Reasons for Rim Area Variability in Scanning Laser Tomography

James C. H. Tan,1,2 David F. Garway-Heath,1,2 Fred W. Fitzke,2 and Roger A. Hitchings1,2

PURPOSE. To determine reasons for rim area variability in scanning laser tomography.

METHODS. Regional rim area variability from testing in same and different visits and by same and different observers was characterized in 30 normal and 42 glaucomatous eyes. Variations in (1) optic nerve head (ONH) surface geometry (center of gravity: X, Y, Z), (2) image tilting (horizontally and vertically), and (3) position of the reference plane in relation to the ONH (REF) were analyzed by multiple regression analysis. Whether and how much these factors explain rim area variability was studied in cross-sectional and longitudinal data by using two different reference planes.

RESULTS. Variability was higher in glaucoma and in testing by different observers in separate visits. Across a range of eyes, approximately 40% of variability in single-topography images and 60% of variability in mean-topography images was explained. In individual image series, a median 85% of variability in mean-topography images was explained in cross-sectional and longitudinal data by using two different reference planes.

CONCLUSIONS. A large proportion of rim area variability was explained by variation in the topographical features studied, especially REF and Z. Reference plane definition also influenced variability. Variation in the position of the reference plane in relation to the ONH can affect rim area measurements and should be considered when evaluating the progression of glaucoma. (Invest Ophthalmol Vis Sci. 2005;44:1126–1131) DOI:10.1167/iovs.01-1294

Scanning laser tomography of the optic nerve head (ONH) is reproducible,1,2 accurate,3 and potentially useful for assessing progression of glaucoma. Progression is expected to reduce topographical height, but height may appear to change because of variability in the measurements. Topographical height in images is measured by the height of a reference ring in the image periphery which serves as a zero reference, and can then be analyzed by a reference plane to determine topographical parameters such as rim area. Parameters could be affected if positions of the reference ring, reference plane, and pixels defining the ONH shift in relation to each other from image to image. Because these positional relationships are crucial to determining parameters such as rim area, their study could lead to ways to compensate for variability.

METHODS

Criteria for Selecting Subjects

Subjects were selected from the ocular hypertension and early glaucoma research clinic at Moorfields Eye Hospital to undergo the same protocol of repeat testing. They were experienced with tests, having previously undergone scanning laser tomography and perimetry at least six times. 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 who were spouses or friends of hospital patients, hospital staff, or members of external nonmedical social organizations. They had (1) intraocular pressure (IOP) by tonometry that was repeatedly found to be less than 22 mm Hg, (2) serially normal and reliable visual fields (Humphrey 24-2; Humphrey Systems, Dublin, CA) with Advanced Glaucoma Intervention Study (AGIS)3 visual field scores of zero, (3) no concurrent ocular disease, (4) no family history of glaucoma, (5) refractive errors less than ±2.0 D, and (6) age of more than 40 years. ONH appearance was not taken into account for entry into the study. Patients with glaucoma had pretreatment IOP of more than 21 mm Hg on at least two separate occasions, reproducible and reliable visual field defects (Humphrey 24-2; Humphrey Systems) with AGIS scores of more than zero, open anterior chamber angles, and no known ocular disease other than glaucoma. All patients with glaucoma were treated medically and had IOP less than 22 mm Hg at the time of testing. Neither the appearance of the ONH nor severity of visual field abnormality was used to restrict entry into the study.

Imaging

Seventy-two age-matched subjects—30 normal and 42 with glaucoma—underwent test–retest imaging by the same operator in the same visit (intrainvisit–intraoperator). Of the 72, 21 normal and 21 subjects with glaucoma also underwent test–retest imaging by different operators in separate visits (intervisit–interoperator). Their demographics are shown in Table 1. Variability in longitudinal data was assessed in single-topography image series in 20 normal subjects.

Intrainvisit–intraoperator and intervisit–interoperator test–retest rim area variabilities were evaluated. Testing was conducted in two test visits 6 to 8 months apart. Each visit had two imaging sessions separated by at least 1 hour. Two experienced operators conducted scanning; one in both sessions of the first visit, the other in the second visit. The order of imaging sessions was random. At each session, three well-centered 10° topography images were acquired of subjects’ eyes in random order (Heidelberg Retina Tomograph [HRT], software ver. 2.01; Heidelberg Engineering, Heidelberg, Germany). Corneal curvature, scan depth, and focus settings were kept constant, and pupils...
were not dilated. Mean-topography images from one randomly selected eye of subjects with glaucoma and normal subjects were analyzed. The mean images were generated from triplets of single-topography images and used if mean pixel standard deviation was less than 50 Hm. A contour line was drawn on the inner margin of the scleral ring of Elschnig in mean images of each subject (all JT), as shown in Figure 1B. Stereoscopic optic disc photographs were referred to if needed. Contour lines were exported to images of each eye. Reproducibility of drawing and exporting the contour line is reported elsewhere.6,7 Rim area variability was assessed globally and regionally. Variability was then investigated in longitudinal series of single-topography images of normal eyes. Each series was analyzed separately and had more than 20 images obtained during 3 years. Images in each series had been acquired by three to six different experienced and inexperienced operators because of the turnover of clinic technical staff, reflecting the realities of longitudinal testing.

**Image Analysis**

Rim area was analyzed by the standard reference plane set 50 μm posterior to the mean of contour line heights between 350° to 356° on the contour line (HRT software ver. 1.11-2.01; Heidelberg Engineering), and the 320-μm reference plane offset 320 μm posterior to the mean height of the reference ring (HRT software ver. 1.09-1.10; Heidelberg Engineering). The reference ring is centered on the image frame and located in its periphery and has an outer diameter that is 94% and width that is 3% of the image size as shown in Figure 1A.

Factors potentially influencing rim area variability (RIMVAR) were studied: position of the reference plane in relation to the ONH margin.

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932918/)
(REF), orientation of the scanned ONH (HTILT, VTILT), and ONH surface geometry (X, Y, Z). Both the two latter factors are independent of reference planes. Analysis in pairs of mean-topography images was as differences (diff), and in series of single topography images as standard deviations (sd).

1. RIMVAR: rim area variability; analyzed as RIMVAR$_{intr}$ and RIMVAR$_{inter}$
2. REF: variability in the z-axis height between the ONH margin and reference plane, calculated as $|H_{ref} - MHC|$; where $H_{ref}$ is the z-axis distance between the reference plane and mean reference ring height, and MHC is the mean height of the contour line measured from the reference ring. MHC represented the ONH position on the z-axis as shown in Figure 2. Analysis was for REF$_{intr}$ and REF$_{inter}$
3. HTILT and VTILT. HRT software tilts each image horizontally (HTILT) and vertically (VTILT) to compensate for inclination of the peripapillary retinal plane. Variation in tilt-compensation was taken to represent the variable orientation of the ONH and peripapillary retina. Analysis was for HTILT$_{intr}$ and VTILT$_{intr}$ and HTILT$_{inter}$ and VTILT$_{inter}$
4. X, Y, Z: variability in the distance between the centers of gravity of the ONH and contour line along each of the topographical axes (as x, y, and z). Centre of gravity is the weighted geometrical center of pixel heights (Zinser G, personal communication, 2000). Geometrical relationships reflected by distances between centers of gravity were studied. Images were tilt-corrected and pixel heights measured by the reference ring. Analysis was for X$_{intr}$, Y$_{intr}$ and Z$_{intr}$ and X$_{inter}$, Y$_{inter}$ and Z$_{inter}$

Statistics

How well RIMVAR was explained by six independent variables, REF, HTILT, VTILT, X, Y, and Z, was investigated by multiple regression analysis. Variables were explanatory if statistically significant ($P < 0.05$). Noncontributory variables ($P > 0.05$) were excluded by backward elimination. Multicolinearity indicated that independent variables were interrelated. Adjusted coefficients of determination (adjusted $R^2$) reflected how well each model explained RIMVAR.

In the first part of the study normal and glaucomatous eyes were analyzed to investigate differences in intravisit–intraoperator and intervisits–interoperator variabilities, and then all eyes were grouped (taken to represent a continuum of morphology) and studied to explain variability. Positive or negative signs indicated the direction of change on repeat testing. For positive values in RIMVAR$_{intr}$, the initial test value exceeded the retest value; REF$_{intr}$ distance decreased in the retest image; HTILT$_{intr}$ and VTILT$_{intr}$ there was net downward angular displacement in the image’s top left-hand corner; X$_{intr}$, Y$_{intr}$, and Z$_{intr}$ there was net nasal shift, inferior displacement, and depression, respectively.

In the second part of the study, the relationship between RIMVAR$_{inter}$ and independent variables was studied in each image series to try to explain variability in sequential imaging. The range of differences for explanatory variables (for example REF$_{intr}$) was calculated to give an idea of the size of variation.

![Figure 2. Surface plot of an ONH illustrating the concepts of contour line, reference plane, reference ring, HRef, MHC, and REF. REFht is the distance between mean contour line height and HRef in the article text, variability in REFht is referred to as REF. All distances are measured on the topographical z-axis.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932918/ on 11/18/2018)

**Figure 3.** Polar plots of the 95% confidence intervals of test–retest differences for sector rim area in normal (A) and glaucomatous (B) eyes for the standard (A) and 320-μm reference planes (B). Each plot: n = 21. Solid line: intravisit–intraoperator variability; (●) intervisits–interoperator variability; 0°, temporal; 90°, superior; 180°, nasal; 270°, inferior.

To characterize regional variability, agreement intervals$^{19}$ (95% confidence intervals of differences and SD) for 30° sectors were graphed in polar plots. The Wilcoxon signed-rank test was used to test for significance in nonparametric data. Statistical analyses were conducted on computer (SPSS ver. 9 for Windows; SPSS Inc; Chicago, IL).

**RESULTS**

**Part 1: Test–Retest Variability**

Figures 3A1 and 3B1 show that rim area variability was not uniform around the ONH in normal eyes but tended to peak temporally (0–90°, 270–360°) with either reference plane. Agreement intervals widened with intervisits–interoperator than intravisit–intraoperator testing, especially temporally and with the standard reference plane. Increased differences were significant ($P < 0.05$) in three sectors in standard reference plane data and two sectors in 320-μm reference plane data for normals.

Figures 3A2 and 3B2 show generally wider agreement intervals in glaucoma than in normal eyes. Very high nasal variability in glaucoma was seen with the standard but not the 320-μm reference plane. This pattern was reproducible, being seen in intravisit–intraoperator and intervisits–interoperator testing. Taken globally, normal and glaucomatous eyes had similarly increased variability after intervisits–interoperator testing (approximately 50%). Variability was not correlated with rim area ($P > 0.05$).

Table 2 shows adjusted $R^2$ for single- and mean-topography images. In single-topography images, 40% and 43% of RIMVAR was explained in standard and 320-μm reference plane data respectively. In mean-topography images, even more RIMVAR was explained: 60% and 58% in standard and 320-μm reference plane data respectively.

REF and Z were the most frequently significant determinants of RIMVAR ($P < 0.05$, Table 2) for either reference plane in both mean- and single-topography images. Standardized regression coefficients for REF and Z were also generally larger than other variables indicating that they contributed more to RIMVAR. Models describing RIMVAR differed between reference planes, suggesting that each reference plane had a different influence on rim area variability. Multicolinearity was only
found between X_{sd} and Y_{sd} in single-topography images analyzed by the standard reference plane ($r^2 = 0.46, P = 0.000$), but neither variable contributed to the model. REF was significantly more in standard than 320-μm reference plane data in mean-topography images ($P = 0.027$; median REF_{diff}; standard reference plane = 16 μm; 320-μm reference plane = 12 μm) and single-topography images ($P = 0.000$; median REF_{all}; standard reference plane = 50 μm, 320-μm reference plane = 20 μm). Z_{diff} and Z_{sd} did not differ between reference planes ($P > 0.05$).

### Part 2: Variability in Longitudinal Data

Table 3 shows adjusted $R^2$ in a range of 0.62 to 0.95 for the standard reference plane (median, 0.84) and a range of 0.61 to 0.97 (median, 0.86) for the 320-μm reference plane. A substantial proportion of RIMVAR_{all} was thus explained: median of 84% in standard reference plane data and 86% in 320-μm reference plane data.

As in test-retesting, RIMVAR_{all} within image series was most frequently explained by REF_{all} and Z_{all} ($P < 0.05$). With the 320-μm reference plane, REF_{all} was significant in 19 (95%) of 20 eyes, and Z_{all} was significant in 16 (80%) of 20 eyes. With the standard reference plane, REF_{all} was significant in 20 (100%) of 20 eyes and Z_{all} in 19 (95%) of 20 eyes. [H_{heft} − MHC] height varied by as much as 726 μm in analysis by the standard reference plane (median = 150 μm; Q1, Q3 = 110, 332 μm), significantly more than analysis by the 320-μm reference plane ($P = 0.000$, Wilcoxon test); in the latter, the [H_{heft} − MHC] height varied by only as much as 166 μm (median = 116 μm, Q1, Q3 = 74, 140 μm). Within series, Z_{diff} varied by as much as 285 μm (median = 78 μm, Q1, Q2 = 59, 125 μm; not reference plane dependent). Other variables that were repeatedly significant were X_{sd} (8/20 [40%] of eyes with either reference plane) and Y_{sd} (7/20 [35%] of eyes with the standard reference plane). Explanatory models for RIMVAR differed between reference planes, as seen in Table 2. No multicollinearity was found.

### Discussion

Between 40% and 60% of rim area variability in intravitreal test-retest images could be explained. In image series, even more variability—a median of approximately 85%—was explained. REF and Z almost always contributed to the models and can be considered important causes of rim area variability. The nature of variability differed between reference planes. These factors may underlie our observations that variability was not uniform around the ONH, results in testing involving different operators and visits tended to be more variable, and reference planes differed in their patterns of variability.

Variability in image acquisition and analysis has been studied before. The few studies that have evaluated longitudinal data have not sought out reasons for variability; knowing such reasons could help in optimizing reproducibility. We assessed the relevance of topographical variability to rim area variability and studied the influence of different reference planes. A large enough sample was studied to ensure a meaningful exploration of six independent variables over a range of morphologies. Variability in image series was analyzed because it is pertinent to evaluating progression.

Adjusted $R^2$ was higher for mean than single-topography images, indicating that variability in the former was better explained. It is possible that some random fluctuation is eliminated in mean-topography images. However, variability was calculated differently in mean-topography images (differences) and single-topography images (standard deviation) and might have influenced analysis. Variability was even better explained in longitudinal data, where it was almost totally explained in some eyes (80–90% variability explained in 25–35% of eyes; Table 3), probably because analysis in image series was specific to each ONH. By contrast, a broad cross section of ONH morphologies had to be accounted for in analyzing test-retest data. Nevertheless, both cross-sectional and longitudinal models indicate that the independent variables explained a large proportion of rim area variability.

REF and Z almost always explained rim area variability regardless of the reference plane used. This is not surprising, given that rim area is measured where the reference plane intersects the ONH on the z-axis, so that any z-axis shifts between the ONH and reference plane—due to variation in ONH and peripapillary retinal topography, reference plane position, or both—could affect rim area measurement. Reference planes differed in their amount of REF, explanatory models, and patterns of regional variability. REF was significantly higher with the standard than 320-μm reference plane ($P < 0.05$), indicating that the former shifted more in relation to the ONH to cause more variability, as seen in Figure 3. That reference planes differ in variability has been suggested elsewhere.

Shifting in the center of gravity of the ONH in relation to that of the contour line reflects geometrical variation along separate vectors, as depicted in Figure 4. Center-of-gravity shifts on the z-axis (Z) significantly explained rim area vari-

### Table 2. Summary of Multiple Regression Models for Testing in Study Part 1

<table>
<thead>
<tr>
<th>Image Type and Reference Plane</th>
<th>$R^2$</th>
<th>Independent Variables of Statistical Significance†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean topo. 320-μm ref. plane</td>
<td>0.58</td>
<td>$Z_{diff}$: -0.43, $P = 0.000$; REF_{diff}: -0.55, $P = 0.000$; HTILT_{diff}: 0.24, $P = 0.005$; X_{diff}: -0.21, $P = 0.012$; Y_{diff}: -0.21, $P = 0.012$; HTILT_{sd}: 0.22, $P = 0.016$</td>
</tr>
<tr>
<td>Single topo. 320-μm ref. plane</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Mean topo. standard ref. plane</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Single topo. standard ref. plane</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>

Topo, topography; Ref. plane, reference plane; Stand. Coeff., standardized coefficient.

* Adjusted $R^2$.
† $P < 0.05$. 

Downloaded From: https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932918/ on 11/18/2018
cause surface geometry to vary asymmetrically and unpre-

sumably in 80% (320-μm reference plane) and 95% (standard reference plane) of image series. Z was not inconsiderable, having a median of 78 μm and range of 285 μm. There may be several reasons for this. First, centering of the ONH in images may vary. HRT software centers the reference ring on the image frame, not on the ONH, and so the location of the ONH in relation to the ring may vary between images, to influence the referencing of pixels. Figures 1A1 and 1A2 show a slight transverse shift in the ONH in relation to the reference ring. Second, the surface of the ONH may be oriented differently between images. Rim area variability in some eyes could be explained by image tilt, indicating that tilt compensation by software was mostly, but not always, adequate. Whether compensation is adequate probably de-

Table 3. Summary of Multiple Regression Models for Individual Image Series in Study Part 2

<table>
<thead>
<tr>
<th>Subject</th>
<th>$R^2$</th>
<th>Significant Variable</th>
<th>Subject</th>
<th>$R^2$</th>
<th>Significant Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.61</td>
<td>REF (−0.54, 0.000), X (−0.57, 0.001), Z (−0.38, 0.015)</td>
<td>1</td>
<td>0.95</td>
<td>REF (0.82, 0.000), X (−0.40, 0.000)</td>
</tr>
<tr>
<td>2</td>
<td>0.94</td>
<td>REF (−0.49, 0.000), Y (−0.17, 0.003), Z (−0.89, 0.000)</td>
<td>2</td>
<td>0.84</td>
<td>Z (−0.60, 0.000)</td>
</tr>
<tr>
<td>3</td>
<td>0.97</td>
<td>REF (−0.23, 0.000), Z (−0.85, 0.000), VTILT (0.11, 0.000)</td>
<td>3</td>
<td>0.83</td>
<td>REF (−0.39, 0.000), Z (−0.88, 0.000)</td>
</tr>
<tr>
<td>4</td>
<td>0.82</td>
<td>REF (−0.06, 0.000), X (−0.39, 0.000)</td>
<td>4</td>
<td>0.91</td>
<td>X (−0.30, 0.009), Z (−0.34, 0.002), REF (−0.86, 0.000)</td>
</tr>
<tr>
<td>5</td>
<td>0.71</td>
<td>REF (−0.55, 0.000), Z (−0.67, 0.000)</td>
<td>5</td>
<td>0.70</td>
<td>REF (1.11, 0.000), VTILT (0.28, 0.019), Z (−1.20, 0.000)</td>
</tr>
<tr>
<td>6</td>
<td>0.92</td>
<td>REF (−0.56, 0.000), Z (−0.41, 0.000), Y (0.18, 0.047)</td>
<td>6</td>
<td>0.62</td>
<td>Z (−0.94, 0.000), REF (−0.36, 0.022)</td>
</tr>
<tr>
<td>7</td>
<td>0.70</td>
<td>REF (−0.33, 0.018), Z (−0.80, 0.000), X (0.36, 0.018)</td>
<td>7</td>
<td>0.89</td>
<td>X (0.61, 0.000), Z (−0.81, 0.000), REF (−0.76, 0.000)</td>
</tr>
<tr>
<td>8</td>
<td>0.86</td>
<td>REF (−0.68, 0.000), Z (−0.40, 0.000)</td>
<td>8</td>
<td>0.90</td>
<td>Z (−0.50, 0.000), REF (−0.92, 0.000)</td>
</tr>
<tr>
<td>9</td>
<td>0.90</td>
<td>REF (−0.48, 0.000), X (0.17, 0.029), Y (0.18, 0.037), Z (0.96, 0.000)</td>
<td>9</td>
<td>0.90</td>
<td>X (0.15, 0.025), Z (−0.69, 0.000), REF (−0.95, 0.000)</td>
</tr>
<tr>
<td>10</td>
<td>0.68</td>
<td>REF (−0.50, 0.000), X (0.43, 0.001), Y (−0.40, 0.002), Z (−0.34, 0.007)</td>
<td>10</td>
<td>0.82</td>
<td>X (0.26, 0.019), Z (−0.18, 0.040), REF (−0.69, 0.000)</td>
</tr>
<tr>
<td>11</td>
<td>0.94</td>
<td>REF (−1.01, 0.000), Z (−0.41, 0.000)</td>
<td>11</td>
<td>0.86</td>
<td>X (−0.48, 0.000), Z (−0.27, 0.000), REF (−0.96, 0.000)</td>
</tr>
<tr>
<td>12</td>
<td>0.69</td>
<td>REF (−0.58, 0.000), X (−0.48, 0.000), Z (−0.31, 0.031), HTILT (−0.28, 0.019)</td>
<td>12</td>
<td>0.62</td>
<td>HTILT (−0.24, 0.045), REF (−0.79, 0.000)</td>
</tr>
<tr>
<td>13</td>
<td>0.95</td>
<td>REF (−0.72, 0.000), X (0.18, 0.037), Z (−0.23, 0.007), HTILT (−0.16, 0.099)</td>
<td>13</td>
<td>0.66</td>
<td>Z (−0.65, 0.001), HTILT (−0.36, 0.022), REF (−0.55, 0.003)</td>
</tr>
<tr>
<td>14</td>
<td>0.80</td>
<td>REF (−0.442, 0.000), X (−0.21, 0.032), Z (−0.77, 0.000)</td>
<td>14</td>
<td>0.77</td>
<td>Z (−0.68, 0.000), REF (−0.58, 0.000)</td>
</tr>
<tr>
<td>15</td>
<td>0.49</td>
<td>Z (−0.67, 0.000)</td>
<td>15</td>
<td>0.76</td>
<td>Z (−0.72, 0.000), REF (−0.63, 0.000)</td>
</tr>
<tr>
<td>16</td>
<td>0.76</td>
<td>REF (−0.75, 0.000), Y (0.27, 0.015), VTILT (−0.26, 0.026)</td>
<td>16</td>
<td>0.88</td>
<td>Y (0.15, 0.050), Z (−0.16, 0.019), HTILT (−0.019, 0.010), VTILT (−0.18, 0.019), REF (−0.76, 0.000)</td>
</tr>
<tr>
<td>17</td>
<td>0.70</td>
<td>REF (−0.35, 0.045), Y (0.73, 0.000)</td>
<td>17</td>
<td>0.77</td>
<td>X (−0.52, 0.007), Y (0.48, 0.003), Z (−0.28, 0.036), REF (−0.62, 0.000)</td>
</tr>
<tr>
<td>18</td>
<td>0.94</td>
<td>REF (−0.52, 0.000), Z (−0.51, 0.000), VTILT (0.15, 0.04)</td>
<td>18</td>
<td>0.89</td>
<td>X (0.33, 0.001), Z (−0.55, 0.000), REF (−0.42, 0.000)</td>
</tr>
<tr>
<td>19</td>
<td>0.82</td>
<td>REF (−0.87, 0.000), Z (−0.72, 0.000)</td>
<td>19</td>
<td>0.85</td>
<td>Z (−0.77, 0.000), REF (−0.83, 0.000)</td>
</tr>
<tr>
<td>20</td>
<td>0.89</td>
<td>REF (−0.47, 0.000), Y (−0.43, 0.000), Z (−0.81, 0.000)</td>
<td>20</td>
<td>0.92</td>
<td>Y (−0.29, 0.000), Z (−0.52, 0.000), REF (−0.75, 0.000)</td>
</tr>
</tbody>
</table>

Data in parentheses are standardized coefficient and probability.
* Adjusted $R^2$.
FIGURE 4. Surface plots of six topographies from the same emetropic eye imaged on the same day, all shown sharing a common aspect. Central concavity is the cup (left: temporal; right: nasal) and the flatter periphery is the peripapillary retina. Y-axis: depth values in relation to the mean reference ring height (z0 = 0 μm; z1 = −500 μm); x-axis: transverse distance (all the same scale). There is variation in the shape of the cup, the depth of the cup (shown in relation to the z1 mark), and the contour of peripapillary retina where the reference ring is located.

fluctuations significantly affect rim area variability but keeping the positional relationships stable could help improve reproducibility.

Acknowledgments
The authors thank Ed White and Ian Thrasher for technical support.

References