Reference Plane Definition and Reproducibility in Optic Nerve Head Images

James C. H. Tan and Roger A. Hitchings

Purpose. To describe and evaluate a new experimental reference plane for measuring rim area in scanning laser tomography.

Methods. The experimental reference plane was positioned so that (1) it always lay entirely below the margin of the optic nerve head (ONH), (2) it remained at a set z-axis distance below the ONH in images of each eye, and (3) it was at a level where variability in rim area is least. Twenty normal control subjects and 20 patients with glaucoma underwent test–retest scanning laser tomographic imaging by same and different operators during same and separate visits. Control subjects had image series spanning at least 3 years. The effect of the positioning of the reference plane on global and regional rim area variability was assessed in intra- and intervisit test–retest images and longitudinal image series and compared with the standard and 320-μm reference planes.

Results. Variability in the experimental reference plane was less in test–retest images and longitudinal data (P < 0.05) and more uniform around the ONH than with other reference planes. Variability in the former was not appreciably affected by testing involving different operators and visits, or by the presence of glaucoma.

Conclusions. Variability in rim area by the experimental reference plane was significantly less, more uniform around the ONH, not affected by different operators and visits, and less affected by glaucomatous morphology than other reference planes. This difference was pronounced in sequential data and affected by glaucomatous morphology than other reference planes, and less affected by different operators and visits. CONCLUSIONS.

METHODS

Reference Plane Analysis

Figure 1 illustrates the different reference plane concepts compared in this study. The standard and 320-μm reference planes have been used widely in studies.

New Experimental Reference Plane. For each eye, a mean topography image was selected to be the baseline image in which the position of the reference plane was determined, calculating the position as follows:

\[ LOW_{5\%} \]

The contour line’s lowest region was calculated \( LOW_{5\%} \). Heights 1° apart (360 data points) on the contour lines of single topography images (used to derive the baseline mean topography image) were read from the HRT software. Heights were ranked in each single topography image, from which the mean of the lowest 5% of heights was calculated. The means of the lowest 5% of heights for each of the three single topography images were averaged to arrive at \( LOW_{5\%} \).

\( R \). The reference plane was positioned to ensure that it lay beneath the entire circumference of the contour line. A reference plane lying above part of the contour line underestimates adjacent rim tissue. To determine \( R \), variability was analyzed in longitudinal image series of normal control eyes. \( R \) was the distance of the reference plane beneath \( LOW_{5\%} \), where rim area variability was least. This was held constant once calculated.

\( REF_{dist} \). Once positioned at \( R \), the z-axis distance of the reference plane below mean height of the contour line (MHC; which is the mean height of locations on the contour line measured in relation to the reference ring) was calculated for each ONH and called \( REF_{dist} \). MHC was used as a marker of the topographical z-axis position of the ONH. \( REF_{dist} \) is unique to each ONH and, once calculated in the baseline image, is kept constant in all images of an eye. By keeping \( REF_{dist} \) constant, we sought to keep the height relationship between the ONH and reference plane constant in image series of the same eye. \( REF_{dist} \) can be expressed as:

\[ REF_{dist} = LOW_{5\%} + R \]  

(1)

\( REF_{per} \). Position of the reference plane, or \( REF_{per} \), can thus be expressed as:

\[ REF_{per} = \text{image height} \]
Referring to the mean contour line height between 350° and 356° on the contour line (HRT software versions 1.11–2.01, and HRT II). The reference ring centered on the image frame and located in its periphery, had an outer diameter of 94% and a width of 3% of the image size, and is the zero-referencing region for pixel height.9

The 320-μm Reference Plane. This plane was offset 320 μm posterior to the mean height of the reference ring (HRT software versions 1.09–1.10). The reference ring is centered on the image frame and located in its periphery, had an outer diameter of 94% and a width of 3% of the image size, and is the zero-referencing region for pixel height.9

Subjects and Imaging

Variability in rim area was studied in test–retest images of age-matched subjects with glaucoma and normal control subjects, and in longitudinal series of images of normal eyes. Normal subjects and those with glaucoma attended the Ocular Hypertension and Early Glaucoma Research Clinic at Moorfields Eye Hospital and underwent the same protocol of repeat testing. All had previously undergone scanning laser tomography and perimetry at least six times. Table 1 shows subjects’ demographic information. This study adhered to the tenets of the Declaration of Helsinki and had appropriate Institutional Review Board approval and the subjects’ informed consent.

Normal subjects were volunteers comprising spouses or friends of hospital patients, hospital staff, or members of external nonmedical social organizations. They had IOP persistently less than 22 mm Hg; normal and reliable serial visual fields (Humphrey 24-2; Humphrey Systems) with AGIS scores of 0; no concurrent ocular disease; no family history of glaucoma; and refractive errors less than ±6 D. All were more than 40 years of age. The appearance of the ONH was not taken into account for entry into the study. Patients with glaucoma had had pretreatment IOP higher than 21 mm Hg on at least two occasions; reproducible field defects (24-2 program; Humphrey Systems) with AGIS scores higher than 0; open anterior chamber angles; and no known ocular disease other than glaucoma. All patients were treated medically and had IOP lower than 22 mm Hg at the time of testing. Neither ONH appearance nor severity of visual field abnormality was used to restrict entry into the study.

Test–retest scanning laser tomography (HRT software ver. 2.01; Heidelberg Engineering) of both eyes of 20 normal and 20 age-matched subjects with glaucoma was conducted by experienced operators. Three well-centered 10° single topography images were acquired at each session. Corneal curvature, scan depth, and focus settings were kept constant. Pupils were not dilated. Normal subjects attended two test visits separated by 6 to 8 months. Each visit comprised two imaging sessions separated by at least 1 hour. The same operator performed the scans in both imaging sessions of the first visit and in one session of the second visit. A second operator performed the scans in the other imaging sessions of the second visit. Patients with glaucoma underwent test–retest imaging in only one visit, performed by the same operator. Imaging sessions were randomly scheduled. Test–retest variability was analyzed for testing by: the same operator in the same visit (same operator-same visit) and by different operators in separate visits (different operator-different visit). This is relevant because longitudinal imaging in chronic glaucoma is likely to be performed in many different visits over time by different operators.

Nineteen normal subjects had longitudinal image series from at least six imaging visits over at least 3 years. Different operators with various levels of experience had acquired these images because of the turnover of research clinic technical staff. Each series was analyzed separately.

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932918/ on 10/31/2018)

**Figure 1.** Different positions of reference planes. Top: experimental reference plane; middle: standard reference plane; bottom: 320-μm reference plane.

\[
\text{REF}_{\text{pos}} = \text{MHC} + \text{REF}_{\text{dis}} \quad (2)
\]
\[
= \text{MHC} + \text{LOW}_{\text{5%}} + R \quad (3)
\]

**Standard Reference Plane.** This plane was set 50 μm posterior to the mean contour line height between 350° and 356° on the contour line (HRT software versions 1.11–2.01, and HRT II).5,9

**The 320-μm Reference Plane.** This plane was offset 320 μm posterior to the mean height of the reference ring (HRT software versions 1.09–1.10). The reference ring is centered on the image frame and located in its periphery, had an outer diameter of 94% and a width of 3% of the image size, and is the zero-referencing region for pixel height.9

**Table 1.** Demographic Data for Normal and Glaucoma Groups

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Glaucoma</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>66.5 ± 9.2</td>
<td>68.8 ± 11.5</td>
<td>0.70</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Right eyes</td>
<td>13</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Visual field MD (dB)</td>
<td>+0.11 ± 1.2</td>
<td>-4.6 ± 1.9</td>
<td>0.0000</td>
</tr>
<tr>
<td>Visual field CPSD (dB)</td>
<td>0.89 ± 0.75</td>
<td>3.3 ± 1.8</td>
<td>0.0000</td>
</tr>
<tr>
<td>Mean SD (μm) of mean images</td>
<td>22.6 ± 8.1</td>
<td>25.9 ± 9.65*</td>
<td>0.17*</td>
</tr>
<tr>
<td></td>
<td>22.9 ± 10.2†</td>
<td>27.2 ± 10.5†</td>
<td>0.16†</td>
</tr>
</tbody>
</table>

*Image reliability data from the first sessions of the first test visit† and second visit‡ are shown. MD, mean deviation; CPSD, corrected pattern standard deviation.*
Image Analysis and Statistics

Mean topography images from one randomly selected eye of subjects in the glaucoma and normal groups were analyzed by the three reference planes. Mean images were generated from triplets of single topography images and used if mean pixel standard deviation was less than 50 μm, with pixel height measured by the reference ring. A contour line was outlined corresponding to the inner margin of the scleral ring of Elschnig on a mean topographic image of each subject (all performed by JCHT). Stereoscopic optic disc photographs were referred to if needed. Contour lines were then exported to related test–retest mean topography images. Rim area was evaluated globally and regionally. Patterns of variability around the ONH were examined by assessing regional rim area in 30° sectors around the ONH (0–360°).

Variability in test–retest data was represented by agreement between pairs of images, expressed in graphs as the width of agreement intervals (95% confidence intervals of differences). For regional variability, analysis was modified so that agreement intervals for rim area sectors were presented in bar graphs by angle (0–360°).

Variability in longitudinal image series was represented by standard deviation, which was used to estimate variability so that R could be derived and in longitudinal data. The width of the intervals for the 5th to 95th percentiles and median standard deviations for each sector were plotted in bar graphs. Significance testing in nonparametric data was by the Wilcoxon matched-pairs test (signed rank sum test) for comparing paired data. Statistical analysis was conducted by computer (SPSS ver. 9.0 for Windows; SPSS Inc, Chicago, IL).

RESULTS

Derivation of R

Figure 2 shows a trend toward decreasing rim area variability as the reference plane descended from 0 to 100 μm below LOW%. Variability was least between 100 and 120 μm but increased when the reference plane was more than 120 μm below LOW%. R of 100 μm was chosen for subsequent analysis.

Variability in Rim Area in Test–Retest Data

Figure 3 shows that 95% confidence intervals of differences (agreement intervals) for global variability in normal eyes were narrower with the experimental reference plane than standard and 320-μm reference planes. All agreement intervals tended to be wider with different-operator–different-visit testing than same-operator–same-visit testing. This tendency was less, however, in experimental reference plane data than data from other reference planes: widening of agreement intervals with
the experimental reference plane was 23% and 17% less than with the standard and 320-μm reference planes, respectively.

Figure 4 shows polar plots of agreement intervals for regional rim area variability in normal and glaucomatous eyes. In normal control subjects, agreement intervals tended to be wider temporally (0°–90° and 270°–360°) than nasally in all reference planes. In glaucomatous eyes, agreement intervals tended to be widest temporally with the experimental and 320-μm reference planes but widest nasally with the standard reference plane. Same-operator–same-visit regional variability was more uniform around the ONH with the experimental reference plane (left) than with other reference planes (middle and right). Unlike other reference planes, the variability profile of experimental reference plane data did not change appreciably with different-operator–different-visit testing or in testing glaucomatous eyes.

**Variability in Rim Area in Longitudinal Series of Images**

Figure 5 shows that the patterns of regional variability differed between reference planes. The 5th- to 95th-percentile intervals of sector variability were wider in standard and 320-μm reference plane data than in experimental reference plane data for every sector. Agreement intervals for the standard and 320-μm reference planes were widest temporally and nasally. Intervals in the former were particularly wide nasally and in keeping with findings in standard reference plane test-retest data. Again, experimental reference plane variability was more uniform around the ONH than in standard and 320-μm reference plane data.

Figure 6 shows rim area sectors in longitudinal image series with significantly less variability (P < 0.05), when measured by the experimental reference plane compared with the standard (Fig. 6, left) and 320-μm (Fig. 6, right) reference planes. The pattern of sector variability differed between standard and 320-μm reference plane data.

Figure 7. Description of rim area by the standard (top left) and 320-μm (bottom left) reference planes in the same image. Adjacent to the images are renderings of the ONH in cross-section: profiles: height along the contour line marking the ONH margin; top horizontal line: MHC; bottom horizontal line: reference plane. With the standard reference plane (top), rim area is represented as expected. With the 320-μm reference plane (bottom), the plane is situated above the temporal margin of the ONH, causing rim area to be grossly underestimated. The variation in rim area due to different reference plane positions is not uniform around the ONH but much greater temporally than nasally. This is probably because the surface of nasal rim is relatively high (profiles between 90° and 270°) with its adjacent cup walls steeper.
DISCUSSION

Increased variability in rim area can be expected if the ONH and reference plane shift in relation to each other from image.\textsuperscript{12} Analyzed by the experimental reference plane, rim area variability tended to be less than in standard or 320-\textmu{m} reference plane analysis. Variability around the ONH was also more variable, was not appreciably affected by glaucomatous morphology or different test conditions, and was significantly ($P < 0.05$) less than other reference planes in longitudinal analysis.

No two ONHs are identical, and thus analysis should adapt to suit morphologic variations. The experimental reference plane was devised to lie entirely beneath the margin of the ONH by factoring the height profile of each contour line into calculations. Figure 7 illustrates why this is necessary. LOW\textsubscript{5\%} was the level in each ONH above which the reference plane should not rise. The value for LOW\textsubscript{5\%} was not preselected but was derived from the lowest 5\% of contour line heights of each ONH and averaged from multiple topographies to minimize the effect of randomly outlying values. R of 100 \textmu{m} (100 \textmu{m} below LOW\textsubscript{5\%}) was relatively deep and compatible with least variability. With this R, the reference plane is more likely to remain entirely below the ONH margin despite topographical variability. The cup is probably steeper here as well. Rim area is affected more when the reference plane shifts on a gradually sloping than a steep cup, as suggested by Figures 1 and 7. With R deeper than 120 \textmu{m}, variability tended to increase, possibly because of proximity to major vascular trunks and the lamina cribrosa, especially in shallow cups. REF\textsubscript{xos} was derived from all heights on the contour line so that the reference plane’s position would not rely on localized regions or presume selectivity in the pattern of change.

Unlike other definitions, the experimental reference plane compensates for positional changes between the reference plane and ONH. This is the main reason for its reduced variability.\textsuperscript{12,15,16} Testing involving different operators and visits probably exaggerates these positional changes. The 320-\textmu{m} reference plane does not cater well to morphologic variations and may lie above parts of the ONH margin,\textsuperscript{5} as depicted in Figure 7. The standard reference plane is superficial in the temporal ONH (50 \textmu{m} below the surface), where cup slope is gradual and susceptible to variability if the reference plane shifts. In glaucoma, the nasal ONH surface may be depressed and come to lie beneath the standard reference plane, which is fixed temporally. Because the standard reference plane is positioned by a small 6° section of the inferotemporal ONH where it pivots, tilting of the image or shifts in reference plane may cause variability opposite the pivot nasally.

Reference planes have usually been positioned by landmarks presumed to be relatively unaffected by glaucoma, although whether this is true has been debated.\textsuperscript{17} The experimental reference plane differs in being positioned by a landmark that is expected to change in glaucoma. But there are reasons that this position is useful. First, experimental reference-plane data were more reproducible than other reference planes and not appreciably influenced by varied test conditions or glaucomatous morphology. Variability that changes with repeat testing and advancing damage can confound analysis. Second, the experimental reference plane compensated for shifts of the ONH that can affect analysis were the reference plane not to shift in tandem, as illustrated in Figure 8. Changed rim area should reflect disease rather than such shifts. Third, positioning the reference plane at the same distance below the ONH surface allowed standardized measurement of rim area in imaging series. The ONH may shift in relation to external landmarks, such as the reference ring, which if used to anchor the reference plane can result in measurement artifact (Fig. 8).
Bias in measuring height and volume by the experimental reference plane can be expected, but this is less so with rim area because it is estimated perpendicular to the z-axis. Fourth, detecting progression depends on the system’s signal-to-noise ratio. The z-axis position of the ONH fluctuated considerably in image series: 50% had a range of MHC exceeding 120 μm, with this range greater than 200 μm in some. Normal human retinal nerve fiber layer (RNFL) at the ONH margin is 310 to 406 μm thick,18 and so MHC variability is considerable in relation to normal RNFL thickness. MHC variability may even exceed RNFL thickness in glaucoma if significant axons are already lost by the time visual field defects appear. Empiric data suggest that the benefit of significantly reduced variability should outweigh the possibility that MHC is affected by glaucoma. Figure 8 shows that experimental reference plane analysis is sensitive to subtle rim area progression despite markedly reduced MHC.

Variability in rim area was less and more uniform around the ONH when the z-axis distance between the experimental reference plane and ONH was held constant. More uniform variability should be simpler to account for and help limit bias in detecting localized progression. Application of the experimental reference plane in progressive glaucoma is outside the scope of the present study but will be the subject of a future study.

References


