

Eye motion during the exposure

Another limitation on the accuracy of the method is the motion of the eye during a single exposure. We used a 4-ms exposure to give us the largest signal per flash while keeping images that were visibly blurred by eye motion to fewer than about 5%. Riggs, Armington, and Ratliff (1954) reported median retinal image motion as a function of stimulus duration from about 15 ms to 1 s. Extrapolation of their data down to 4 ms predicts a median retinal image motion of 1.5 arcsec, which is one twentieth of the size of a foveal cone. Because we rejected trials in which there was visible motion blur corresponding to saccades, we conclude that retinal image motion is not an important limitation.

Head stabilization and axial alignments

The fixation stimulus and the CCD should be aligned so that they occupy conjugate planes and are in focus on the retina simultaneously. An error in focus of one relative to the other will introduce errors in estimating the point of fixation because of parallax if the head translates relative to the optical system. The angular parallax, Θ , is given by

$$\Theta = \arctan(x \bullet |\Delta D|) \quad (1)$$

where x is the lateral displacement of the head in meters and ΔD is the dioptric difference in focus between the stimulus and the CCD. Pupil displacements when sitting in the bite bar are highly subject dependent. Makous (1998) reported the pupil of experienced subjects clenching a bite bar typically stays within 50 μm of its intended position. We did not obtain records of pupil movement, so assume conservatively a standard deviation for pupil movement of 200 μm . For a conservative focus error as large as 0.1 diopters, the parallax at the retina would be only about 0.07 arcmin, or one eighth of the diameter of a foveal cone. Parenthetically, we used monochromatic light of the same wavelength for both imaging and fixation to avoid additional parallax caused by head movements in conjunction with the chromatic aberration of the eye.

Combining the errors associated with cross-correlation, eye motion during the exposure, and head instability on the bite bar, the total error in the measurement of the fixational stability is at most 0.08 arcmin, or one sixth the size of a single foveal cone photoreceptor. This error corresponds to about 400 nm at the retina, which is less than the wavelength of our stimulus. This accuracy could be improved if the demands required it by more careful head stabilization and alignment procedures, briefer imaging flashes, and a more sophisticated method of locating image cross-correlation peaks.

Lateral alignment error

There is one additional source of error, which is the accuracy with which the fixation stimulus is registered with respect to the CCD array. Assuming a conservative error as large as 0.5 of a retinal image pixel, this would cause an error of approximately

0.06 arcmin, or about one eighth of the diameter of a foveal cone. This is a systematic error in our ability to determine absolute fixation position and does not result in any error in determining the relative shift in position from one fixation to the next. This error combined with the other sources of error results in a total error in determining the absolute position of a stimulus on the retina of about 0.1 arcmin, or approximately one fifth of the diameter of a foveal cone.

Fixation stability

Figure 3 shows fixation locations for 83 trials superimposed on the foveal cone mosaic for one subject (J.P.). The cross shows the mean fixation position and the ellipses correspond to one and two standard deviations.

Similar results were obtained on the other four subjects. The standard deviation ranged from 2.1 to 6.3 arcmin (10.2–30.9 μm) in the vertical and horizontal directions, with an average of 3.6 arcmin (17.8 μm) in the horizontal direction and 3.2 arcmin (15.6 μm) in the vertical direction. Table 1 shows the data for all five subjects. Except for one instance (D.G., vertical direction) fixation points were normally distributed in both the vertical and horizontal dimension, based on the Kolmogorov-Smirnov

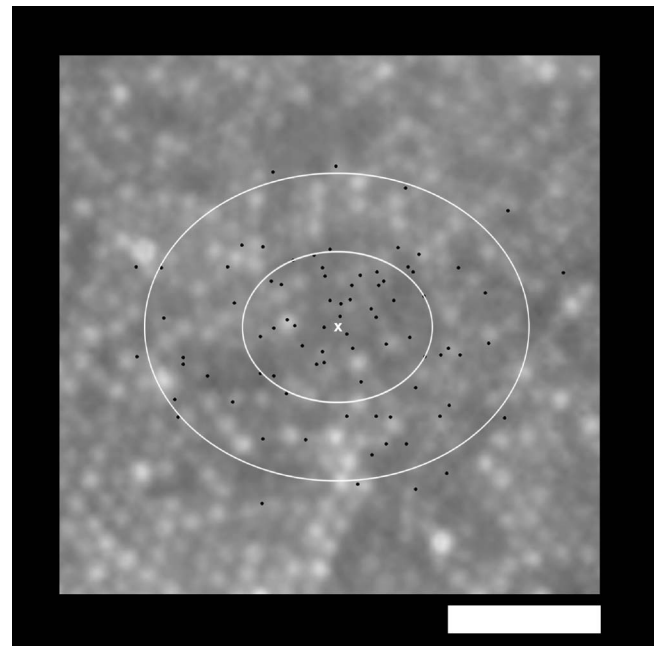


Figure 3. The foveal cone mosaic for one subject (J.P.) with the location of 83 fixations superimposed (small black dots). The size of the dots corresponds to the error of measurement, which is estimated to be about 5 arcsec. The white cross is the average fixation position. The foveal center (area of highest cone density) does not appear in this image, but resides 59.5 μm temporal inferior (up and to the right) of the average fixation position. White ellipses represent ± 1 and ± 2 standard deviations for fixation. Scale bar is 20 μm (4.03 arcmin).

Subject	Horizontal standard deviation of fixation (arcmin : μm)	Vertical standard deviation of fixation (arcmin : μm)	Mean standard deviation of fixation (arcmin : μm)	Peak cone density (cones/mm ²)	Deviation of center of fixation from the foveal center (μm)
J.P.	2.56 : 12.7	2.06 : 10.2	2.31 : 11.5	148,825	36.48 nasal, 47.00 superior
N.P.	6.30 : 30.9	3.29 : 16.1	4.80 : 23.5	—	—
D.G.	3.18 : 15.0	5.49 : 25.9	4.33 : 20.5	—	—
A.L.	3.26 : 16.1	2.58 : 12.7	2.92 : 14.4	114,963	18.49 temporal, 44.40 superior
J.C.	2.62 : 14.5	2.38 : 13.2	2.50 : 13.8	226,929	44.08 temporal, 12.80 inferior
Average	3.58 : 17.8	3.16 : 15.6	3.37 : 16.7	163,572	—

Table 1. Summary of data relating fixational stability and the relationship between cone density and the center of fixation.

test for normality ($p > .10$). This is consistent with previous findings (Steinman, 1965).

These results are in agreement with previous reports that used a variety of methods. For example, Barlow (1952) estimated a standard deviation of approximately 5 arcmin. Ditchburn (1973) reported the standard deviation ranges from 1.4 to 3.2 arcmin for subjects fixating at a distant target, and Steinman et al. (1973) reported standard deviations ranging from approximately 2 to 5 arcmin during maintained fixation. These earlier reports of the stability of gaze were based on measurements of the front of the eye. The data reported here have the advantage that the retina itself was directly tracked. The agreement between the front and the back of the eye tracking methods tends to suggest that both approaches generate accurate estimates of the stability of gaze.

Fixation location and cone topography

Despite the miniature eye movements that characterize the fixating eye, the prevailing view is that the eye has a quite small and stable preferred retinal locus of fixation (Barlow, 1952; Steinman, 1965). Steinman (1965) reported that the location of fixation can shift about 2 arcmin depending on target size, color, and luminance but concluded that these small shifts did not vitiate the notion of a stable retinal location for fixation. An assumption that is almost universally adopted is that the center of fixation corresponds to the anatomical center of the fovea (Polyak, 1949). Displacements between the two are generally associated with vision loss, as in the development of a pseudofovea in macular degeneration (Timberlake et al., 1986; von Noorden & Mackensen, 1962; White & Bedell, 1990). In normal eyes, however, the terms *center of fixation* and *center of the fovea* are often used interchangeably in psychophysical experiments. High-resolution imaging with adaptive optics provides an accurate measurement of whether the center of fixation actually does lie at the location of maximum cone density.

Figure 4 shows scatter plots of fixation superimposed on the cone mosaic for three of the five subjects. The remaining two subjects are not included because of the difficulty resolving cones at the foveal center (and thereby impeding accurate estimates of cone density). The black squares show the center of the area of highest cone density for each subject. Peak cone density values were the following: J.P. = 148,825 cones/mm²; A.L. =

114,963 cones/mm²; J.C. = 226,929 cones/mm². The dashed and solid lines are contours representing a 5% and 15% increase in cone spacing, respectively. Note that for each subject, the mean fixation position is displaced from the anatomical foveal center, defined by cone density. The displacements are the following: J.P., 59.5 μm (11.26 arcmin) nasal superior; A.L., 48.1 μm (9.75 arcmin) temporal superior; J.C., 45.9 μm (8.29 arcmin) temporal from the foveal center. Table 1 gives the two-dimensional vector displacements for each subject. Depending on the observer, the center of fixation lies approximately three to five times further from the point of highest cone density than the standard deviation of fixation. The direction of the displacement does not appear to be systematic, although more subjects would be required to confirm this.

Besides the location of maximum cone density, there are other anatomical features that can be used to define the foveal center, such as the foveal pit, the avascular zone, the rod-free zone, and the tritanopic zone. Curcio et al. (1991, 1990) reported that neither the rod-free zone nor the tritanopic zone is perfectly centered on the location of peak cone density. Bedell (1980) and Zeffren, Applegate, Bradley, and van Heuven (1990) reported that fixation position is not always symmetrically placed within the foveal avascular zone. Although Bedell (1980) reported a deviation of 0.6–0.8° in one eye, Zeffren et al. (1990) found that on average, the center of fixation deviated $66.5 \pm 49.5 \mu\text{m}$ from the center of the avascular zone.

Under the somewhat dubious assumption that acuity is reciprocally related to cone spacing near the fovea (Green, 1970; Marcos & Navarro, 1997), acuity will have declined by 8.2%, 4.4%, and 10.1% for J.P., A.L., and J.C., respectively, at the center of fixation compared with the anatomic center of the fovea. The displacement results, therefore, predict relatively small losses in acuity. Although it is thought that acuity generally falls in all directions away from the center of fixation, there have been few measurements that address whether this holds true within the central foveal region (Clemmesen, 1944; Jones & Higgins, 1947; Weymouth, Hines, Acres, Raaf, & Wheeler, 1928). Weymouth et al. (1928) mapped grating acuity in 11 arcmin steps throughout the fovea in three observers and did not report that the location of maximum acuity was displaced from fixation. However, blurring by the eye's optics reduces foveal visual acuity somewhat below the cone Nyquist frequency (Marcos & Navarro, 1997), which tends to obscure the influence of cone density. It would be of some interest to revisit the relationship between the spatial variation in

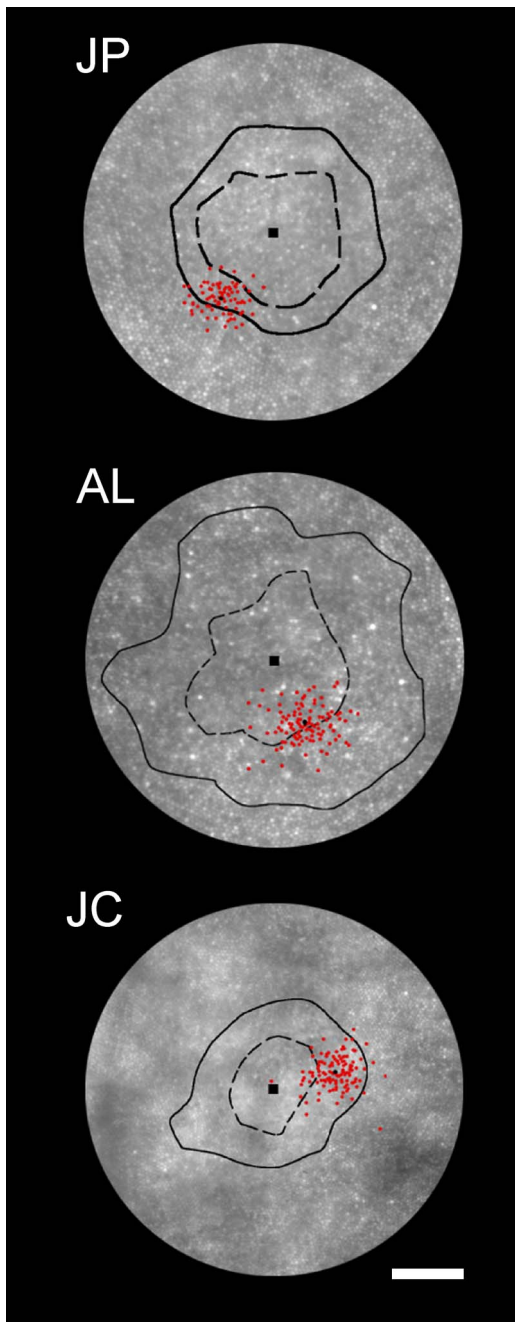


Figure 4. Area of highest cone density is not always used for fixation. Shown are retinal montages of the foveal cone mosaic for three subjects. The black square represents the foveal center of each subject (see the Methods section for how this was derived). The dashed black line is the isodensity contour line representing a 5% increase in cone spacing, and the solid black line is the isodensity contour line representing a 15% increase in cone spacing. Red dots are individual fixation locations. Scale bar is 50 μm .

acuity and cone density across the central fovea using interference fringe stimuli that are immune to optical blur.

Although the eye's optical blur may relax the pressure to select a center of fixation precisely at the location of highest cone density, the possibility remains that other factors may drive the

fixation locus. The variation in optical quality of the cornea and lens with retinal eccentricity is not a viable candidate for driving fixation because it changes so slowly (Jennings & Charman, 1981; Navarro, Artal, & Williams, 1993; Williams, Artal, Navarro, McMahon, & Brainard, 1996). It seems hard to escape the conclusion that the foveal pit and its associated avascular zone evolved to provide superior optical quality to foveal cones (Polyak, 1949; Weale, 1966), despite the fact that a difference in optical quality has proven difficult to measure (Artal & Navarro, 1992; Williams, Brainard, McMahon, & Navarro, 1994). It is conceivable that fixation coincides with the bottom of the foveal pit, and that both can be shifted from the cone density peak. Alternatively, the relatively small offsets observed here might simply reflect the insensitivity of the biological process with which fixation is established. If this were true, then left unexplained is the small standard deviation of fixation. The deleterious consequences of motion of the retinal image caused by fixation variability, such as the loss of vision during saccades (Dodge, 1900), may drive the visual system to keep fixation variability small.

Applications of tracking retinal position with subphotoreceptor accuracy

The present study capitalizes on the high spatial resolution afforded by adaptive optics to provide an instantaneous estimate of eye position with accuracy of about 5 arcsec, or one fifth of the diameter of a foveal cone. This error is lower than previous methods of measuring eye position. For example, the accuracy of dual Purkinje eye tracking is about 1 arcmin (Cornsweet & Crane, 1973; Crane & Steele, 1978). In this method, displacements of the lens during saccades interfere with direct access to the motion of the image with respect to the retina (Deubel & Bridgeman, 1995). Scleral search coils are subject to movement, and the accuracy is also on the order of 1 arcmin (De Bie, 1985). The method described here has the advantage shared with all retinal tracking methods (e.g., Cornsweet, 1958; Crossland & Rubin, 2002; Hammer et al., 2003) that it tracks the retina directly, rather than other features of the eye. Our method also has the advantage of revealing the absolute position of the stimulus on the retina.

So far, we have been able to record retinal location only for brief instants in time. However, Roorda et al. (2002) have coupled adaptive optics to a scanning laser ophthalmoscope with higher temporal bandwidth. Recently, they have demonstrated that they can use displacements of microscopic features such as photoreceptors from frame to frame to reconstruct the record of eye movements with much greater fidelity than previously possible (Stevenson, Raghunandan, Frazier, Poonja, & Roorda, 2004). This method of tracking the retina could also be used to increase precision in laser retinal surgery, such as photocoagulation used to treat diabetic retinopathy, resulting in less damage to healthy retinal tissue and ultimately, better outcomes for the patient.

There are many applications that would benefit from a more accurate method of measuring fixation. For example, it would be

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