaging. For a fixed entrance pupil position, the focal plane is rapidly modulated to image the guided light in various axial planes. For cones with different pointing directions, this focus diversity will cause a shift in their apparent position, allowing for their relative pointing to be determined. For four normal human subjects, retinal images were acquired, registered and the positions of individual cones tracked throughout the dataset. Variation in cone tilt was 0.02 radians, agreeing with other objective measurements on the same subjects at the same retinal locations.

The Stiles-Crawford Effect of the First Kind (SCE-I) [1] describes how light rays entering near the center of the eye's pupil appear brighter to an observer than rays incident near the periphery. This is due to the waveguide properties of the cone photoreceptors [2, 3], and it has been demonstrated that various eye conditions such as myopia [4] and various retinal disease [5–10] have an altered SCE-I function.

Psychophysical SCE-I measurements require long experimental sessions plus a high degree of cooperation from the test subject, and so are impractical as a routine clinical measure. Reflectometry methods analyze the intensity distribution of the reflected light at the exit pupil [11–15] and are more time efficient. The two methods give the same mean direction towards which the cones are pointing, however the directionalities (ρ values) are greater when measured using reflectometry rather than psychophysically [16, 17]. Both methods measure the properties of an ensemble of cones.

Roorda and Williams used an AO flood-illuminated fundus camera to measure the pointing of individual cone photoreceptors in two normal human subjects [18]. Their measurements confirmed that the disarray in the pointing direction of individual cones was small, as suggested by previous studies [19, 20], and showed that the angular tuning properties of an individual cone was the same as that measured from an ensemble. The technique requires

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images of the same retinal location taken with the illumination entering through multiple pupil locations. The variation in a cone's reflected intensity with entrance location allows its pointing direction to be measured.

Here we present a novel technique that provides a rapid, objective measurement of individual cone pointing that may be clinically applicable as a diagnostic tool. It consists of rapidly acquiring retinal images at different focal depths and measuring the apparent positions of the cones. The imaging technique is similar in principle to that described by Shahidi et. al. (21). Cones with different pointing angles will appear to shift relative to each other as the imaging depth is changed. The main advantages of the focus diversity (FD) method are: (i) it is objective, (ii) requires no modifications to existing hardware, and (iii) speed, the data acquisition takes a few seconds, similar to SLO and OCT cone pointing measurements [22,23]. The same information takes several minutes with the moving entrance pupil (MP) method [18, 24, 25] and around 40 minutes if measured psychophysically. A further benefit of the FD method is that it does not depend on the intensity of the image pixels, hence bleaching of the retina is not required prior to imaging.

The reason why a tilted beam emitted from the object plane appears to move with defocus can be understood from Fig. 1. Consider an imaging system designed such that points on the object plane (retinal plane, RP) are conjugate to points in the nominal focal plane, F_0 . When an imaging camera is placed in the F_0 plane, point sources on RP will appear 'in focus' with the most compact PSFs. In Fig. 1 (a), the narrow beam (shaded) is parallel with the overall optical axis and arrives at the focal plane still parallel with the optical axis. This is true for all RP points since the imaging system is designed to be jointly telecentric in the object and image spaces. If the camera were 'defocused' by moving it to another plane, say F_1 or F_2 , the beam's image would become broader, but not shift in lateral position. The situation is different when the narrow beam leaves RP in a tilted direction, Fig. 1(b). The image is still formed at the same position at F_0 , but in other defocused planes the image is both broadened and shifted. Note this effect cannot be seen if the cone beam width at the pupil, d , is broader than the pupil diameter, D . A cone beam illuminating a smaller region than the entire pupil ($d < D$) will result in an image that is larger than the diffraction-limit ($\delta x = \lambda F_0 / d$). In this case, the beam can be tilted by a range of angles and still pass through the pupil. The smallest detectable tilt depends on the range, quality and SNR of the cone images.

In our experiments, the FD was introduced by modifying the Shack-Hartman WFS reference centroid locations on a flood illuminated AO camera [25]. Once the AO loop was closed, the DM corrected for the subject's ocular aberration while closing to a defocus offset.

The plane of best focus was first found by adjusting the axial position of the imaging camera with the AO loop closed to zero defocus offset on the WFS. The camera position was then held fixed and the FD introduced to move the focal plane in the anterior retinal direction. To maximize image quality and contrast, light was introduced at the subject's SCE-I peak and each was bleached (550 nm, 47×10^6 Td-s) prior to imaging. During a single acquisition trial, images with a 0.5° field of view were rapidly acquired for 12 defocus steps with 5 images being acquired at each step (60 total images). This corresponded to focus depth range of 67 μm relative to the subject's retina (± 220 nm in the Zernike defocus coefficient [26]) as seen

by the WFS). The 60 images were then registered together and the 5 images at each focal depth were averaged. The final stack consisted of 12 images, each corresponding to a different focus depth relative to the retina. Several such trials were acquired for each subject.

In the overall montaged image, created by averaging the 12 images in the defocus stack, the apparent positions of the cones were identified. These positions are the starting points for the estimation of the cones' apparent motion with defocus. Since the apparent cone positions can change with defocus in an initially unknown direction, we used an iterative selection and fitting technique to find the most consistent linear shift with defocus for each cone. Firstly, cones were selected in each of the twelve defocused images that fell within 5 pixels of the mean position. This initial set does not include any tilt and possibly excludes cone images for highly-tilted beams or incorrectly includes images of a different cone. We fitted the initial set with a three-dimensional line and found a new set of cone images that fell within 3 pixels of the first linear fit. This process was repeated 3 times to optimize the number of cones images included in the linear fit. Locations in the montage with fewer cones images in a linear fit tended to be near the margins of individual defocus montage images. The image processing, peak picking and line fitting was carried out using an automated MATLAB® routine. Since the images were registered to the average position, the slopes of the fitted lines describe the shift in the position of each cone relative to the mean; i.e. the average absolute pointing into the pupil plane is lost.

Four normal subjects (N1, N2, N3 and N4), aged between 20 and 42 years old were recruited. All had a corrected Snellen acuity of 20/15, with refractive errors not exceeding ± 3.0 D sphere or ± 0.5 D cylinder. The presence of abnormal ocular media and retinal disease was ruled out by a conventional eye exam, including slit lamp examination and ophthalmoscopy. Individual axial lengths were measured and used to scale the defocus steps. The tenets of the Declaration of Helsinki were observed and the protocol was approved by the Institutional Review Board of The Ohio State University. Written informed consent was obtained after all procedures were fully explained to the subjects and prior to any measurements. Pupils were dilated with one drop each of 1% tropicamide and 2.5% phenylephrine. All imaging was performed over a 6 mm exit pupil in the right eye using 680 nm light at 4° in the temporal retina (TR).

Fig 2 shows the cone pointing measurement. All twelve registered, average retinal images spanning the 67 μm range are shown. The location of two cones is indicated by either a red or yellow circle. The pointing of these cones is indicated by the red and yellow dashed arrows. The same phenomenon is evident in the composite images shown in Fig. 3. Here, the composite images in the XZ and YZ planes are constructed from stacked line sections taken from the same retinal location at each focal depth, where Z is out of the plane. The vertical and horizontal dashed lines show the location of the XZ and YZ plane of the composite images. The intersections of these lines show the position of the two cones identified in Fig. 2.

Fig. 4 shows the scatter plot of the X and Y slopes of the cones for each of the 4 subjects. Only slopes calculated over 7 or more defocus planes are included. The outer ring represents the slope of the line from the origin to the edge of the 6 mm exit pupil assuming an eye with

a nominal focal length of 17 mm. The blue ellipse axes are 2 std. dev. The disarray in X and Y (standard deviation of the cone pointing in radians, σ_x and σ_y) between cones is (0.012, 0.014) for N1; (0.013, 0.017) for N2; (0.013, 0.020) for N3 and (0.014, 0.020) for N4. For each subject, the cone pointing is directional, occupying a small portion of the exit pupil, as expected from the SCE-I effect. There is a significant asymmetry between the horizontal and vertical cone tilts measured by the FD method. Cones are tilted more along the vertical axis.

For subjects N1 and N2 the cone pointing at 4 TR was also measured by the moving pupil (MP) method (23 - 25). Briefly, the MP method uses the same AO fundus camera and varies the position of the entrance pupil on the eye (the exit pupil is fixed). Images of the same photoreceptors are obtained at varying entrance pupil locations and hence are imaged with incident light of varying angles. The reflected light intensity for an individual cone as a function of entrance pupil location can then be fitted to a Gaussian function that describes the profile in the pupil plane. The peak of the Gaussian function is taken to be the pupil intercept for that cone.

Retinal images taken by the FD and the MP method were registered and 47 (N1) and 79 (N2) common cones were found in the two datasets. The relative pointing in the pupil plane of the cones common to both methods is shown in Fig. 5 (green – FD, red – MP). The FD data was projected into the pupil plane using subjects axial length measurements. The outer circle shows the 6 mm exit pupil with the inner ellipses encompassing 95% (2 std. dev.) of the cones. The distribution of cone pointing measured by the FD and MP methods did not differ significantly. The mean difference in the pointing distribution of the common cones ($\delta\sigma_x$ and $\delta\sigma_y$ in mm) assessed by FD and by the MP methods is (0.17, 0.17) for N1 and (0.15, 0.21) for N2.

The agreement between the two methods was examined by linear regression of the difference of the cone tilts determined by the FD and MP methods versus the mean of these measurements, Fig 5 – right. A positive slope, between 0.50 and 1.25 was found in the X slopes of N1 and X and Y slopes of N2 showing a trend whereby larger tilts are over-estimated by the FD method. This may be due to inaccurate scaling of the tilts between the two methods.

Fig. 6 illustrates the variation in the distribution of cone pointing in the pupil plane for all 4 subjects. Error bars are ± 1 std. dev. with three trials for N1, N2 and N3 and two trials for N4. For all subjects, the estimation error in cone pointing increased when 3 or more defocus planes were used to calculate cone tilts. The FD method is susceptible to image noise, which has a more significant impact when the cone tilts are calculated over fewer defocus planes.

The average distribution of cone pointing estimates from both methods was less than 0.2 mm (0.01 rads) in both the X and Y directions. Cones were tilted more along the vertical than the horizontal, demonstrating the anisotropy of cone directionality observed by Applegate et. al. and Gorrand et al., (27, 28). The distribution of relative cone pointing was generally higher in the FD method than the MP approach, Fig 5. While the FD method is not reliant on cone intensity (as in the case with the MP method), it is sensitive to changes in the axial position of the eye and the quality of the image registration. The light was introduced

at the SCE-I peak to a bleached retina solely to maximize the quality and contrast of the images. FD data sets acquired through the center of the pupil to an unbleached retina resulted in a slight increase in the tilt measurement error which was reflected in larger σ values. The flood AO system employed here used an 8 msec exposure, but acquired image stacks at 12 Hz and has reduced contrast due to accepting scattered light from all retinal layers. The AO loop minimizes errors introduced by residual accommodation and axial motion, but using a faster imaging modality with improved image contrast (such as scanning laser ophthalmoscopy) would allow the data to be collected in <1 second. Furthermore, since the scatter in the slopes would be reduced by including images over the full defocus range, such a modality would provide better results. Eye motion would be a challenge in these systems where the exposure time for one frame is ~33 msec. However, the strip based registration approaches advocated by Stevenson et al. (REF) would be a solution when using the FD method in an AOcSLO. The relative importance of estimation error vs. actual pointing disarray will become apparent when the method is extended to studying cone disarray in diseased eyes.

In summary, the FD method allows for a very rapid measurement of the pointing direction of individual cones in the field of view of an AO-corrected fundus camera. The computation cost and algorithmic complexity is not significantly increased and the accuracy of the method is comparable to previous cone pointing techniques.

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Fig 1.

Principle of the FD technique. For a camera placed at the focal plane, F_0 , point sources on RP will appear ‘in focus’ with the most compact PSFs. At F_1 or F_2 , the image of the beam (shaded) (a) traveling parallel to the optic axis would become broader and would not shift, and (b) traveling at an angle to the optic axis would be both broader and shifted laterally.

Fig. 2. Image stacking and cone pointing (N1, 4 TR). The locations of two cones are indicated by red and yellow circles in all planes. The red and yellow arrows show the pointing of the cones.

Fig 3.

Building the composite images. The retinal image acquired at a defocus plane of 25 μm is shown. The two cones identified in Figure 2 are at the intersection of the horizontal, X, and vertical, Y, red and yellow dashed lines. The cross-sections of the two cones are constructed in the XZ and YZ planes from stacked line sections taken from the same retinal location at each focal depth, where Z is out of the plane. Retinal images were acquired at 4 TR.

Fig. 4.

Plot of the X and Y slopes of each cone analyzed for the 4 subjects. The radii of the ellipses (blue) indicate $2\sigma_x$ (minor) and $2\sigma_y$ (major). The number of cones displayed is given above each plot.

N1—26 cones

N2—85 cones

Fig. 5.

Left: Variation of cone pointing in the pupil plane assessed by FD (green) and the MP method (red). Right: comparison of the distribution of cone tilt in X (blue) and Y (red) assessed by the MP and FD methods for N1 (top) and N2 (bottom). The mean and ± 2 std are plotted along the trend line for each case.

Fig. 6.

Cone pointing distribution in the pupil plane. Cone tilts were calculated using at least 7 or 3 defocus planes. Error bars are ± 1 std. dev. with three trials for N1, N2 and N3 and two trials for N4.