Improved scanning laser fundus imaging using polarimetry

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Received August 30, 2006; revised November 24, 2006; accepted December 15, 2006; posted January 9, 2007 (Doc. ID 74515); published April 11, 2007

We present a polarimetric technique to improve fundus images that notably simplifies and extends a previous procedure [Opt. Lett. 27, 830 (2002)]. A generator of varying polarization states was incorporated into the illumination path of a confocal scanning laser ophthalmoscope. A series of four images, corresponding to independent incoming polarization states, were recorded. From these images, the spatially resolved elements of the top row of the Mueller matrix were computed. From these elements, images with the highest and lowest quality (according to different image quality metrics) were constructed, some of which provided improved visualization of fundus structures of clinical importance (vessels and optic nerve head). The metric values were better for these constructed images than for the initially recorded images and better than averaged images. Entropy is the metric that is most sensitive to differences in the image quality. Improved visualization of features could aid in the detection, localization, and tracking of ocular disease and may be applicable in other biomedical imaging. © 2007 Optical Society of America

OCIS codes: 120.5410, 180.1790, 110.3000, 120.3890, 170.3880, 330.4460

1. INTRODUCTION
The reliability of ophthalmic diagnoses of retinal diseases and abnormalities is strongly dependent on the quality of fundus images. This quality is limited by the aberrations of the eye, intraocular scatter, and the properties of the light reflected from the fundus structures. Although longer wavelengths and confocal pinholes reduce image degradation due to scattered light in scanning laser ophthalmoscopes (SLOs), scatter still reduces the contrast of the imaged structures. Other techniques to enhance the quality of fundus images include the averaging of images, correction of the ocular aberrations, and deconvolution procedures.

The human eye has complicated polarization properties that are used for clinical diagnosis. The use of simple polarization elements has been shown to enhance the view of endothelial cells, discontinuities in the crystalline lens, and retinal structures. Although the double-pass point-spread function is nearly independent of the state of polarization of the incident light, depolarized light images can improve the contrast of retinal blood vessels and subretinal features. Commercially available polarimetric devices are used mainly for glaucoma diagnosis and rely on linear polarization. The image associated with depolarized light provided by a commercial instrument has also been reported to provide enhanced fundus images in pathological eyes. We will use the complex differential polarization properties of fundus structures to improve the visualization of clinically important fundus structures with a confocal scanning laser ophthalmoscope (CSLO).

While complete Mueller matrix systems have been
built to extract the ocular polarization properties\cite{21,32-35} and to reduce noise and improve contrast in CSLO images,\cite{36} reduced versions can capture much of the information\cite{37,38} and can improve images with less effort.\cite{39} In the current paper we have extended and simplified our previously reported method\cite{36} by reducing the number of recorded images from 16 to 4. This is based on the fact that only the elements of the first row of the Mueller matrix are needed to construct the intensity image. Images corresponding to only four independent incoming polarization states in the generator unit are used to compute the spatially resolved elements of the first row of the Mueller matrix of the eye and instrument combination. Moreover, in this simpler method, the analyzer unit in the recording pathway and the calibration procedure are not required.

The present study uses data from our simplified polarimeter and the global image quality metrics signal-to-noise ratio (SNR),\cite{40} entropy,\cite{41} and acutance\cite{42} to assess the quality of polarimetric images of blood vessels and the optic nerve head (ONH). Previously, only SNR and contrast have been used to quantify polarimetric improvements.\cite{27,28,36,39} Intersubject and intrasubject variations in the polarization properties of the retinal nerve fiber layer (RNFL) near the ONH are important for ophthalmic diagnosis,\cite{22,29,43} as is the morphology of the ONH.\cite{44-46} We will demonstrate that our method improves the imaging of different parts of the healthy ONH.

2. METHODS

A. Experimental System and Procedure

A schematic diagram of the polarimetric CSLO used in this work is depicted in Fig. 1. The experimental system incorporates a generator of polarization states in the illumination arm (Fig. 1). This generator unit (GU) is composed of a fixed vertical linear polarizer (P) and a rotating quarter-wave plate (QWP). The size of a 633 nm collimated laser beam is dependent on aperture AP1. This beam passes through the GU and is scanned in two dimensions by a rotating polygon mirror (horizontal X scan) and a galvanometer (vertical Y scan). The beam enters the eye and is focused onto the fundus. This incident beam is 2.5 mm in diameter when using the configuration for a 10° field image and is 3.3 mm in the 15° field configuration. In the recording pathway, the light emerging from the eye is descanned and reaches the recording arm composed of a collector lens (CL), a confocal pinhole (AP2), and a photomultiplier tube (PMT). The return pathway caused little alteration in the light polarization,\cite{36} and the PMT had similar sensitivity to different polarizations.

To minimize misalignments during recording, the subjects used a bite bar mounted on a three-axis positioning stage. Alternating eyes of four young-adult subjects with no ocular pathologies and normal vision were imaged in 10° or 15° fields. When necessary, lenses focused the light onto the fundus plane of interest. Each eye’s pupil was dilated using 0.5% tropicamide. Measurements were made at two different locations: (1) a retinal area with visible blood vessels and (2) the ONH. Retinal areas containing blood vessels between the fovea and the ONH for subjects 1 and 2 and anterior to the ONH in subject 4 were imaged. All subjects provided informed written consent. These experiments received ethics approval from the University of Waterloo Human Ethics Committee.

Images were viewed in real time and streamed to a computer disk at 28.5 Hz. For each condition, a reference raw background image (taken of an empty field) was chosen and additional background frames with the highest correlation to this reference image were determined. The average of these eight background frames was subsequently subtracted from each selected raw frame of a subject’s eye. For each experimental ocular imaging condition, an initial frame was selected. After background subtraction, this frame and the additional frames with maximum correlation were aligned, rotated, and averaged. Images used to calculate the Mueller matrix elements were the average of four or eight frames. Averages of 32 frames taken with linearly polarized light were also used for comparison.

In a given fundus location, video segments corresponding to four independent incoming polarization states, including linear, circular, and elliptical polarizations, were recorded. These polarization states are achieved in the GU by rotating the fast axis of the QWP to −45°, 0°, 30°, and 60° as described elsewhere.\cite{36,47} From these four images, the elements of the top row of the combined “eye plus instrument” Mueller matrix are computed as a function of pixel position (see Appendix A). These four elements are enough to compute (by means of linear combinations) the resulting retinal image for any incoming totally polarized state (see Subsection 2.B). Resulting images with the minimum and maximum values of three image quality metrics were chosen to be compared with the original images. This is explained in detail using the Mueller matrix formalism in Appendix A and in the following section.

![Fig. 1. Schematic diagram of the polarimetric CSLO: P, linear polarizer; QWP, rotating quarter-wave plate; BS, beam splitter; CL, collector lens; AP1 and AP2, pinholes; PMT, photomultiplier detector; GU, generator unit.](image-url)
B. Image Construction

Once the elements of $M_0$, the first row of the Mueller matrix, are known, we constructed (approximately) 2592 images, $I^{\text{OUT}}$, as a function of pixel position $(x,y)$, corresponding to a set of incident Stokes vectors, $S_{\text{IN}}(\chi,\varphi)$, covering the Poincaré sphere in increments of 5° in longitude (i.e., $2^\circ\text{azimuth}=2^\circ\chi$) and latitude (i.e., $2^\circ\text{ellipticity}=2^\circ\varphi$), by means of

$$I^{\text{OUT}} = \left( \begin{array}{cccc} m_{00}^{(C)} & m_{01}^{(C)} & m_{02}^{(C)} & m_{03}^{(C)} \end{array} \right) \cdot \begin{pmatrix} 1 \\ \cos(2\chi) \cdot \cos(2\varphi) \\ \sin(2\chi) \cdot \cos(2\varphi) \\ \sin(2\varphi) \end{pmatrix}$$

$$= M_0 \cdot S_{\text{IN}}. \quad (1)$$

Three image quality metrics (SNR, entropy, and acutance) were used to quantify the image quality of all constructed images. Since $I^{\text{OUT}}(x,y)$ is the intensity of each individual pixel, SNR is defined as the ratio of the mean $I^{\text{OUT}}(x,y)$ to the standard deviation of $I^{\text{OUT}}(x,y)$ across the entire image. A good quality image is expected to have a large SNR. Entropy is not the traditional image processing definition but rather Shannon entropy (MATLAB, The Math Works, Inc.), which depends on the absolute values of the intensity levels. The larger the number of bright pixels in an image, the higher the entropy value. Acutance is a metric of sharpness, that is, the presence of high spatial-frequency details in an image is expected to provide a high acutance value. Moreover, the acutance will be zero for a constant intensity image regardless of the gray level. This metric has previously been used to characterize breast tumors, and fundus images with high acutance have been postulated to show enhanced views of the RNFL. Further information on these metrics can be found in a companion paper in this issue.

The analysis routine evaluates the image quality metrics across the averaged, experimentally measured images (corresponding to four different input polarization states) and those produced by calculation using Eq. (1). The calculated (constructed) images corresponding to the highest and lowest values of each metric, as well as the corresponding (theoretical) Stokes vectors $S_{\text{IN}}$, were saved and compared with the four measured (four- or eight-frame-averaged) images. Maximizing an image quality metric is a well-known method of producing better image quality. In our case, the images with minimum values for the metrics appeared to give additional information. The Stokes vectors give the relative weighting of the Mueller matrix elements in the best and worst constructed images. This method produces images, $I^{\text{OUT}}(x,y)$, representing many different input polarization states that may not be easily obtained in an experimental system.

Moreover, the constructed images were also compared with those obtained from averaging 32 frames measured with linearly polarized light. This controls for the possibility that image improvement is simply due to a larger number of initial images being combined. Images corresponding to nonpolarized incident light were also computed for comparison. This image corresponds to an autocorrelated image of the element $M_{00}$ of the Mueller matrix. All image processing was performed in MATLAB.

3. RESULTS

A. Effect of Frame Averaging on Fundus Images

Figure 2 shows the result of frame averaging in fundus images of blood vessels recorded with the CSLO for three different subjects. Single frames and the averages of four frames are presented. By direct inspection, it can be observed that, in all cases, there is a noticeable improvement in the image quality. In images of blood vessels, here we observe that contrast increases, especially in the originally darker areas, and that some local details that are difficult to observe in the original images become visible. SNR and entropy both increase with frame averaging, consistent with the observed image improvement. Acutance however, decreases with frame averaging, in spite of the subjective improvement.

![Fig. 2. Improvement in CSLO images by frame averaging: single frames (upper row) and averages of four frames (bottom row). Left, middle, and right panels correspond to subjects 1, 2, and 3, respectively. Images subtend 3.3°.](image-url)
B. Image Quality Improvement through Polarization Imaging

1. Blood Vessel

Polarimetric images of subject 2 for a retinal area of 5.6° containing blood vessels between the fovea and the ONH are depicted in the upper row of Fig. 3. For this subject, the measured image with the largest values of SNR and entropy corresponded to circularly polarized light, while that for the largest value of acutance corresponded to elliptically polarized light. The four spatially resolved elements of the Mueller matrix computed from these images are given in the bottom row of Fig. 3.

Figure 4 shows the images with the minimum and maximum values of the three image quality metrics obtained using the computational procedure described in Subsection 2.B. For comparison, we also include an average of 32 initial images taken with linearly polarized light and the constructed image corresponding to incident non-polarized light. The metric values corresponding to the 32-frame-averaged image were between the highest and the lowest values obtained with our procedure.

Figure 5 shows results for blood vessels in subject 4 for a larger area (7.7°). The top row of the Mueller matrix is shown as well as experimental and constructed images. These images correspond to vessels located anterior to the ONH. For each of the three metrics, the highest metric value for the initially recorded images corresponded to linearly (vertically) polarized light. This initial image is...
shown with constructed images for maximum values of SNR and entropy and the average of 32 initial images taken in linearly polarized light.

For both subjects and retinal regions, the improvements in the images obtained using this polarization method were noticeable. When SNR and entropy were maximized, the images of blood vessels appeared to improve and some small fundus features were much better defined. The results are less clear for acutance (Fig. 4). For this reason, Fig. 5 emphasizes results from SNR and entropy. For all metrics across subjects, except SNR in subject 4, the maximum metric values for constructed images were higher than the metric values for an average of 32 frames (taken with linear polarization). The images for nonpolarized incident light gave good apparent quality in each subject, with metric values higher than those of the initial images taken with linearly polarized light but with values of each image quality metric lower than the maximum value for constructed images.

For a quantitative comparison, the improvement in both SNR and entropy (in percent) are depicted in Fig. 6. The improvement depended on each subject. For acutance, entropy, and SNR, the maximum metric value for the constructed polarization image was higher than those of all initial images and higher than those of the unpolar-
ized light images. For all but one case of SNR, the constructed polarization image with the maximum metric value was also better than that corresponding to averaging 32 frames (taken at linear polarization). Entropy appears to give a larger and more consistent improvement than SNR.

Finally, Fig. 7(a) shows the Poincaré sphere, with the Stokes vectors providing the maximum and minimum values for SNR, entropy, and acutance for blood vessels for subject 2. In general, maximizing or minimizing different metrics corresponds to a Stokes vector on a different part of the sphere. Figure 7(b) shows the coordinates on the Poincaré sphere of the Stokes vectors for two other subjects’ blood vessel images, subject 1 (images not shown) and subject 4. These Stokes vectors also vary with subject.

2. Optic Nerve Head

The upper panels in Fig. 8 depict a set of CSLO polarimetric images for the ONH of subject 3. From these images, the first four elements of the spatially resolved Mueller matrix were calculated (Fig. 8). Some blood vessels and certain structures of the ONH, including the lamina cribrosa and neural retinal rim, can be seen in elements $M_{00}$, $M_{01}$, and $M_{02}$. However, the element $M_{03}$ shows less detail. The constructed images providing the

Fig. 8. CSLO images for the ONH of subject 3 corresponding to the average of four frames in each of the four polarization states in the GU (upper panels) and spatially resolved elements of the first row of the Mueller matrix (bottom panels). Each image subtends 7°. Numbers at the bottom-left corner of the upper images indicate the orientation of the fast axis of the rotating QWP with respect to the linear polarizer in the GU. The gray-level code for the Mueller matrix is shown at the bottom. The area inside the square on the first image will be used for later comparisons.

Fig. 9. Constructed images for the ONH of subject 3. The figure shows constructed images of maximum SNR (a), minimum SNR (b), maximum entropy (c), minimum entropy (d), maximum acutance (e), minimum acutance (f), and the image for incident nonpolarized light (g).
maximum and minimum values for SNR, entropy, and acutance are presented in Fig. 9.

For this subject, the constructed images in Fig. 9 provide detail not available in the original images. The lamina cribrosa pore structure is more visible in images with minimum SNR [Fig. 9(b)] and minimum entropy [Fig. 9(d)]. Note the similar details among images with maximum SNR [Fig. 9(a)], maximum entropy [Fig. 9(c)], and the image for depolarized light [Fig. 9(g)]. Acutance images provide a differing view of the ONH structures.

We centered our attention on the lamina cribrosa, a subsection of the ONH images (that marked on Fig. 8). Figure 10 shows the corresponding elements of the top row of the Mueller matrix as well as the constructed images for maximum and minimum SNR. The Mueller matrix contains information about the lamina structure. Minimum and maximum SNR give differing views of the pore structure.

Results for the ONH of an additional eye (subject 2) are presented in Figs. 11 and 12. The initial images and top row of the Mueller matrix are in Fig. 11. Values for the element $M_{03}$ are close to zero, but some features are clearly visible in elements $M_{00}$, $M_{01}$, and $M_{02}$. In Fig. 12, the constructed images with maximum and minimum metric values are presented. The normalized image $M_{00}$, as well as the 32-frame-averaged image for the linear polarized incoming light image, are also shown. A direct observation again shows that lamina pore structure is best for images of minimum SNR [Fig. 12(b)] and minimum entropy [Fig. 12(d)]. Maximum SNR [Fig. 12(a)] and entropy [Fig. 12(c)] provide good images of the neural retinal rim. The image for nonpolarized incident light [Fig. 12(g)] gave similar views. Minimum and maximum acutance emphasize differing features and provide potentially useful information. As expected, the metric values of the original images fall between the maximum and the...
minimum values of images constructed by using the present method. Elliptically polarized light (Fig. 8) gave the highest initial values of all metrics. For acutance, entropy, and SNR, the maximum metric values for the constructed polarization images of this subject were higher than those of all initial images, higher than those of the nonpolarized incident light images, and also higher than those corresponding to averaging 32 frames taken at linear polarization [Fig. 12(h)]. The minimum metric values for constructed images (which also give important views of ONH structures) were lower than those of all initial images and lower than those of the images for incident nonpolarized light. For SNR and entropy they were also lower than those corresponding to averaging 32 frames (taken at linear polarization). This averaged image, however, gave a value of acutance lower than the lowest value for a polarimetric initial image. Entropy values for the constructed images differ by larger percentages from the initial, averaged, and incident nonpolarized light images than do SNR values. Again, the amount of change in image quality metrics in the polarimetric images depends on each subject.

The location on the Poincaré sphere of the Stokes vectors producing the images with maximum and minimum SNR values for the two analyzed areas of the ONH in subject 3 (the whole ONH image (Fig. 9; 10° across, circles) and a small area within the ONH (Fig. 10; triangles).}

Fig. 13. Poincaré sphere with the Stokes vectors corresponding to the constructed images with maximum (solid symbols) and minimum (open symbols) SNR values in subject 3 for the whole ONH image (Fig. 9; 10° across, circles) and a small area within the ONH (Fig. 10; triangles).

Fig. 14. Longitude (2*Azimuth, solid symbols) and latitude (2*Ellipticity, open symbols) on the Poincaré sphere of the Stokes vectors providing the images with maximum and minimum SNR, entropy (E), and acutance (A) for ONH images of subjects 2 (squares) and 3 (circles).

4. DISCUSSION

A. Polarimetric Imaging

We have reported a simplified and extended polarimetric technique that improves the objective quality of fundus images. This is a modification of our previous method that reduces both image recording and processing times.
Illumination in the registered images increases over our previous technique, as a polarization analyzer unit is no longer required. The series of four images measured (each corresponding to an independent incoming polarization state) and the resulting elements of the top row of the Mueller matrix contain information additional to that normally present in CSLO and SLO images, which use linearly polarized light. The improvements in image quality observed are due to spatial differences (across pixels) in the Mueller matrix elements that we have used to construct improved images. Although the constructed images for nonpolarized incident light were also of good quality, the presence of contrast in all Mueller matrix elements indicates that vessels and ONH structures interact differentially with both linearly and circularly polarized light. We have utilized this interaction to produce improved images. The images produced could not easily be directly measured. The method may be generally applicable to biomedical images of tissues with spatially varying polarization properties. The metrics indicate, in the majority of cases, an improvement of the constructed images over images taken with linearly polarized light and that metric values are better for constructed images than for initially measured images.

Bueno and Vohnsen\textsuperscript{39} have developed a polarimetric high-resolution CSLO to maximize the contrast across retinal blood vessels of small retinal areas. The instrument used fixed incoming linear polarization and modulated the light emerging from the eye to reconstruct Stokes vectors. This configuration clearly differs from the present one, although both have a common goal, to improve fundus image quality. The present configuration, modulating the incoming polarization state, has the advantage of higher light intensity in the images.

B. Image Quality Metrics

Previously, we used a polarimetric method in conjunction with maximizing the SNR.\textsuperscript{35} Here we have compared the results obtained using SNR, entropy, and acutance. As expected, maximizing image quality metrics produces subjectively better images of some structures. Minimizing these image quality metrics also appears to be useful, producing subjectively better images of different structures. This is consistent with the polarization properties that are spatially dependent, differing among fundus features. Images with maximum values of SNR and entropy appear similar, but the improvement in and range of entropy values are larger and more consistent. Elsewhere, we have found that entropy better differentiates CSLO images taken in differing conditions.\textsuperscript{11} Although acutance has previously been proposed as a metric for improving RNFL images,\textsuperscript{42} it has yet to be applied successfully. Here, although it does not improve with frame averaging, maximizing or minimizing acutance values provides differing and potentially useful views of fundus structures. In imaging the ONH, both maximum and minimum values of entropy and acutance are useful in enhancing differing ONH structural details, including those important to the diagnosis of glaucoma. There were differences in the Stokes vectors giving maximum or minimum image quality among the metrics.

The metrics described here objectively quantified improvements in quality in our constructed images. Subjectively, the images produced also appear to enhance different fundus features. Hutchings and colleagues\textsuperscript{49} have recently begun to correlate the subjective quality of CSLO fundus images, obtained through polarimetry, with these objective measures.

C. Comparison with Frame Averaging

Like others,\textsuperscript{11–15,36} we have used image correlation, registration, and frame averaging to improve CSLO images. Frame averaging improves the quality of images recorded with a confocal scanning laser system, since it reduces random noise. This improvement can be obtained independent of the polarization of the reflected light and has been recently reported in samples providing specular, diffuse, and partially depolarizing reflections.\textsuperscript{51} Entropy and SNR improved with averaging but indicated that, except for one case, the improvements with the polarimetric method were larger than those obtained by averaging the same total number of frames taken with only linearly polarized light. The observed decrease in acutance with frame averaging (in spite of the subjective improvement), also shown elsewhere,\textsuperscript{14} might be due to a reduction in speckle noise.\textsuperscript{11}

D. Enhancement of Different Fundus Features

We chose to maximize and minimize the quality of the whole image, as images with maximum acutance have previously been suggested to enhance the visualization of the RNFL.\textsuperscript{42} We applied the method to extended images of different areas of the fundus. In diagnosing disease, larger field images with multiple features, for example, of the ONH, are often used. The optimization procedure can also be applied to different areas of interest in the images in order to enhance them separately. The polarization states of the Stokes vectors that enhanced different structures differed. There were also differences in the degree to which the Stokes vectors produced maximum or minimum image quality among subjects.

The results presented here on blood vessel images have shown that our method can increase the quality of retinal and ONH blood vessel images and their visibility. We were able to construct polarimetric images with higher values of image quality metrics compared with any of the four original images and compared with the $M_{00}$ image. The subject dependence of the blood vessel results agrees with our previous results using the full Mueller matrix and SNR as the metric.\textsuperscript{36}

Constructed $M_{00}$ images of blood vessels were also of better quality than the (eight-frame-averaged) original images taken with linearly polarized light, consistent with improvements reported by Weber et al. for their depolarized light images.\textsuperscript{37} However, in one subject, for all three metrics tested here, image quality metric values were always worse in this $M_{00}$ image than the highest values among images initially taken. According to all image quality metrics, the best constructed images were always better than the $M_{00}$ image for these subjects.

Polarimetric imaging was also successful in improving images of the ONH. Additional information is available on the structures within the ONH by the use of both circu-
larly and linearly polarized light. Different metrics and their maximums and minimums produce subjectively better images depending on whether vessels, lamina, or a subregion of the ONH was considered. Here, manipulating acutance highlighted combinations of structures, different from those given by SNR and entropy. Within an individual subject, the Stokes vectors for a metric maximum or minimum also depend on the analyzed region. This suggests that these methods may allow improvement of details within a specific image dependent on the actual physical properties of retinal features. In addition, an analysis of some of the individual Mueller matrix elements might also help in identifying small structures and features. Once specific features (blood vessels and lamina cribrosa) are more visible, automated segmentation of an image might be easier and both objective and subjective diagnosis could improve.

The polarization states (Stokes vectors) that gave the best and the worst metric values for the ONH images show that there are again differences among subjects (Fig. 14), which presumably reflect the different diattenuation properties across the image. It can also be seen that in most cases for a given metric, the Stokes vectors providing the images corresponding to the maximum and minimum metric values fall far apart on the Poincaré sphere. In the future, once the range of Mueller matrix values of the ONH and vessels among normal and pathological eyes is better known, the number of computations required to produce the enhanced images may be reduced.

E. Polarized and Nonpolarized Light Images

For ONH images in these subjects, values of entropy and SNR were always higher in the image for nonpolarized incident light than in some of the original images taken with polarized light. However, for some subjects, values of acutance were lower in this $M_{00}$ image. This suggests that $M_{00}$ images might provide information not present in the images taken with polarized light. Although, for all metrics, images could always be constructed with polarized light to give metric values higher than those of $M_{00}$ images, further experiments (mainly with older and pathological eyes) may reveal that a combination of both types of images would be useful in clinical diagnosis. Our technique has the advantage that it allows us to construct the image corresponding to both polarized and nonpolarized incident light.

5. CONCLUSIONS

The combination of Mueller matrix polarimetry and CSLO imaging of the fundus has been shown to enhance images of many different structures. This method provides a step forward in improving CSLO fundus imaging, since more structural details and small features, which were not discernible in the original images, can be observed. Since the study and treatment of retinal diseases are based mainly on the direct observation of the fundus, poor image quality can lead to a failure of diagnosis or to an inability to treat. Although this improved imaging procedure has been tested only in healthy normal eyes, it may also aid in visualization and in monitoring and quantifying changes in the fundus structures (for example, the lamina cribrosa and blood vessels) associated with pathologies. Technically, an implementation with liquid crystals or photoelastic modulators could speed up the recording time. This would be very useful in a clinical environment.

APPENDIX A: MUeller MATRIX THEORY

For each independent polarization state emerging from the GU, $S_G^{(i)}=[S_{00}^{(i)}, S_{10}^{(i)}, S_{20}^{(i)}, S_{30}^{(i)}]^T$ ($i=1,2,3,4$), the Stokes vector associated with the light reaching the PMT, $S_F^{(i)}$, is given by

$$S_F^{(i)} = M_C \cdot S_G^{(i)} = M_{SYST}^{(2)} \cdot M_{eye} \cdot M_{SYST}^{(1)} \cdot S_G^{(i)}, \quad (A1)$$

where $M_C = m_{1i}^{(C)}$ ($i,m=0,1,2,3$) is the Mueller matrix of the system (eye plus instrument); $M_{eye}$ is the Mueller matrix of the eye, including the two passes; and $M_{SYST}^{(1)}$ and $M_{SYST}^{(2)}$ are the Mueller matrices of the confocal instrument itself (lenses, scanning unit, beam splitter) for the first and second passages, respectively. Although $M_{eye}, M_{SYST}^{(1)}$, and $M_{SYST}^{(2)}$ are not known, we will be able to obtain the first row of the combined Mueller matrix $M_C$.

The first element of the $S_F^{(i)}$ in Eq. (A1), $I_F^{(i)}$, corresponds to the intensity of the recorded pixel. Since we have four incident $S_G^{(i)}$, then this can be expressed as

$$ I_F = \begin{pmatrix} I_F^{(1)} \\ I_F^{(2)} \\ I_F^{(3)} \\ I_F^{(4)} \end{pmatrix} = \begin{pmatrix} m_{00}^{(C)} & m_{01}^{(C)} & m_{02}^{(C)} & m_{03}^{(C)} \\ m_{10}^{(C)} & m_{11}^{(C)} & m_{12}^{(C)} & m_{13}^{(C)} \\ m_{20}^{(C)} & m_{21}^{(C)} & m_{22}^{(C)} & m_{23}^{(C)} \\ m_{30}^{(C)} & m_{31}^{(C)} & m_{32}^{(C)} & m_{33}^{(C)} \end{pmatrix} \begin{pmatrix} S_{00}^{(1)} \\ S_{00}^{(2)} \\ S_{00}^{(3)} \\ S_{00}^{(4)} \\ S_{10}^{(1)} \\ S_{10}^{(2)} \\ S_{10}^{(3)} \\ S_{10}^{(4)} \\ S_{20}^{(1)} \\ S_{20}^{(2)} \\ S_{20}^{(3)} \\ S_{20}^{(4)} \\ S_{30}^{(1)} \\ S_{30}^{(2)} \\ S_{30}^{(3)} \\ S_{30}^{(4)} \end{pmatrix} $$

$$= M_0 \cdot M_G, \quad (A2)$$

where $M_0$ is the row vector whose elements are the first row of the Mueller matrix $M_C$ and $M_G$ is a $4 \times 4$ auxiliary matrix containing the four incident Stokes vectors. Finally, $M_0$ is obtained by inversion of Eq. (A2):

$$ M_0 = I_F \cdot (M_G)^{-1}. \quad (A3) $$

Equation (A3) applies at every position on the image that can have spatially varying polarization properties. Elements of $M_0$ can be calculated for each image pixel, and this is referred to as the spatially resolved $M_0$.

ACKNOWLEDGMENTS

We thank S. Guthrie and Q. Liang for useful discussions and L. Epps for his assistance with data analysis. This work was supported by the Ontario Centre of Excellence, the Center for Photonics, and National Sciences and Engineering Research Council of Canada. J. J. Hunter was supported by an Ontario Graduate Scholarship.
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