Longitudinal Measurement Variability of Corneal Birefringence and Retinal Nerve Fiber Layer Thickness in Scanning Laser Polarimetry With Variable Corneal Compensation

Thế Anh Mai, MD; Hans G. Lemij, MD, PhD

Objective: To investigate the longitudinal corneal birefringence (corneal polarization axis [CPA] and corneal polarization magnitude [CPM]) variability in scanning laser polarimetry with variable corneal compensation and its effect on retinal nerve fiber layer measurements.

Method: We analyzed scanning laser polarimetry images obtained every 6 months for 3.2 years in 16 healthy eyes, 38 eyes with ocular hypertension, and 53 eyes with glaucoma in 107 white participants. Differences in values between each intraeye CPA and CPM measurement and the first measurement were used to investigate the variability and any trend with time, and any association with age or diagnosis. We also calculated the percentage of these values within the range of ±5° or ±5 nm, respectively. Any effect of corneal birefringence variability on the retinal nerve fiber layer measurements was also evaluated.

Results: The CPA and CPM measurement variability showed no trend with time and did not differ between diagnostic groups. It did not appear to be affected by age. With more than 90% of the CPA and CPM measurement variability within the range of ±5° or ±5 nm, no significant effect on the retinal nerve fiber layer measurements was observed.

Conclusions: The CPA and CPM measurement variability did not differ between groups, showed no trend over time, was independent of subject age, and did not seem to systematically affect retinal nerve fiber layer reproducibility.

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CANNING LASER POLARIMETRY (SLP) is one of several imaging modalities that enable documentation of peripapillary retinal nerve fiber layer (RNFL) thinning caused by, for example, primary open-angle glaucoma, an age-related degenerative neuropathy resulting from accelerated loss of retinal ganglion cells and their axons. This technique measures the retardation of the polarized light double passing the presumed form birefringent RNFL, a property that correlates with RNFL thickness. In SLP, the total signal is derived from the combined birefringence of the anterior segment and the RNFL. Proper compensation and removal of the anterior segment birefringence, largely that of the cornea (for brevity, called corneal compensation), is, therefore, necessary to obtain an accurate assessment of the RNFL. In addition, the widely variable range of corneal birefringence values (either corneal polarization axis [CPA] or corneal polarizati-
justed CPA and CPM variability (whether biological or the result of measurement errors) over time may affect the RNFL measurements. Any such variability needs to be explored. To our knowledge, to date there is only 1 study on the measurement variability of anterior segment birefringence during 1 year. 50

The purpose of our study was to investigate, using the GDx VCC, the intraeye measurement variability of corneal birefringence (both CPA and CPM) during 3 years in healthy eyes, eyes with ocular hypertension (OHT), and eyes with glaucoma, and any general effect of age. In addition, we also explored the effects of CPA and CPM measurement variability on RNFL measurements in healthy eyes.

METHODS

SUBJECTS

One hundred seven white participants, consisting of 16 subjects with healthy eyes, 38 with OHT, and 53 with glaucoma, were entered in the study. During a mean (SD) of 3.2 (0.4) years, approximately every 6 months all subjects underwent an ophthalmologic examination including slit-lamp biomicroscopy, intraocular pressure measurement using Goldmann applanation tonometry, and a white-on-white standard automated perimetry visual field (VF) test (Humphrey Field Analyzer II for Glaucoma; Carl Zeiss Meditec, Inc.). The VF testing strategy that we used was either 24-2 full-threshold (82 subjects) or 24-2 standard Swedish interactive testing algorithm (SITA-standard) (25 subjects). Only 1 eye per subject was randomly selected if both eyes were eligible.

Baseline criteria for inclusion in the study were (1) no history of ocular disease except glaucoma in the glaucoma group, laser-assisted in situ keratomileusis, intraocular surgery except uncomplicated cataract surgery, or any significant coexisting systemic diseases with possible ocular involvement such as diabetes mellitus or arterial hypertension; (2) best corrected visual acuity of 20/40 or better; (3) any refractive error within a range between −7.0 diopters (D) and + 3.0 D (spherical equivalent); and (4) VFs that met preset reliability criteria, that is, for healthy individuals and subjects with OHT fixation loss of not more than 25%, false-negative and false-positive responses did not exceed 20% for the full-threshold test strategy and 7% for SITA test strategy. For the subjects with glaucoma, the same criteria for inclusion of the VFs were applied except that up to 33% of false-negative responses for the full-threshold and 12% or less for the SITA paradigms were considered acceptable.

All protocols and methods used in the present study adhered to the tenets of the Declaration of Helsinki and were approved by the institutional human experimentation committee. Informed consent was obtained after the participants were informed about possible adverse effects of participation in the study.

Healthy subjects were consecutively recruited from an ongoing follow-up study at The Rotterdam Eye Hospital. All subjects had unremarkable findings at slitlamp examination, open angles at gonioscopy, intraocular pressure of 21 mm Hg or less OU, normal findings at VF testing using standard automated perimetry, a healthy-looking optic disc (no diffuse or local rim thinning or cupping and no optic disc hemorrhages), and no ocular abnormalities. A normal VF test result was defined as a mean deviation and a pattern standard deviation within 95% of the normal range and results of a glaucoma hemifield test within normal limits. None of our healthy subjects reported having first- or second-degree family members with glaucoma. In all subjects with OHT, eye pressure was 22 mm Hg or greater and 32 mm Hg or less OU. All had a normal VF test result using standard automated perimetry. The appearance of the optic disc was not a selection criterion.

The patients with glaucoma had, in the selected eye, an optic disc that demonstrated glaucomatous features (diffuse or local rim thinning or cupping, possibly with optic disc hemorrhages), a glaucomatous standard automated perimetry VF defect confirmed at 2 consecutive examinations, open angles at gonioscopy, and no evidence of secondary glaucoma. A VF defect in the present study was considered glaucomatous if it had 2 or more adjacent points at a P < .01 level or deeper, or 3 or more adjacent points at a P < .05 level or deeper in the total deviation plot, or glaucoma hemifield test results outside normal limits not attributable to causes other than glaucoma. The eyes with glaucoma were classified by the severity of VF defects described by Hodapp et al. 20 Of the eyes with glaucoma, 29 (54.7%) were considered to have mild to moderate VF defects and 24 (45.3%) to have severe VF defects.

MEASUREMENTS

All eyes were imaged using a commercially available SLP (GDx VCC; software version 5.4.0; Carl Zeiss Meditec, Inc). Details of its working principle are described elsewhere. 21 In brief, the GDx VCC 2 linear retarders in rotating mounts to adjust both the retardance and axis of the unit as required. The variable corneal compensation enables eye-specific compensation of the anterior segment birefringence based on the macular retardance profile. 19

In the present study, imaging with the GDx VCC in both eyes of all subjects was performed by 3 trained and experienced technicians through undilated pupils in ambient light. The subjects were asked to keep their head still during the entire session, with their faces resting on the face mask to enable the best alignment between the instrument’s anterior segment compensator and the position of the eyes. At each visit, corneal birefringence was determined. The RNFL retardation (in nanometers) was calculated by taking the retarder-adjusted eye-specific CPA and CPM into account and was converted into thickness values (in micrometers) based on a fixed conversion factor of 0.67 nm. 2 Only typical images of high quality were selected: those that were well focused, without any motion artifacts, with a centered optic disc evenly and adequately illuminated throughout the image, with an intact quality scan score of 7 or more, and with a support vector machine-derived typical scan score of 80 or higher 22 (range, 0-100). We did not select images with a typical scan score lower than 80 because they typically contain retardation patterns that do not correspond with the expected RNFL morphology. They have been shown to adversely affect the diagnostic accuracy of SLP. 12,24

STATISTICAL ANALYSIS

The intraeye CPA and CPM measurement variability in each group and also in all eyes together was investigated by means of the variation between each measurement compared with the earliest measurement. This approach enabled us to standardize the CPA and CPM measurement variability in all eyes while preserving any potential trend of change in the CPA or CPM values over time. The resulting intraeye CPA and CPM measurement variability values were used to determine the mean variability range and to investigate any trend in CPA and CPM over time by means of linear and quadratic regression analysis. Multivariate analysis of variance for repeated mea-
measurements was performed to explore any effect of age and diagnosis (ie, healthy eyes or eyes with OHT or glaucoma) on the CPA or CPM variability. It was also used to test any systematic effect of the CPA or CPM measurement variability on the RNFL measurements in healthy eyes. We did not perform such analyses in the eyes with OHT or glaucoma because it was a priori impossible to determine whether any observed change in RNFL measurements over time might have resulted from CPA and CPM measurement variability or from glaucomatous change.

The intraeye corneal birefringence in all eyes and the RNFL measurement variability with time in healthy eyes were expressed both in within-subject standard deviation (Sw) and in a 2-way mixed intraclass correlation coefficient (ICC). The Sw was defined as the square root of the within-subject variance, calculated by dividing the within-subject sum of squares by its degrees of freedom and taking the square root of the result.25

The 95% confidence intervals (CIs) of Sw were approximated with the formula 95% CI = 1.96 × SE, with SE being the standard error of Sw. We also calculated the repeatability coefficient, defined as the range in which 95% of the within-subject measurement variability falls; it was calculated as 1.96 × Sw.26

The ICC may be viewed as a measure of agreement between the within-subject measurements relative to the between-subjects and total variability. The ICC was calculated using the following formula: ICC = (m × SSB − SST)/(m × SSB + SST), where SSB and SST are the between-subjects and total sum of squares, respectively, and m is the number of measurements per subject.27

The 6 RNFL parameters used in this study were (1) temporal-superior-nasal-inferior-temporal average (TSNIT Avg), (2) inferior average (InfAvg), (3) superior average (SupAvg), (4) TSNIT standard deviation (TSNITSD), and (5) nerve fiber indicator, and (6) intereye symmetry.

In the present study, a P value of .05 was considered statistically significant. The statistical analyses were performed using commercially available software (SPSS version 14.0.2 for Windows; SPSS, Inc, Chicago, Illinois, and Microsoft Excel 2000, version SR-1; Microsoft Corp, Redmond, Washington).

### RESULTS

**DEMOGRAPHIC CHARACTERISTICS OF STUDIED EYES AT BASELINE**

Clinical characteristics at baseline in our patients are given in Table 1. There were some statistically significant differences in intraocular pressure, mean deviation, pattern standard deviation, and central corneal thickness values between the 3 subject groups.

### EFFECT OF DIAGNOSIS AND AGE ON CHANGE IN CORNEAL COMPENSATION

We found no statistically significant association between the diagnosis and the intraeye CPA or CPM measurement variability over time (P = .70 and .04, respectively). We, therefore, pooled the data for all eyes to investigate any association between age and intraeye CPA and CPM measurement variability over time. This association was found to be not statistically significant (P = .22 and .51 for CPA and CPM, respectively) (Figure). Linear and quadratic regression analysis for each individual eye did not reveal any trend of CPA or CPM change over time.

### LONGITUDINAL STABILITY OF CORNEAL BIEREFRINGENCE

The CPA and CPM measurement variability, expressed as the repeatability coefficient, was 5.7 (range, −7.4 to 12.5) and 4.7 (range, −11.0 to 7.0), respectively. The ICC for the CPA and CPM measurement variability was 0.97 (ranges, 0.96-0.98 and 0.96-0.97, respectively) (P < .001) each. In 95.3% (102/107) of the studied eyes, the mean CPA measurement variability was within the range of ±5°, and 93.5% (100 of 107 eyes) had mean CPM variability within the range of ±5 nm (Figure).

### LONGITUDINAL STABILITY OF RNFL PARAMETERS

The mean (SD) RNFL thickness parameter values in healthy eyes varied from 1.7 (0.2) μm (TSNITSD) to 2.6 (0.4) μm (InfAvg). Across all parameters, the ICC values were similar for SupAvg and TSNIT average but were slightly lower for TSNITSD and intereye symmetry. In general, we found no statistically significant correlation between CPA and CPM measurement variability with time and the RNFL parameters (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Eyes (n=16)</th>
<th>Eyes With OHT (n=38)</th>
<th>Eyes With Glaucoma (n=53)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, No. (%)</td>
<td>9 (56.3)</td>
<td>16 (42.1)</td>
<td>31 (58.5)</td>
<td>.29</td>
</tr>
<tr>
<td>Right eyes, No. (%)</td>
<td>8 (50.0)</td>
<td>17 (44.7)</td>
<td>24 (45.3)</td>
<td>.93</td>
</tr>
<tr>
<td>Age, mean±SD, y</td>
<td>64.2±6.9</td>
<td>61.3±10.1</td>
<td>64.5±11.8</td>
<td>.32</td>
</tr>
<tr>
<td>Follow-up, mean±SD, y</td>
<td>3.3±0.2</td>
<td>3.1±0.5</td>
<td>3.3±0.3</td>
<td>.08</td>
</tr>
<tr>
<td>Spherical equivalent refraction, mean±SD, D</td>
<td>3.0±1.3</td>
<td>1.9±2.9</td>
<td>1.7±2.8</td>
<td>.36</td>
</tr>
<tr>
<td>IOP, mean±SD, mm Hg</td>
<td>16.1±2.3</td>
<td>22.9±2.5</td>
<td>14.8±4.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CCT, mean±SD, μm</td>
<td>558.4±34.5</td>
<td>574.1±34.3</td>
<td>540.3±34.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mean deviation, mean±SD, dB</td>
<td>0.4±1.3</td>
<td>0.2±1.4</td>
<td>−10.4±8.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PSD, mean±SD, dB</td>
<td>1.8±0.5</td>
<td>1.7±0.4</td>
<td>8.2±4.1</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CCT, central corneal thickness; D, diopter; IOP, intraocular pressure; OHT, ocular hypertension; PSD, pattern standard deviation.

Table 1. Baseline Demographic and Clinical Characteristics in the Study Patients

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measurement reproducibility. In SLP, accurate RNFL measurements can be achieved only if the corneal birefringence is adequately compensated. If the corneal birefringence remained stable over time, its initial value may, in theory, be used for subsequent measurements. But one may be concerned, however, about any effects other factors may have on corneal birefringence. These include age,17 eye movements at each session, laser-assisted in situ keratomileusis–associated change,18 or change in alignment between the scanned eye and the backscattered light beam.

By determining the corneal birefringence at each visit and its compensation, we found no significant trends in the latter over time in any of our 3 studied groups (healthy eyes, eyes with OHT, and eyes with glaucoma), nor did we note any systematic trends with age. Our finding of a relatively stable CPA was similar to that of Greenfield and Knighton,19 who found, using a slitlamp-mounted device equipped with 2 crossed linear polarizers and an optical retarder, a mean (SD) intersession CPA measurement standard deviation of 4.1° (3.1°). Their study was limited, however, to only 1 year of follow-up, whereas ours was considerably longer. Furthermore, we found that well over 90% of the long-term measurement variability of both CPA and CPM was within ±5° and ±5 nm, respectively. Using a GDx VCC prototype, Reus et al16 demonstrated that, in healthy eyes and eyes with glaucoma, even a slightly inadequate CPA compensation of 5° may result in an apparent change in RNFL retardation patterns. Although most eyes in the present study demonstrated a smaller measurement variability than this, it is unclear what the exact erroneous effects these inappropriate corneal compensations may have on the overall measurements. Because the alignment between the GDx device and the scanned eye is likely to vary between imaging sessions, we think that one should therefore determine the corneal birefringence at each follow-up measurement session in individual eyes to enable optimal corneal compensation. In the present study, we did not systematically examine the effects of applying the initial (baseline) corneal compensation during follow-up visits. Such an approach, however, would have demonstrated the extent of constant but potentially inadequate corneal compensation on the RNFL measurements.

![Scatterplots show the intraeye measurement variability for corneal polarization axis (CPA) and corneal polarization magnitude (CPM), compared with the first measurement, in all eyes during 3.2 years. In more than 90% of the eyes, the mean CPA and CPM measurement variability was between ±5° and ±5 nm, respectively (102 of 107 studied eyes [95.3%] for CPA and 100 of 107 studied eyes [93.5%] for CPM). Some studied eyes showed significant measurement variability, most probably owing to change in alignment between the scanned eye and the backscattered light beam or to movement of the eye during image acquisition.](image)

### Figure

Table 2. Retinal Nerve Fiber Layer (RNFL) Measurement Variability Over Time

<table>
<thead>
<tr>
<th>RNFL</th>
<th>Sw±1.96 × SE (RC)</th>
<th>ICC (95% CI)</th>
<th>P Value for Association With Mean Change in CPA</th>
<th>P Value for Association With Mean Change in CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSNIT, µm</td>
<td>1.5±0.2 (2.9)</td>
<td>0.94b (0.89-0.97)</td>
<td>.25</td>
<td>.57</td>
</tr>
<tr>
<td>Sup, µm</td>
<td>2.1±0.3 (4.1)</td>
<td>0.94b (0.89-0.98)</td>
<td>.75</td>
<td>.38</td>
</tr>
<tr>
<td>Inf, µm</td>
<td>2.6±0.4 (5.1)</td>
<td>0.88b (0.79-0.95)</td>
<td>.68</td>
<td>.31</td>
</tr>
<tr>
<td>TSNIT, µm</td>
<td>1.7±0.2 (3.4)</td>
<td>0.78b (0.63-0.90)</td>
<td>.03</td>
<td>.48</td>
</tr>
<tr>
<td>NFI</td>
<td>2.6±0.4 (5.2)</td>
<td>0.90b (0.82-0.96)</td>
<td>.41</td>
<td>.15</td>
</tr>
<tr>
<td>Intereye symmetry</td>
<td>0.05±0.01 (0.11)</td>
<td>0.77b (0.62-0.89)</td>
<td>.18</td>
<td>.19</td>
</tr>
</tbody>
</table>

Abbreviations: Avg, average; CI, confidence interval; CPA, corneal polarization axis; CPM, corneal polarization magnitude; ICC, intraclass correlation coefficient; Inf, inferior; NFI, nerve fiber indicator; RC, repeatability coefficient; SD, standard deviation; Sup, superior; Sw, within-subject standard deviation; TSNIT, temporal-superior-nasal-inferior-temporal; 1.96 × SE, 95% confidence intervals of the Sw where SE is standard error of the Sw.

- Statistical significance level, tested by multivariate analysis of variance for repeated measurements.
- Statistically significant at P<.001.
results were of the same order of magnitude as reported by Medeiros et al. These investigators measured 31 healthy eyes with the GDx VCC every 12 months over a period mean (SD) of 26.0 (8.9) months and found a repeatability coefficient that varied from 3.21 to 4.97 µm for RNFL thickness parameters and from 3.9 to 6.2 µm for the nerve fiber indicator.

Traditionally, the ICC has been used to express either short-term or long-term stability of the measurements (test-retest measurement repeatability and measurement reproducibility, respectively). The ICC expresses the weight of the between-subjects variability contributing to total variability and, therefore, indirectly reflects the within-subject variability. It is also independent of the used unit of measure and, therefore, is useful for comparing various parameters with different units of measure. Despite this advantage, it does not intuitively reflect the magnitude of the within-subject variability of the measurements. The ICC may therefore be, in our opinion, potentially misleading to clinicians.

In this study, its values for SupAvg and TSNITavg were both 0.91, which may give the impression that both measurement errors were the same. However, when expressed with the Sw, the measurement variability of SupAvg was greater than the TSNITavg (Table 3). To adequately express both the magnitude of measurement variability and the variability across parameters, we believe that both the ICC and the Sw, as well as their 95% CIs, should be provided.

In conclusion, the long-term intraeye corneal birefringence measurement variability measured with the GDx VCC did not differ between healthy eyes, eyes with OHT, and eyes with glaucoma. It showed no systematic trend with time and was independent of age. When CPA and CPM were adequately compensated at each visit, their measurement variability showed no systematic effect on the reproducibility of RNFL measurements. We believe that corneal birefringence should be determined and appropriately compensated at each visit. In addition, the position of the patients’ faces on the GDx VCC face mask should be maintained between determining the corneal birefringence and obtaining RNFL measurements to minimize any malalignment of the corneal compensation.

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<table>
<thead>
<tr>
<th>RNFL Parameter</th>
<th>Sw±1.96 × SE, Mean (SD)</th>
<th>RC</th>
<th>ICC (95% CI) a</th>
<th>P Value for Association With Mean Change in CPA</th>
<th>P Value for Association With Mean Change in CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSNITavg, µm</td>
<td>1.5 (0.2)</td>
<td>2.9</td>
<td>0.94 (0.89-0.97)</td>
<td>.25</td>
<td>.57</td>
</tr>
<tr>
<td>Superioravg, µm</td>
<td>2.1 (0.3)</td>
<td>4.1</td>
<td>0.94 (0.89-0.98)</td>
<td>.75</td>
<td>.38</td>
</tr>
<tr>
<td>Inferioravg, µm</td>
<td>2.6 (0.4)</td>
<td>5.1</td>
<td>0.88 (0.79-0.95)</td>
<td>.68</td>
<td>.31</td>
</tr>
<tr>
<td>TSNITtop, µm</td>
<td>1.7 (0.2)</td>
<td>3.4</td>
<td>0.78 (0.63-0.90)</td>
<td>.03</td>
<td>.48</td>
</tr>
<tr>
<td>Nerve fiber indicator</td>
<td>2.6 (0.4)</td>
<td>5.2</td>
<td>0.90 (0.82-0.96)</td>
<td>.41</td>
<td>.15</td>
</tr>
<tr>
<td>Intereye symmetry</td>
<td>0.95 (0.01)</td>
<td>0.11</td>
<td>0.77 (0.62-0.89)</td>
<td>.18</td>
<td>.19</td>
</tr>
</tbody>
</table>

Abbreviations: Avg, average; CI, confidence interval; CPA, corneal polarization axis; CPM, corneal polarization magnitude; ICC, intraclass correlation coefficient; RC, repeatability coefficient; RNFL, retinal nerve fiber layer; Sw, within-subject standard deviation; TSNIT, temporal-superior-nasal-inferior-temporal; 1.96 × SE, 95% confidence intervals of the Sw where SE is standard error of the Sw.

aStatistically significant at P<.001, multivariate analysis of variance for repeated measurements.

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Role of the Sponsor: Carl Zeiss Meditec had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

REFERENCES


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**Correction**

Errors in Abstract and Text. In the Clinical Trials article titled “Canadian Glaucoma Study: 2. Risk Factors for the Progression of Open-angle Glaucoma,” by Chauhan et al, published in the August issue of the Archives (2008;126[8]:1030-1036), the Trial Registration identifier, which was listed in 2 places, was incorrect. On page 1030, the Trial Registration portion of the “Abstract” should have appeared as follows: “clinicaltrials.gov Identifier: NCT00262626.” On page 1031, the third sentence of the “Methods” section should have appeared as follows: “The CGS is a multicenter Canadian study involving 3 hospital-based university departments and is registered with the ClinicalTrials.gov Protocol Registration System (identifier NCT00262626).” Online versions of this article on the Archives of Ophthalmology Web site were corrected on August 11, 2008.
in patients with significant bleb dysesthesia that is not relieved with time and simple lubrication.

Although conjunctivoplasty alone is effective at reducing the symptoms of dysesthetic blebs, we feel that, particularly in eyes with thick interpalpebral blebs, simply limiting subconjunctival dissection of aqueous humor into this area may have a limited effect in some eyes, as subconjunctival scar tissue will still contribute to bleb height and discomfort. Our experience suggests that removing this tissue can have a striking effect on patient comfort, and our case series demonstrates that this maneuver can be routinely added to conjunctivoplasty without adversely affecting long-term IOP control.

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REFERENCES


Correction

Mislabeled Subsection. The article titled “Longitudinal Measurement Variability of Corneal Birefringence and Retinal Nerve Fiber Layer Thickness in Scanning Laser Polarimetry With Variable Corneal Compensation,” by Mai and Lemij, published in the October Archives (2008;126[10]:1359-1364), should not have been labeled “Surgeon’s Corner.”