**Purpose.** To investigate the accuracy of ocular misalignment measurement, using corneal reflections.

**Methods.** Corneal reflex positions were measured relative to two landmarks, the limbus center and the entrance pupil center, using high-resolution digital images for cyclopean gaze angles from 0° to 18.8° (34.04 prism diopters; PD) to the right and to the left in 10 subjects. Distance \( h \) from the center of the corneal curvature to each landmark was determined from linear regressions and showed significant differences between both conditions: mean \( h_{\text{limbus}} \) was 5.243 mm, and mean \( h_{\text{pupi|}} \) was 4.884 mm. From these, limbus- and entrance-pupil-center-related Hirschberg ratios were determined as 11°/mm (19.43 PD/mm) and 11.82°/mm (20.92 PD/mm), respectively; and ocular alignment was calculated for both conditions. Simulated angles of strabismus were calculated as the **binocular** difference between ocular alignment of the right and left eyes (condition 1), and as the **monocular** difference between the ocular alignment of right eyes and left eyes separately (condition 2).

**Results.** Condition 1: Errors in simulated angles of strabismus were approximately twice as large for entrance pupil center compared with those in limbus-center-based evaluation; in the primary position, the 95% pupil-related confidence interval of the binocular difference was ±5.217° (9.1 PD), compared with ±3.174° (5.5 PD) for the limbus-related option. Condition 2: Errors were approximately equal.

**Conclusions.** The entrance pupil center is a less reliable landmark than is the limbus center for measuring ocular alignment by using corneal reflections, because of unreliable positions of the entrance pupil center; different mean Hirschberg ratios should be used in both conditions. Invest Ophthalmol Vis Sci. 1997; 38:2597-2607.

A number of corneal reflex techniques for measuring ocular alignment have been suggested and analyzed. However, the relative merits of these techniques have, to our knowledge, never been studied experimentally by direct comparison with the same alignment data. Most techniques may, in principle, be derived from equation 1 by adequate substitutions or approximations:

\[
\phi = \arcsin \left( \frac{x}{h} \right) \quad (1)
\]

where

- \( \phi \): rotation angle of the corneal axis relative to the light source,
- \( x \): displacement of corneal reflex relative to an axially located ocular landmark
- \( h \): distance of the axially located landmark to the center of the cornea curvature,
- \( \text{as illustrated in Figure 1. Equation 1 results from a paraxial approximation, given a cyclopean eye with a spherical cornea. It holds best for eye rotation angles that are not large (less than 20°) and in conditions in which the light source is not close to the cornea—that is, for distances larger than one third of a meter.}^{19}

A simplified relationship uses the Hirschberg ratio (HR) in a linear approximation of equation 1. That is:

\[
\phi \approx \eta = \text{HR} \times x \quad (2)
\]

where \( \eta \): is the eye rotation angle calculated with the HR.
The HR can be gained empirically (HR_{empir}) from linear fits of gaze angle \( \beta \) versus measured corneal displacement \( x \). It may also be calculated (HR_{calc}) from equation 1 by setting \( x = 1 \) mm for any appropriate chosen or given distance \( h \) and dividing the resulting value \( \phi_{HR} \) by 1 mm.5

The determining parameter for the theoretically derived HR_{calc} is the distance \( h \) that is the separation between the center of anterior corneal curvature and the reference plane in which the landmark lies, regarding which the decentration \( x \) of the corneal reflex may be specified (Fig. 1). This implies that the HR varies with ocular components and with the landmark chosen.

Influence of various parameters on the HR was examined previously. A dependence of HR on the corneal radius may be predicted from Figure 1, in that the distance \( h \) depends on the position of the center of curvature of the anterior corneal surface, and hence on its corneal radius \( r \). Because of peripheral flattening of the cornea, the values for the corneal radius \( r \) and the distance \( h \) increase; consequently, the HR decreases in the periphery.21

There is widespread agreement that, within the limits of measurement accuracy, a single HR of 21 to 22 PD/mm may suffice3,4,6,12-16,18,19 to measure ocular alignment for clinical and scientific purposes, regardless of individual factors. However, large variations of the individual HR of the order of up to 100% relative to the mean HR have to be expected.5,12,19,18

In securing higher accuracy, little attention has been paid to the role of \( h \) or, in other words, to the proper choice of the reference plane and landmark. Most often, the corneal reflex position was measured relative to the entrance pupil center. The entrance pupil is the virtual, enlarged image of the physical pupil, which appears a little closer to the observer than the physical pupil.23

For a quick, clinical estimation of corneal reflex position (for example, with the Hirschberg test), the entrance pupil center is probably the only viable landmark relative to which the corneal reflex symmetry between both eyes may be judged, in that there is usually a bright, easily observable contrast between the entrance pupil and the corneal reflex. The entrance pupil center is also used as a landmark for ocular alignment measurement in recently developed digital imaging devices for refractive screening.24-26

The limbus margins27 or the center of the limbus were used as alternative landmarks, for instance, because the margins of the entrance pupil were more difficult to locate in the presence of a dark iris than was the limbus with visible light.3,6 This was combined with computation of eye alignment from ratios of distances—for instance, the ratio of corneal reflex displacement to the width of the limbus.17,20 No calibration for measurement of distances in images would then be necessary; consequently, the examination distance did not have to be controlled.

The movement of the limbus or of its center with eye rotation is readily described through the movement of the limbus in the normal plane of the observer’s direction of gaze as \( h \cdot \sin \phi \) of the eye rotation angle. The entrance pupil center, however, may undergo a prismatic effect with eye rotation, because of its refraction through the cornea. Hence, its motion in the normal plane of the observer’s imaging axis would be more difficult to predict.
Accuracy of Ocular Alignment Measurement

The distance of the central fixation target to the subject's pupillary plane was adjusted to 50 cm.

Therefore, the use of the limbus center as a landmark might offer advantages over the use of the entrance pupil center—more so, because the latter is known to vary significantly between subjects and is prone to unpredictable asymmetric shifts with pupil constriction, occurring with changes of accommodation, vergence, ambient light levels, or neurovegetative tonus.

Therefore, the object of this study was to examine to what extent the choice of the limbus or entrance pupil center as a landmark influences the experimentally found FR, and which of the two choices leads to a more accurate measurement of the ocular alignment.

METHODS

Experimental

In the design of the experiments, care was taken to minimize nonsystematic errors for analysis of systematic differences between different measurement conditions and evaluation procedures. Therefore, a head and chin rest, similar to that used for slit lamp examinations, was used to control head position and distance to the recording apparatus. Subjects were instructed to keep their heads still throughout the measurements in all gaze directions. However, the head was not completely immobilized (for instance, with a bite bar or a strap). All values measured or calculated in degrees (°) were converted into prism diopters (PD) by the equation PD = 100 • tan°.

The apparatus (Fig. 2) consisted of a horizontal fixation bar located 50 cm from the eyes. Fixation targets were positioned at cyclopean gaze angles of ±18.8° (34.04 PD), ±10° (17.63 PD), ±5° (8.75 PD), and 0° (0 PD). Two charge-coupled device cameras (SDT 3440, Seinter Datentechnik, Seefeld, Germany), one for each eye, equipped with extension tubes and lenses, were used for imaging the anterior eye segments at a 50-cm distance. A photo flash unit (Tumax A16) mounted 10 cm vertically under the central 0° fixation target was used to generate a bright corneal reflex in both eyes. Two additional units, positioned 8.8 cm to the left and right of the central light source, were used to generate additional Purkinje images for further studies.

For each gaze direction, three pictures were taken and stored. The horizontal positions of the corneal reflex were measured in 10 young, orthotropic subjects for all gaze directions from 0° (0 PD) to 18.8° to the right and to the left (Fig. 3). Informed consent was obtained from all subjects, and the studies were conducted in accordance with the tenets of the Declaration of Helsinki.

The video signal was digitized (512×512 pixels, 8-bit gray level depth) with two frame grabbers (Matrox PIP 1024, Dorval, Canada) and was stored in a desktop computer. The digitized images were transferred to a digital image workstation and were evaluated interactively with NIH Image 1.58 software (National Institutes of Health, Bethesda, MD). The imaging system was calibrated with a millimeter grid, and the pixel-to-millimeter ratio was determined as 21.5 pixels/mm.

The horizontal positions P of ocular structures involved in the measurement of the horizontal corneal reflex decentrations relative to the limbus center (xlimbus) and to the entrance pupil center (xpup). The horizontal entrance pupil center position (centerpup) was calculated as [P(right pupil margin) + P(left pupil margin)]/2. The horizontal limbus center position (centerlimbus) was calculated as [P(right limbus) + P(left limbus)]/2. Consequently, in the right eye, x_pup = P(corneal reflex) - center_pup, and x_limbus = P(corneal reflex) - center_limbus. In the left eye, x_pup = -P(corneal reflex) - center_pup, and x_limbus = -P(corneal reflex) - center_limbus. These definitions were chosen to have a positive sign of x for a nasal shift of the corneal reflex, consistent with a corresponding positive angle k, and vice versa for a temporal shift.
with no significant bias between central and peripheral portions of the image.

The examiner took the \( x,y \) coordinates of the lateral limbus margins, the entrance pupil margins, and the corneal reflex in each eye. As illustrated in Figure 3, the quantities calculated from this data were the horizontal limbus diameter (LD), the positions of the limbus center and the entrance pupil center, and the horizontal distance of the corneal reflex relative to the entrance pupil center \((x_{\text{up}i})\) and the limbus center \((x_{\text{limb}u})\). A nasal decentration \( x \) carried a positive sign, and a temporal decentration \( x \) carried a negative sign.

Three examiners participated in the evaluation of the image data. There were six subjects with light irides and four subjects with dark irides. On average, the localization of the horizontal position of the nasal and temporal limbus appeared subjectively a little more difficult than the localization of the nasal and temporal entrance pupil margins, because of the presence of a gray-level transition zone from the iris to the sclera that represented the position of the limbus, whereas there was a sharp contrast between entrance pupil and iris, unless the subject had a dark iris.

Error levels associated with image evaluation were assessed in preliminary trials, and the typical discrepancy in the measurement of the corneal reflex position between examiners and between reevaluations of the same image by the same examiner was 0 or 1 pixels.

The overall reproducibility of the measurement of the distance between ocular landmark and corneal reflex in the study with all 10 subjects was assessed in the following way: In the binocular pictures taken in the primary position, the mean and standard deviation of the difference of measured corneal reflex position \( x \), within each set of three images taken of one eye in one gaze direction, was calculated in all 10 subjects for entrance pupil center and limbus center, separately. The standard deviation, \( \text{SD}(x) \), of the corneal reflex position \( x \) in a set of three images served as an indicator of its reproducibility in each subject.

Therefore, the 20 standard deviations, \( \text{SD}(x) \), obtained for all right and left eyes and each landmark (entrance pupil–limbus center), were averaged and their standard deviations calculated: mean of \( \text{SD}(x_{\text{limb}u}) = 0.048 \text{ mm} \) with a SD of \( \text{SD}(x_{\text{limb}u}) = 0.031 \text{ mm} \), and mean of \( \text{SD}(x_{\text{up}i}) = 0.044 \text{ mm} \) with a SD of \( \text{SD}(x_{\text{up}i}) = 0.031 \text{ mm} \). There was no statistically significant difference between the mean values of pupil- and limbus-based measurement of the corneal reflex position. Therefore, the reproducibility of corneal reflex position was the same for pupil-based and limbus-based measurement. The slightly smaller value of the mean \( \text{SD}(x_{\text{up}i}) \), compared with \( \text{SD}(x_{\text{limb}u}) \) is in keeping with the examiners’ subjective impressions that the margins of the entrance pupils were easier to locate than the somewhat less well defined limbi: In general, entrance pupil margins had a sharp contrast with the iris, whereas the limbus could be described as a pixel region with a transition from one gray level to another. In the latter instance, the examiner had to delimit the middle of the transition zone to mark the limbus position, which appeared less well defined than the entrance pupil margins.

The same checks were executed for secondary gaze positions and for monocular images; and again, no significant difference between pupil- and limbus-based measurements were observed in the reproducibility of the corneal reflex position.

The 0.05-mm magnitude of the mean reproducibility roughly equaled the pixel size; therefore, it can be concluded that the determination of the corneal reflex position was limited by the minimum pixel (1-pixel) error in this setup, for either condition. Bear in mind that measurement of the corneal reflex position involved three measurements, each of which could be affected by, for instance, variations of fixation, and by the pixel error (error inherent to digitized images) that could accompany the determination of the nasal and temporal entrance pupil margins or of the nasal and temporal limbus and of the corneal reflex position. This could have entailed the maximum pixel error (3 pixels), but the current data indicate that the reproducibility was far better than that.

In the workup of the patients, the keratometer readings and their objective autorefractions (RK-1; Canon Europe, Amstelveen, The Netherlands) were taken. The keratometer readings were converted to give the corneal curvature in the horizontal meridian. Most subjects were moderately myopic (mean spherical equivalent [SE] approximately -2 diopters [D]; minimum SE -5.6 D, maximum SE +1.7 D). The largest measured astigmatic component was 2.5 D.

Data Evaluation

In the first step of the data analysis, the measured displacements \( x \) were plotted against the sine of the gaze angle \( \beta \) to determine the experimental value of \( h \) and the offset \( x_s (\beta = 0^\circ) \) compared with the relationship \( x = h \cdot \sin(\beta) + x_s \) (derived from equation 1). This was done for each subject, for the entrance pupil center and the limbus center reference, and in both eyes separately. The values of \( h \) in the 10 subjects for each reference center were plotted against the measured corneal radius in the horizontal meridian for right eyes and left eyes separately (Figs. 4A, 4B, 5A, 5B). Mean corneal radius in the right eyes \((r = 8.06 \pm 0.26 \text{ mm [SD]}) \) and in the left eyes \((r = 8.07 \pm 0.30 \text{ mm}) \) were slightly above the average adult value of \( r = 7.8 \text{ mm} \), in keeping with the slight predominance of myopic subjects with larger globes participating in the study. From this data, a mean \( h_{\text{pupil}} = 4.884 \text{ mm} \)
The calculated eye rotation angles $\eta_{RE, pupil}$ and $\eta_{LE, pupil}$ (where RE = right eye and LE = left eye) were computed, using HR$_{pupil}$ mean, and equation 2. In the same manner, $\eta_{RE, limbus}$ and $\eta_{LE, limbus}$ were computed using HR$_{limbus}$ mean. Two ways of interpreting eye alignment data may be used clinically to measure ocular misalignment. These two options were simulated with our data.

Condition 1, binocular approach: The angle of strabismus was obtained by subtracting the value of $\eta_\beta$ for any gaze direction $\beta$ from the value $\eta_\beta$ ($\beta = 0^\circ$) of the fellow eye in the primary position. This should be a good estimate of the manifest ocular misalignment under binocular viewing conditions. However, differences in the offsets $x_0$ of the corneal reflex in the primary position in the right and left eye could lead

(right eye: $4.840 \pm 0.320 \text{ mm}$; left eye: $4.927 \pm 0.347 \text{ mm}$) and a mean $h_{limbus} = 5.243 \text{ mm}$ (right eye: $5.235 \pm 0.290 \text{ mm}$; left eye: $5.253 \pm 0.308 \text{ mm}$) were derived, and linear fits were obtained, one for right and one for left eyes.

The HRs were calculated as $(180^\circ/\pi) \cdot \arcsin(1 \text{ mm/h})/1 \text{ mm}$, as explained above. The differences of the mean HR$_{pupil}$ and HR$_{limbus}$ were found to be statistically significant for right eyes and left eyes, separately. After averaging between right and left eyes, we found HR$_{pupil}$ mean = $11.82^\circ/\text{mm}$ (20.92 PD/mm), and HR$_{limbus}$ mean = $11^\circ/\text{mm}$ (19.43 PD/mm). These HRs were used in the rest of the study to calculate $\eta$ from observed corneal reflex positions.

For comparison, the linear fits of Figures 4A, 4B, 5A, and 5B were used to calculate the HR for the average corneal radius in adults, $r = 7.8 \text{ mm}$. This resulted in $h_{limbus} (r = 7.8 \text{ mm}) = 5.165 \text{ mm}$, and HR$_{limbus} (r = 7.8 \text{ mm}) = 11.16^\circ/\text{mm}$ (19.73 PD/mm), and $h_{pupil} (r = 7.8 \text{ mm}) = 4.854 \text{ mm}$, with HR$_{pupil} (r = 7.8 \text{ mm}) = 11.89^\circ/\text{mm}$ (21.05 PD/mm), with both HRs falling in the range of reported HRs.6,12-15,18,19

The calculated eye rotation angles $\eta_{RE, pupil}$ and $\eta_{LE, pupil}$ (where RE = right eye and LE = left eye) were computed, using HR$_{pupil}$ mean, and equation 2. In the same manner, $\eta_{RE, limbus}$ and $\eta_{LE, limbus}$ were computed using HR$_{limbus}$ mean. Two ways of interpreting eye alignment data may be used clinically to measure ocular misalignment. These two options were simulated with our data.

Condition 1, binocular approach: The angle of strabismus was obtained by subtracting the value of $\eta_\beta$ for any gaze direction $\beta$ from the value $\eta_\beta$ ($\beta = 0^\circ$) of the fellow eye in the primary position. This should be a good estimate of the manifest ocular misalignment under binocular viewing conditions. However, differences in the offsets $x_0$ of the corneal reflex in the primary position in the right and left eye could lead...
to errors in this approach. The differences \( \Delta \eta_{\text{binoc}}(\beta) = \eta_{\text{binoc}}(\beta) - \eta_{\text{monoc}}(\beta = 0^\circ) \), \( \Delta \eta_{\text{limbus}}(\beta) = \eta_{\text{limbus}}(\beta) - \eta_{\text{limbus}}(\beta = 0^\circ) \), with \( \beta = \pm 5^\circ \) (8.75 PD), \( \pm 10^\circ \) (17.63 PD), and \( \pm 18.8^\circ \) (34.04 PD) were used to simulate angles of strabismus of the same size. They were computed in the right and left eyes of the 10 subjects, together with their mean values and SDs. These followed a normal distribution, and the confidence intervals for the measured angle of strabismus were calculated as twice the standard deviation.

Condition 2, monocular approach: The angle of strabismus was obtained by subtracting the value of \( \eta(\beta) \) for any gaze angle \( \beta \) from the value \( \eta(\beta = 0^\circ) \) of the same eye in the primary position. This equals, or at least comes close to, the orthoptic simultaneous prism and cover test procedure. The differences, \( \Delta \eta_{\text{monoc}}(\beta) = \eta(\beta) - \eta(\beta = 0^\circ) \), with \( \beta = \pm 5^\circ \) (8.75 PD), \( \pm 10^\circ \) (17.63 PD), and \( \pm 18.8^\circ \) (34.04 PD), should equal angles of strabismus of the same size. The advantage of this approach is that the offset \( \chi \) of the corneal reflex in the primary position is automatically eliminated — that is, the angle \( \chi \) or any other individual offset does not bias the measurement. The simulated angles of strabismus \( \Delta \eta_{\text{monoc}}(\beta) \) were computed in the right and left eyes of the 10 subjects with their mean values and SDs. Using the same procedure as for condition 1, assessment was made to determine whether the simulated angles of strabismus could be treated as normally distributed. The data were found to be normally distributed for both conditions, and the confidence intervals for the differences between calculated and expected angles of strabismus were calculated as the mean difference plus twice the SDs to assess the error levels.

Finally, to illustrate the variability of the position of the entrance pupil center relative to the limbal center, the horizontal distances of the entrance pupil center relative to the limbus center, \( \text{center}_{\text{pupil}} - \text{center}_{\text{limbus}} \), were plotted against the sine of the gaze angle \( \beta \) for all 10 subjects in the right and left eyes.

**RESULTS**

Four twin box plots (Figs. 6A, 6B, 7A, 7B, 8A, 8B, 9A, 9B) allow for a comparison of accuracy of misalignment measurement with entrance pupil and limbus center reference. In the box plots, the ordinate indicates the difference between measured and expected angle of strabismus. Boxes show 25% and 75% confidence intervals, and T bars indicate 95% confidence intervals; data points outside the 95% confidence interval are shown separately. The boxes represent the seven simulated angles of strabismus, from right \((-18.8^\circ, -34.04\) PD) to left \((+18.8^\circ, +34.04\) PD). Note that the central 0° box refers to the differences of measured angles in the primary position, below which a manifest strabismus may not be distinguished from alignment data occurring in orthotropic subjects. In other words, 0° data delimit the threshold for detection of a manifest misalignment in the primary position.

Condition 1 simulated binocular angles of strabismus: Figures 6A and 6B show box plots of the differences between expected and simulated angles of strabismus (limbus center), for (A) right eye and for (B) left eye misaligned, for six simulated angles of strabismus calculated from limbus center reference data. Figures 7A and 7B show the box plots of the difference between calculated eye rotation angles \( \eta_{\text{limbus}} \) and \( \eta_{\text{limbus}}(\beta = 0^\circ) \) of the fellow eye, (A) right eye misaligned and (B) left eye misaligned, for six simulated angles of strabismus calculated from limbus center reference data.

**FIGURES 6 TO 9.** Box plots (for Figures 6 to 9) of differences between calculated and expected angles of strabismus for binocular condition 1 and monocular condition 2. Central bar indicates median, upper and lower box borders refer to 25% to 75% confidence interval limits, and T-bars refer to 95% confidence intervals. Data points outside these limits are shown as individual points.

**FIGURE 6, Condition 1:** box plots of the difference between calculated eye rotation angles \( \eta_{\text{limbus}} \) and \( \eta_{\text{limbus}}(\beta = 0^\circ) \) of the fellow eye, (A) right eye misaligned and (B) left eye misaligned, for six simulated angles of strabismus calculated from limbus center reference data.
Condition 2 simulated monocular angles of strabismus: Figures 8A and 8B show box plots of the difference between expected and simulated angles of strabismus (limbus center), for (A) right eyes and for (B) left eyes. In the primary position, the maximum 95% confidence intervals for right and left eyes were limbus, ±1.07° (1.9 PD), and entrance pupil, ±1.3° (2.3 PD). Errors were slightly smaller for the limbus option for all simulated angles of strabismus. The 95% confidence levels were, at most, 19.3% (limbus) and 21.9% (entrance pupil) of the amount of the simulated angles of strabismus.

It could be speculated that with a higher camera resolution, systematic differences between pupil- and limbus-based measurement reproducibility could have been noted, if they were present. However, such error levels would, on average, have had to be smaller than or equal to at least 1 pixel. Consequently, any systematic difference between pupil- and limbus-based mean corneal reflex distances that was markedly larger than 1 pixel must be caused by genuine differences between these conditions, in that the image evaluation was done in the same images.

Figure 10 (right eyes only) reveals that the entrance pupil and limbus center positions, plotted against the sine of the gaze angle, varied for different gaze angles. In most subjects, the differences decreased when looking to the left (negative sines). For some of the plots, satisfactory linear regressions could be obtained. At approximately an 11° (19.43 PD) gaze angle to the left, entrance pupil and limbus centers of the right eyes coincided, on average, within a range of ~±0.25 mm. The average distance between entrance pupil center and limbus center in the primary position is indicated by the differences at \( \sin(0°) \): On average, the entrance pupil centers were decentered ~0.18 to 0.23 mm nasally, in keeping with independent data. The difference between both increased with a more temporal position of the eye, because the projected distances between the entrance pupil and limbus centers appeared larger when viewed obliquely, in that the entrance pupil plane is slightly behind the limbus plane (Figs. 4, 5). The data for left eyes (not shown in Fig. 10) suggest similar conclusions. Note, however, that in one subject (top plot, black triangles), the difference was nearly constant and linearity was limited in some of the plots. This could be because of the entrance pupil movement; the limbus movement should be linear with the sine of the gaze angle, in keeping with the eye model in Figure 1. However, it could be speculated that in some
of the cases, the limbus itself was seen through the peripheral portions of the cornea, and hence could be treated as an entrance limbus itself. The movement of its center would then be more difficult to predict.

DISCUSSION

Brodie et al. favored the use of the limbus as a landmark, partly for practical reasons, partly because they suspected that this choice would reduce error levels caused by varying depths of the anterior chamber. In the same study, a nominal value for \( h \) was computed, but an experimental dependence on the corneal radius was not established, and a single HR was advocated regardless of pupil- or limbus-based measurement, confirming previous similar reasoning.

In the eye model shown in Figure 1, the difference between the entrance pupil center and the limbus center as a landmark and the resulting behavior may be explained readily: The entrance pupil center is, on average, \( \sim 0.4 \) mm axially behind the limbus center, and is nasally shifted by approximately \( 0.25 \) mm. This can be adjusted for by using appropriate HRs.

However, there is some variation between right and left eyes, and the scatter of the difference is sometimes large. This suggests that the entrance pupil center is a much less reliable landmark than the limbus center, when used to measure the relative position of the corneal reflex. Unpredictable shifts of the entrance pupil center and variable anterior chamber depths contribute to this unsystematic error.

To examine whether varying pupil size could have been the reason for the lower precision of eye alignment measurement, horizontal entrance pupil and limbus diameter variations were analyzed in the same way as the reproducibility of corneal reflex position (see Methods): For each eye, the mean diameter of the limb of the entrance pupil, and its standard deviation, SD(diameter), were determined from a triplet of images in the primary position. Then the mean SD(diameter) and standard deviation of SD(diameter) of the 20 individual standard deviations of the diameters were computed. Mean standard deviations indicated a significant difference \( (P < 0.0001, \text{paired Student's } t \text{test}) \) between entrance pupil and limbus diameter variation: Mean SD(pupil diameter) = 0.456 mm, with a standard deviation of SD(pupil diameter) = 0.154 mm, mean SD(limbus diameter) = 0.132 mm, with a standard deviation of SD(limbus diameter) = 0.137 mm. Note that the mean SD(pupil diameter) was much larger than the pixel error. This indicated that the reproducibility of diameter measurements was equal for the entrance pupil and for the limbus.

FIGURE 10. Plot of the horizontal distance between entrance pupil center and limbus center, \( \text{center}_{\text{pupil}} - \text{center}_{\text{limbus}} \), versus \( \sin(\beta) \) in the right eyes. In this plot, a linear behavior points to a constant ratio of \( \text{center}_{\text{pupil}} / \text{center}_{\text{limbus}} \). The slopes of the graphs are proportional to individual ratios of \( h \). The differences change approximately linearly with \( \sin(\beta) \), but the positions and slopes of the curves vary significantly between individuals, and in some instances, nonlinear behavior is evident.
The variability of the entrance pupil diameter, however, was threefold, compared with the limbus diameter variability and compared with the measurement reproducibility of the diameters. Similar results were obtained for the other gaze positions.

This means that the most probable explanation for the difference of precision between limbus and entrance pupil measurement of eye alignment in binocular images is given by the larger variations of entrance pupil size, and hence by the associated shifts of the entrance pupil center without an ocular rotation. By contrast, at least in our model (Fig. 1), the limbus center cannot shift in a similar way, because the limbus is a rigid structure. However, the simple anatomic model shown in Figure 1 does not represent all of the real effects that could be expected: Coincidentally, the effects of neglected corneal asphericity and the linearization of the sine in equation 1 compensate each other to some extent, such that the validity of equation 2 is extended to larger angles. Exact ray tracing analysis with software introduced elsewhere and modified to model aspheric corneas, showed that when a spherical shape was taken into account, the limbus must be assumed to be a structure imaged through the peripheral portions of the cornea, lying ~0.5 mm behind the corneal surface. This means that the visible limbus is an “entrance limbus” analogous to the entrance pupil.

The study's maximum error in eye alignment measurement is tangible in the binocular approach (Figs. 6A, 6B, 7A, 7B): Here, the errors of entrance pupil data became approximately twice as large as those of limbus data. This is of considerable clinical relevance, because most measurements of eye alignment are recorded relative to the entrance pupil center as binocular difference of ocular alignment between right and left eyes, in that this does not require pictures with one eye covered.

In the monocular approach (Figs. 8A, 8B, 9A, 9B), any offset in terms of entrance pupil center shift, angle \( \kappa \) or other parameter, whatever its cause, was cancelled, because only the difference of the angle \( \eta \) between two gaze directions was computed. This reduced the error levels such that differences between entrance pupil and limbus center measurements became small, if not negligible. However, this measurement procedure, which requires covering the right eye and the left eye, often cannot be applied in infants, toddlers, and preschool children, in whom screening with an objective ocular alignment technique is most useful.

It is of clinical and practical interest to note that when coaxial infrared lighting screening techniques are used, the entrance pupil margins are easily identifiable structures with simple thresholding algorithms in digitized images. This is because the dominant image portions are the fundus reflexes against which the corneal reflex appears as a single bright reflex and the entrance pupil margins as sharp boundaries of the fundus reflex. The limbal region is imaged with less contrast under these lighting conditions, which may lead to neglect of the limbus center as a landmark. This explains, possibly together with a limited resolution of the CCD camera used in the apparatus, the limited accuracy of 8 to 10 PD (4.6° to 5.7°) for detection of ocular misalignment reported elsewhere. In the current study, despite subjective impression, the limbus could be located with precision interactively. We also showed elsewhere that image processing may be used to automate the detection of limbus structures in digitized images and with nearly the same precision as is possible in detection of the entrance pupil margins.

These results prove that the accuracy of measurement of ocular misalignment depends decisively on the choice of the reference landmark. Different HRs should be used, depending on this choice, because the mean limbus value was 1.5 PD/mm lower than the entrance pupil value in this study. Normative values for \( h \), the distance that, together with the corneal radius and limbus diameter, governs the HR, were established for limbus and entrance pupil center landmarks for an average human adult eye with corneal radius \( r = 7.8 \) mm. Most data in the literature refer to the entrance pupil center only or do not take into account systematic differences between pupil- and limbus-based measurement. For specific populations, in which the corneal radius may be measured, the HR may be determined from our data by interpolation or extrapolation of \( h \), unless the HR is calculated through plots of measured corneal reflex shift versus gaze angle. Using this alternative for evaluation of the present alignment data led to the same systematic difference between pupil- and limbus-based HRs. We conclude that, as a rule, limbus-based HRs are 1 to 2 PD/mm lower in humans than are pupil-based HRs. In animals, similar systematic effects can be predicted.

After the current study, which was conducted solely on control subjects, to minimize errors that would impede the accurate assessment of misalignment measurements, a comparison of methods could be done in strabismic subjects with orthoptic control data. However, care must be taken to avoid subject-related, time-dependent fluctuations of the angle of strabismus when different techniques are compared. Because random errors would be larger than those in this study, the number of strabismic subjects would have to be much higher to ascertain the same amount of systematic difference. Because of the design of the current study, it was possible to examine differences between techniques with a relatively low number of subjects.
Other studies concluded that $h$ was usually independent of age after 3 years of age and that the statistical correlation of the HR with the corneal radius was too weak to be relevant. Although this may be true, the systematic yet small difference in HR related to the choice of the landmark should be taken into account, because it would improve reported insufficient thresholds for ocular misalignment detection.

The highest accuracy of ocular alignment measurement was obtained with the monocular approach, in which the choice of limbus or entrance pupil center did not make a difference. However, such a measurement strategy depends on the cooperation of the subject.

In conclusion, we recommend the use of the limbus center for screening and measuring applications with corneal reflexes, whenever recorded images are used. The threshold for detection of ocular misalignment in the binocular approach, the 95% confidence interval, was $3.174^\circ$ (5.5 PD), compared with nearly twice that error in the widely used entrance pupil center evaluation. To increase the precision of ocular alignment measurement further, measurement with corneal and posterior crystalline lens reflexes from three light sources could be used. Using this methodology and the alignment data of this study, the threshold for detection of ocular misalignment was $2.1^\circ$ (3.7 PD).

If the corneal reflex position is simply estimated during a clinical examination, without recording, we speculate that the limbus option probably does not improve the accuracy of the eye alignment assessment: The errors in the estimation of the asymmetry of the corneal reflex position, especially regarding the limbus center, are probably much larger than the potential increase in accuracy indicated by the results of the current study.

A similar effect can be expected if the spatial resolution of the recorded images is low—that is, on the same order as the inherent errors of binocular corneal reflex position measurement: If the limbus-center-related threshold in the primary gaze is taken from the current data as $3.174^\circ$ (5.5 PD), and a typical HR is $\approx 21$ PD/mm, then a critical limit must be expected for a resolution of $\approx 0.25$ mm/pixel, or 4 pixels per millimeter as a hardware magnification factor (compared with 21.5 pixels per millimeter in this study, with one camera per eye). To image spatial differences in the corneal reflex position of 0.25 mm reliably, the required pixel density must be higher than this limit, depending on the type of hardware used.

Fortunately, 35-mm photographs or slides have much better resolution than 0.25 mm, and most of the currently available low-cost analog CCD cameras and digitizing frame-grabbers frequently used for eye alignment measurement perform well enough to surpass the critical value of 4 pixels per millimeter; some perform well even if only one camera is used to image both eyes at a time.

However, care must be taken not to overestimate the spatial and temporal resolution that can realistically be expected with some CCD cameras: For instance, in the interface mode, half-images are typically read out at 50 to 60 Hz, which can cause motion artifacts (shifted half-images of moving objects), especially in eye-tracking applications; and asynchronous pixel readout or aliasing may cause errors of at least 1 pixel width. The maximum spatial resolution is also limited by the amount of CCD sensor elements per row and number of rows, signal-to-noise ratio, bandwidth of the analog video signal, and the frame grabber resolution. Such effects limit the benefit of any subpixel evaluation technique. These problems may be avoided by using appropriate imaging devices—for instance, analog full-frame-transfer CCD cameras and cameras with a synchronized pixel transfer, or CCD cameras with a digital output, which do not require a frame grabber.

Therefore, in addition to the proper choice of the landmark for corneal reflex position, we recommend that the design of recording devices for screening, clinical, and research purposes be guided by the desired measuring accuracy or threshold for detection and that the hardware be carefully selected and tested to ensure that those criteria are met.

Key Words
accuracy, angle of strabismus, corneal reflex, instrumentation, paraxial ray tracing analysis

References
Accuracy of Ocular Alignment Measurement


