Comparison of Scanning Laser Polarimetry Using Variable Corneal Compensation and Retinal Nerve Fiber Layer Photography for Detection of Glaucoma

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Objective: To compare retinal nerve fiber layer (RNFL) measurements obtained with scanning laser polarimetry (SLP) using variable corneal polarization compensation with standard red-free photography for detection of RNFL damage in glaucoma.

Methods: This observational, cross-sectional study included 1 eye of each of 42 patients with open-angle glaucoma, 32 patients suspected of having glaucoma, and 40 healthy subjects. The RNFL measurements using SLP with variable corneal compensation were obtained within 3 months of red-free photographs. Two independent observers graded RNFL photographs using a standardized protocol. Superior and inferior hemiretinas were scored separately, and a global score was obtained by averaging scores from each hemiretina.

Main Outcome Measures: The RNFL photography scores were compared with RNFL thickness measurements obtained with SLP. The receiver operating characteristic (ROC) curves were constructed to assess the abilities of the different methods to differentiate glaucoma patients from healthy subjects.

Results: The RNFL thickness decreased with increased RNFL damage as assessed by photographs in both hemiretinas ($R^2=15\%-47\%$). The area under the ROC curve for the best SLP parameter, Nerve Fiber Indicator, was significantly greater than the area under the ROC curve for the global RNFL photography score (0.91 vs 0.84, $P=.03$).

Conclusions: A moderate correlation was found between RNFL thickness measurements obtained with SLP and RNFL scores from red-free photographs. Compared with semiquantitative RNFL photography scores, the best SLP parameter had a higher diagnostic accuracy to separate glaucoma patients from healthy subjects.


Retinal nerve fiber layer (RNFL) abnormalities have been shown to precede the development of visual field defects in glaucoma patients. Although RNFL photographs have been established as a standard method for detection of RNFL defects, the qualitative and subjective nature of this assessment, as well as the requirement for maximal pupillary dilation and optimal media clarity, limits its widespread applicability.

Scanning laser polarimetry (SLP) is a diagnostic tool developed to quantitatively measure the thickness of the peripapillary RNFL. It is based on the principle that polarized light passing through the birefringent RNFL undergoes a measurable phase shift, known as retardation, which is linearly related to histologically measured RNFL tissue thickness. Although differences in retardation between healthy and glaucomatous eyes have been previously described, earlier studies evaluating RNFL photographs and SLP in glaucoma showed only modest associations between these 2 methods.

A significant source of error in previous studies evaluating the SLP technology was most likely introduced by the erroneous compensation of anterior segment birefringence. The RNFL is not the only birefringent structure in the eye. The cornea and Henle fiber layer of the macula, and to a lesser extent the lens, also are birefringent. To address anterior segment birefringence, the first commercial SLP had a fixed corneal compensator. The compensator was calibrated based on the assumption that all individuals had a slow axis of corneal birefringence $15^\circ$ nasally downward with a magnitude of retardance of 60 nm. However, there is a wide variation in both the axis and the magnitude of corneal birefringence in healthy and glaucomatous individuals. An improvement of the SLP technology consisting of the variable compensation of an-
terior segment birefringence has been recently described.25 Weinreb et al26 showed that SLP with variable compensation of the axis and magnitude of corneal birefringence results in improvement of the sensitivity and specificity of several parameters to discriminate between healthy and glaucomatous eyes. Further, monkey studies27,28 have suggested that RNFL measures obtained with SLP using variable corneal compensation (VCC) better reflect the RNFL qualitative appearance than measures obtained using SLP with fixed corneal compensation.

To determine the clinical validity of any new technology, it is important to compare it with previously established diagnostic techniques with proven clinical utility. Hence, the purpose of this study was to compare RNFL measurements obtained with SLP using VCC to standard red-free photography for detection of RNFL damage in glaucoma.

**METHODS**

This observational, cross-sectional study included 114 eyes of 114 patients. Forty-two patients had open-angle glaucoma, 32 patients were suspected of having glaucoma, and 40 were healthy. Mean ± SD age of glaucoma patients, patients suspected of having glaucoma, and healthy individuals was 67 ± 11 years, 61 ± 12 years, and 65 ± 11 years, respectively (P = .06, analysis of variance). All patients were evaluated at the Hamilton Glaucoma Center, University of California, San Diego, and retrospectively selected from our research database. These patients were included in a prospective, longitudinal study designed to evaluate optic nerve structure and visual function in glaucoma. All patients who met the inclusion criteria described were enrolled in the current study. Informed consent was obtained from all participants. The University of California, San Diego, Human Subjects Committee approved all protocols, and the methods described adhered to the tenets of the Declaration of Helsinki.

Each subject underwent a comprehensive ophthalmologic examination, including review of medical history, best corrected visual acuity, slitlamp biomicroscopy, intraocular pressure (IOP) measurement using Goldmann applanation tonometry, gonioscopy, dilated funduscopy examination using a 78-diopter (D) lens, stereoscopic optic disc photography, and automated perimeter using 24-2 full-threshold standard automated perimetry or Swedish Interactive Threshold Algorithm (Zeiss-Humphrey field analyzer, Zeiss-Humphrey, Dublin, Calif). To be included, subjects had to have best corrected visual acuity of 20/40 or better, spherical refraction within ±5.0 D, cylinder correction within ±3.0 D, and open angles on gonioscopy. Eyes with coexisting retinal disease, uveitis, or non-glaucomatous optic neuropathy were excluded from the study.

The RNFL photographs were acquired with a TRC-50VT camera (Topcon America Corp, Paramus, NJ) using Kodak Kodak high-contrast film (Eastman Kodak Company, Rochester, NY). Superior and inferior 20° fields were obtained using a set of narrow-pass filters (SE-40 and SE-50, Spectrotech Filter, Saugus, Mass) and a standard Topcon red-free filter. In addition, 35° wide-angle views of the optic disc centered between the superior and inferior regions of the RNFL were obtained. Photographs were printed on 35-mm transparencies and viewed on a light box with an acrylic optical grade magnifier (original magnification, ×4).

Evaluation of the RNFL photographs was completed using a semi-quantitative method described by Niessen et al.30 In this system, photographs were compared with a set of 25 reference photographs, and a point scale was used to score the photographs according to nerve fiber visibility.30,31 A high score of 25 indicated normal, thick, striated fibers, whereas a low score of 1 indicated that no nerve fibers were visible. Superior and inferior arcuate bundles were scored separately. Each photograph was assessed by 2 graders (F.A.M. and K.M.), and the final RNFL score was calculated as the average of the RNFL scores of each grader. Each grader was masked to the subject's identity and the other test results. Disagreements (difference of more than 4 points between the 2 graders) were resolved by consensus of adjudication of a third grader (C.B.). A global severity score (RNFL global) for each eye was created by averaging the superior and inferior final RNFL scores. Poor-quality RNFL photographs, as assessed by consensus of the graders, were discarded. Seventeen patients (13%) of an initial group of 131 eligible patients had poor-quality RNFL photographs and were excluded from the study, leaving 114 subjects for the analysis.

All patients underwent imaging with a commercially available SLP (GDx VCC, software version 5.0.1; Laser Diagnostic Technologies Inc, San Diego, Calif) within 3 months of RNFL photographs. The general principles of SLP have been described in detail elsewhere.5 The GDx VCC is a modified SLP system with VCC. Images of the ocular fundus are formed by scanning the beam of a near infrared laser (780 nm) in a raster pattern. The scan raster covers an image field 40° horizontally and 20° vertically in the eye, covering both the papillary and the macular regions.32 In the GDx VCC, the method of VCC, as described by Zhou and Weinreb,23 has been automated and replaced the original fixed corneal compensator. The VCC in the system consists of 2 identical linear retarders in rotating mounts so that both the retardance and axis of the unit can be adjusted according to requirements. To measure eye-specific corneal polarization axis and magnitude, SLP images of the macula are first acquired without compensation (the retardance of the VCC is set to zero). The radial birefringence of
Table 1. Mean ± SD RNFL Photographic Scores and SLP Parameter Values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glaucoma Patients (n = 42)</th>
<th>Patients Suspected of Having Glaucoma (n = 32)</th>
<th>Healthy Controls (n = 40)</th>
<th>ANOVA P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNFL scoring system</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior hemifield</td>
<td>12.7 ± 6.7</td>
<td>18.3 ± 4.5</td>
<td>19.4 ± 4.4</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Inferior hemifield</td>
<td>9.9 ± 6.3</td>
<td>16.9 ± 4.5</td>
<td>18.2 ± 5.0</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Global</td>
<td>11.3 ± 6.1</td>
<td>17.6 ± 4.3</td>
<td>18.3 ± 4.4</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>SLP parameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nerve Fiber Indicator</td>
<td>49.0 ± 22.3</td>
<td>26.8 ± 14.7</td>
<td>18.6 ± 8.5</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Superior ratio</td>
<td>2.24 ± 0.94</td>
<td>3.10 ± 0.80</td>
<td>3.17 ± 0.99</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Inferior ratio</td>
<td>2.22 ± 0.83</td>
<td>3.20 ± 0.90</td>
<td>3.24 ± 1.18</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Superior-nasal ratio</td>
<td>2.08 ± 0.75</td>
<td>2.41 ± 0.57</td>
<td>2.42 ± 0.58</td>
<td>.03†</td>
</tr>
<tr>
<td>Maximum modulation</td>
<td>1.75 ± 0.89</td>
<td>2.41 ± 0.82</td>
<td>2.45 ± 1.10</td>
<td>.002†</td>
</tr>
<tr>
<td>Ellipse modulation</td>
<td>2.97 ± 1.34</td>
<td>3.76 ± 1.16</td>
<td>3.80 ± 1.49</td>
<td>.01†</td>
</tr>
<tr>
<td>Ellipse SD</td>
<td>16.1 ± 4.79</td>
<td>20.7 ± 4.12</td>
<td>22.1 ± 4.07</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Ellipse average</td>
<td>45.7 ± 8.43</td>
<td>50.6 ± 6.09</td>
<td>54.1 ± 4.20</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Superior average</td>
<td>54.7 ± 11.1</td>
<td>62.2 ± 8.18</td>
<td>66.4 ± 7.0</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Superior maximum</td>
<td>49.7 ± 12.0</td>
<td>58.7 ± 8.8</td>
<td>63.1 ± 7.3</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Inferior maximum</td>
<td>64.0 ± 14.2</td>
<td>72.1 ± 10.3</td>
<td>77.8 ± 7.64</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Normalized superior area</td>
<td>0.097 ± 0.034</td>
<td>0.120 ± 0.025</td>
<td>0.134 ± 0.020</td>
<td>&lt;.001†‡</td>
</tr>
<tr>
<td>Normalized inferior area</td>
<td>0.090 ± 0.035</td>
<td>0.124 ± 0.027</td>
<td>0.138 ± 0.024</td>
<td>&lt;.001†‡</td>
</tr>
</tbody>
</table>

Abbreviations: ANOVA, analysis of variance; RNFL, retinal nerve fiber layer; SLP, scanning laser polarimetry.

*Corresponding pairs (glaucoma and healthy eyes) were significantly different (P<.05).
†Corresponding pairs (glaucoma patients and patients suspected of having glaucoma) were significantly different.
‡Corresponding pairs (patients suspected of having glaucoma and healthy subjects) were significantly different.

The Henle fiber layer in the macula is used as an intraocular polarimeter, and both the Henle fiber layer and corneal retardance can be determined from the macular retardation profile. Next, corneal birefringence-compensated SLP images are obtained using the appropriate eye-specific corneal polarization axis and magnitude values by adjusting the VCC retarders. The GDx VCC measures retardation in units of nanometers. To simplify communications, retardation values are converted into thickness values (micrometers) using a fixed conversion factor of 0.67 nm/µm.32

A baseline image was automatically created from 3 images obtained for each subject and used in each analysis. Only scans of good quality were included. Quality assessment was evaluated by an experienced examiner masked to the subject’s identity and results of the other tests. The hemifield SLP parameters investigated in this study were superior ratio (superior quadrant thickness to temporal quadrant thickness), inferior ratio, superior-nasal ratio, superior maximum (average of the 1500 thickest points in the superior quadrant), inferior maximum, superior average, inferior average, normalized superior area (area under the TSNIT [temporal-superior-nasal-inferior-temporal] curve in the superior quadrant), and normalized inferior area. Global SLP parameters investigated were maximum modulation ([thickest quadrant−thinnest quadrant]/thinnest quadrant), ellipse modulation, ellipse average, ellipse standard deviation, and the Nerve Fiber Indicator (NFI). The NFI is calculated using a support vector machine algorithm based on several RNFL measures (Michael Sinai, PhD, Laser Diagnostic Technologies Inc, written communication, March 2003) and assigns a number from 0 to 100 to each eye. The higher the NFI, the greater the likelihood the patient has glaucoma.

Analysis of variance was used to assess differences in SLP parameters and RNFL photography scores among glaucoma patients, patients suspected of having glaucoma, and healthy individuals. The Fisher least-significant difference test was used to perform post hoc multiple comparisons. The correlation between the semiquantitative RNFL severity scores obtained by assessment of RNFL photographs and the SLP parameters was evaluated by Pearson product moment correlation coefficients. The RNFL scores for each hemiretina were compared with SLP parameters evaluating the corresponding hemiretina, whereas global RNFL scores were compared with global SLP parameters.

Receiver operating characteristic (ROC) curves were used to describe the ability to differentiate glaucomatous from healthy eyes of each GDx VCC software–provided parameter and also of the RNFL photographic scoring system. The ROC curve shows the tradeoff between sensitivity and 1–specificity. An area under the ROC curve of 1.0 represents perfect discrimination, whereas an area of 0.5 represents chance discrimination. The method of DeLong et al33 was used to compare areas under the ROC curve. Minimum specificity cutoffs of 80% and 95% were used for comparing the sensitivity of the SLP parameters and the RNFL photographic scoring method. P<.05 was considered statistically significant. Statistical analyses were performed using SPSS statistical software, version 10.0 (SPSS Inc, Chicago, Ill).

RESULTS

RNFL MEASUREMENTS BY DIAGNOSTIC GROUP

Table 1 gives the mean values of all SLP parameters and RNFL scores in glaucoma patients, patients suspected of having glaucoma, and healthy individuals. Significant differences between glaucoma patients and healthy subjects and between glaucoma patients and patients sus-
expected of having glaucoma were found for all SLP parameters and also for RNFL photographic scores. When patients suspected of having glaucoma were compared with healthy individuals, the SLP parameters NFI, superior maximum, and normalized superior area showed statistically significant differences between the 2 groups.

ASSOCIATION BETWEEN SLP PARAMETERS AND RNFL SCORES

Table 2 gives the correlation coefficients for the associations between SLP parameters and RNFL photographic scores. All correlations were statistically significant with \( P < .001 \). Lower RNFL scores were associated with thinner RNFL thickness measurements as indicated by the SLP parameters. For the superior hemiretina, correlations between superior RNFL scores and corresponding SLP parameters showed \( R^2 \) values ranging from 16% to 40%. For the inferior hemiretina, the \( R^2 \) values ranged from 16% to 37%. For the association between global RNFL scores and global SLP parameters, the \( R^2 \) values ranged from 15% to 47%. The strongest relationship was found between the SLP parameter NFI and the global RNFL score (\( r = -0.683, R^2 = 47\% \), \( P < .001 \), Pearson correlation coefficient). As the SLP parameter NFI increased (more damage), the global RNFL photograph severity score decreased (more damage) (Figure 1).

We also investigated the correlation between the SLP parameters and RNFL scores with the visual field indices MD and PSD. The SLP parameter NFI showed the best correlation with MD and PSD among the SLP parameters. Higher values of NFI were associated with lower values of MD (\( r = -0.618, R^2 = 38\% \), \( P < .001 \), Pearson correlation coefficient) and higher values of PSD (\( r = 0.597, R^2 = 36\% \), \( P < .001 \), Pearson correlation coefficient). For the RNFL photographic scoring system, lower values of the global RNFL score were significantly associated with lower MD values (\( r = 0.565, R^2 = 32\% \), \( P < .001 \), Pearson correlation coefficient) and higher PSD values (\( r = -0.526, R^2 = 28\% \), \( P < .001 \), Pearson correlation coefficient).

ABILITY OF SLP PARAMETERS AND RNFL PHOTOGRAPHIC SCORING METHOD TO DIFFERENTIATE GLAUCOMA PATIENTS FROM HEALTHY SUBJECTS

Table 3 gives the values of the areas under the ROC curves for all SLP parameters and the RNFL photographic scores. For the SLP parameters, ROC curve areas ranged from 0.65 to 0.91, whereas for the RNFL photographic scoring system, the ROC curve areas were 0.80,
Using 30 as a cutoff point, the NFI classified as having abnormal eyes 9 (28%) of the 32 patients suspected of having glaucoma. Six (67%) of these 9 patients had glaucomatous-appearing optic discs, whereas 3 (33%) had ocular hypertension and normal optic discs. Using 8 as a cutoff point, the RNFL global score classified only 1 (3%) of the 32 patients suspected of having glaucoma as having abnormal eyes. This patient had a glaucomatous-appearing optic disc and was also diagnosed as having abnormal eyes by the NFI.

For both the superior and inferior hemifields, as well as for global RNFL measures, we found moderate correlations between the amount of RNFL damage as assessed by RNFL photographs and GDx VCC software--provided parameters. Because semiquantitative RNFL photographic assessment has been shown to precede detectable optic disc damage and visual field defects in glaucoma,1,3 the measurements of RNFL thickness obtained with the GDx VCC may provide clinically relevant information for diagnosing and monitoring glaucoma patients and also for detecting early glaucomatous damage.

The correlations found in our study between the SLP parameters and the Niessen semiquantitative RNFL scoring system ranged from a minimum $R^2$ of 15% to a maximum $R^2$ of 47%, depending on the specific SLP parameter evaluated. These values were generally better than previously reported correlations in studies comparing the same RNFL photographic scoring system with earlier versions of SLP using fixed corneal compensation.12,13 Niessen et al12 found a maximum $R^2$ of 28% for the correlation between cross-section RNFL measurements obtained with a nerve fiber analyzer (GDx Nerve Fiber Analyzer; Laser Diagnostic Technologies Inc) and the semiquantitative RNFL photographic scoring system. In another study, Zangwill et al13 found $R^2$ values ranging from 0.35% to 25.7% for the correlation between GDx Nerve Fiber Analyzer (Laser Diagnostic Technologies Inc) parameters and the Niessen photographic scoring method. These findings agree with recent reports that showed an improvement in the correlation coefficients for associations between SLP parameters and other measures of glaucomatous damage, such as visual field indices or optical coherence tomography RNFL thickness measurements, when VCC rather than fixed corneal compensation is used.34,35

The improvement in correlation coefficients with the use of SLP with VCC seems to be most noticeable for thickness parameters than for ratio and modulation ones. In the study by Zangwill et al,13 which used SLP with fixed corneal compensation, the best correlation using a thickness parameter was found between the superior average parameter and the superior hemifield RNFL score. However, the $R^2$ for this correlation was only 5%, whereas in our study the same association had an $R^2$ of 25%. For the ratio and modulation parameters, the best correlation in the study by Zangwill et al13 was found for the ellipse modulation parameter and global RNFL score, with an $R^2$ of 25.7%. In our study, the same association had a com-

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**Figure 2.** Receiver operating characteristic curves for the scanning laser polarimetry parameter Nerve Fiber Indicator (NFI) and for the global retinal nerve fiber layer (RNFL) photographic score to discriminate glaucomatous from healthy eyes.

**Figure 3.** Sensitivity and specificity values for each cutoff point of the scanning laser polarimetry parameter Nerve Fiber Indicator to discriminate glaucomatous from healthy eyes.
parable $R^2$ of 21.7%. The stronger improvement found for thickness parameters than for ratio and modulation parameters is likely because the latter may already compensate for some of the changes in retardation measurements caused by an inadequate corneal compensation in some patients. This finding is supported by the study by Weinreb et al., which showed that the diagnostic ability of several SLP parameters at classifying eyes as glaucomatous or healthy is improved considerably with SLP using VCC as compared with SLP using fixed corneal compensation, particularly for the thickness parameters.

The best correlation in our study was found between the GDx VCC software–provided parameter NFI and the global RNFL photographic score, with an $R^2$ of 47%. This is not a surprising result, because the NFI is calculated using a sophisticated machine learning classifier that takes into account several parameters of the RNFL and is intended to provide the best measure of the current RNFL status obtained with SLP. Higher values of NFI were also significantly associated with more severe visual field damage, as indicated by the global indices MD and PSD. The correlation between NFI and visual field indices was higher than the association found between the visual field indices and the global RNFL photographic score. Interestingly, the correlation between the 2 methods of RNFL assessment was higher than the correlation between each method and the visual field indices. This is not surprising, since RNFL damage often can precede visual field loss and therefore structure-function correlations are expected to be weak at a given point in time.

We also evaluated the ability of the SLP parameters and RNFL photographic scores to discriminate glaucoma patients from healthy individuals. The best SLP parameter, NFI, had a significantly better diagnostic power than any of the RNFL scores evaluated. This was particularly evident for higher specificities. When the specificity was set at 95%, the sensitivity of the NFI to diagnose glaucoma was 71% compared with only 36% for the global RNFL score. At a moderate specificity (80%), the NFI parameter and the RNFL global score had similar sensitivities (88% and 81%, respectively). In a study comparing the ability of several diagnostic tests to distinguish healthy from glaucomatous eyes, Paczka et al. found that the SLP with fixed corneal compensation had a similar sensitivity compared with a semiquantitative RNFL scoring system, when both methods were evaluated at high-specificity settings.

Besides the apparent better diagnostic ability of the SLP using VCC as compared with the RNFL photographic assessment, other potential advantages of the SLP technology are notable. SLP provides real-time, immediate, and objective assessment of the RNFL and does not require pupil dilation. On the other hand, good-quality red-free RNFL photographs are technically difficult to obtain, requiring maximum pupillary dilation and clear ocular media. In addition, evaluation of RNFL photographs requires special training and remains a subjective method, even when semiquantitative scoring systems are used. However, potential benefits of RNFL photographic assessment exist, namely its lower cost and the fact that this method has been previously validated by longitudinal studies for early detection of glaucomatous damage.

Both the SLP parameters and the RNFL photographic scoring method were able to find significant differences between glaucoma patients and healthy subjects, as well as between glaucoma patients and patients suspected of having glaucoma. However, only the SLP was able to detect significant differences between those suspected of having glaucoma and healthy individuals, with 3 of the investigated parameters showing statistically significant differences between these 2 groups. Accordingly, using a cutoff with 95% specificity, 28% of those suspected of having glaucoma were classified as having normal eyes using the NFI parameter, whereas only 3% of the same subjects were classified as having abnormal eyes using the RNFL global photographic score. If the excess of abnormal eyes in those suspected of having glaucoma over healthy subjects is taken as a measure of true glaucomatous defect among the group with suspected glaucoma, a larger proportion of eyes with early glaucoma damage were identified with the NFI compared with the RNFL photographic scoring method. Although this might indicate an advantage of SLP over conventional RNFL photographic assessment for the identification of early damage in those suspected of having glaucoma, longitudinal studies are still needed to verify this possibility.

In conclusion, a good correlation was found between RNFL assessment using red-free photographs and selected SLP parameters, suggesting that these 2 methods provide comparable information about the status of the RNFL damage in glaucoma. In high-specificity settings, the best parameter from the SLP had better performance than RNFL semiquantitative photographic assessment in the detection of patients with glaucomatous visual field defects and also in the identification of abnormalities in the eyes of patients suspected of having glaucoma.

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