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Morphometric Features of Laminar Pores in Lamina Cribrosa Observed by Scanning Laser Ophthalmoscopy

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Purpose: To describe a method for making morphometric analysis of the pores in the lamina cribrosa with a confocal scanning laser ophthalmoscope (SLO).

Methods: Sixteen consecutive images were acquired with an SLO from the retinal surface to the bottom of the optic disc in +0.25-diopter increments. An He-Ne laser (633 nm) with a 20° field of view was used. The images from each section were processed and combined with the aid of Macintosh software.

Results: Eyes with physiological cupping showed uniformly round or nearly round pores, whereas eyes with primary open-angle glaucoma frequently had compressed pores.

Conclusions: In vivo morphometry of the surface of the internal lamina cribrosa can be performed by this technique, which should be useful for evaluating the progression of glaucomatous changes of the optic nerve head. **Jpn J Ophthalmol 1999;43:415–421**

Key Words: He-Ne laser, lamina cribrosa, laminar pore, primary open-angle glaucoma, scanning laser ophthalmoscope.

Introduction

Vascular[1] and mechanical[2] theories have been proposed and discussed extensively as the two major factors involved in optic nerve damage in glaucomatous eyes. However, neither theory can adequately explain the complex pathogenesis of this blinding disease. The concept of apoptosis[3] has recently attracted attention as a mechanism involved in the death of retinal ganglion cells. Regardless of the mechanism, the optic nerve damage in glaucomatous eyes is considered to develop in the lamina cribrosa.[4] A study of experimental glaucomatous eyes has shown abnormal changes in the morphology of the laminar pores with changes in the structure of the lamina cribrosa.[5] Changes in the size and shape of the laminar pores with progression of glaucoma have also been reported.[6] However, qualitative methods of analyzing the morphology of laminar pores in vivo have not been established.

We have acquired consecutive images of the optic nerve from the surface to the bottom of the disc using a scanning laser ophthalmoscope (SLO). The images were processed using a computer to obtain images of the laminar pores in normal subjects and in patients with glaucoma.

Materials and Methods

Method of Measurement

The ocular fundus was examined with an SLO (Rodenstock 101, Ottobrun, Germany) after mydriasis with Mydrin-P. The imaging conditions were set using an SLO-Control v. 2.0. For recording the cross-sectional images of the optic disc, a long-wavelength He-Ne (633 nm) laser was used as the oscillation wavelength. The field of view was set diagonally at 20°. After output at 100 $\mu W/cm^2$, the focus was adjusted by changing the laser output. The measurement head was then adjusted so that the optic disc was placed in the center of the field. The confocal aperture was C1. The refractive error was corrected so that the maximum image resolution could be obtained on the surface of the peripheral retina of the optic disc. From this site, the refractive correction

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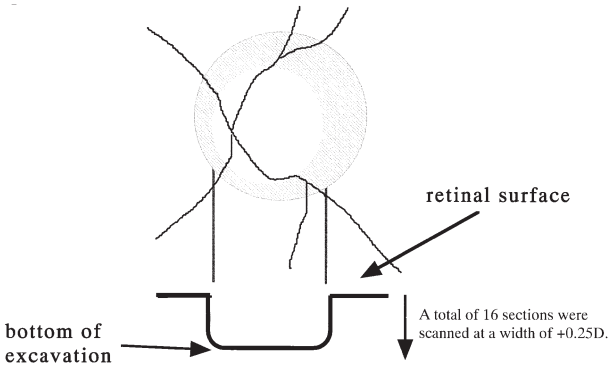

Figure 1. Optic scanning method. Total of 16 sections from retinal surface to cup bottom were automatically scanned at width of +0.25 diopter (D). Data on each slice were recorded by videorecorder and digital videotape.

was advanced by +0.3 diopter (D) steps. All 16 sections were automatically scanned from the retinal surface to the bottom of the cup using the confocal laser with a width of +0.25 D (Figure 1). The image of each section was recorded by videorecorder and digital videotape.

Image Processing

The original image of each slice was displayed on a monitor of a Macintosh computer with 256 × 256 pixels (Figure 2a). To reduce noise, the images were averaged using a median filter and image analysis software (v. 161, NIH Image) (Figure 2b). Subsequently, the averaged image for each slice was added and the original images were subtracted from this averaged image. Minute changes in the pores were enhanced by high-pass processing (Figure 2c). The threshold was determined as the level where the outline of the pores became clearest on the computer, and binarization of the processed images was performed (Figure 2d). The pore outline was traced on the computer, noise was eliminated, and images of the laminar pores were obtained (Figure 2e). The images obtained in each step of this process are shown in Figure 2, and the measurement system is schematically shown in Figure 3.

Experiments on the Model Eye

To determine whether the morphology of the laminar pores can be visualized accurately by this

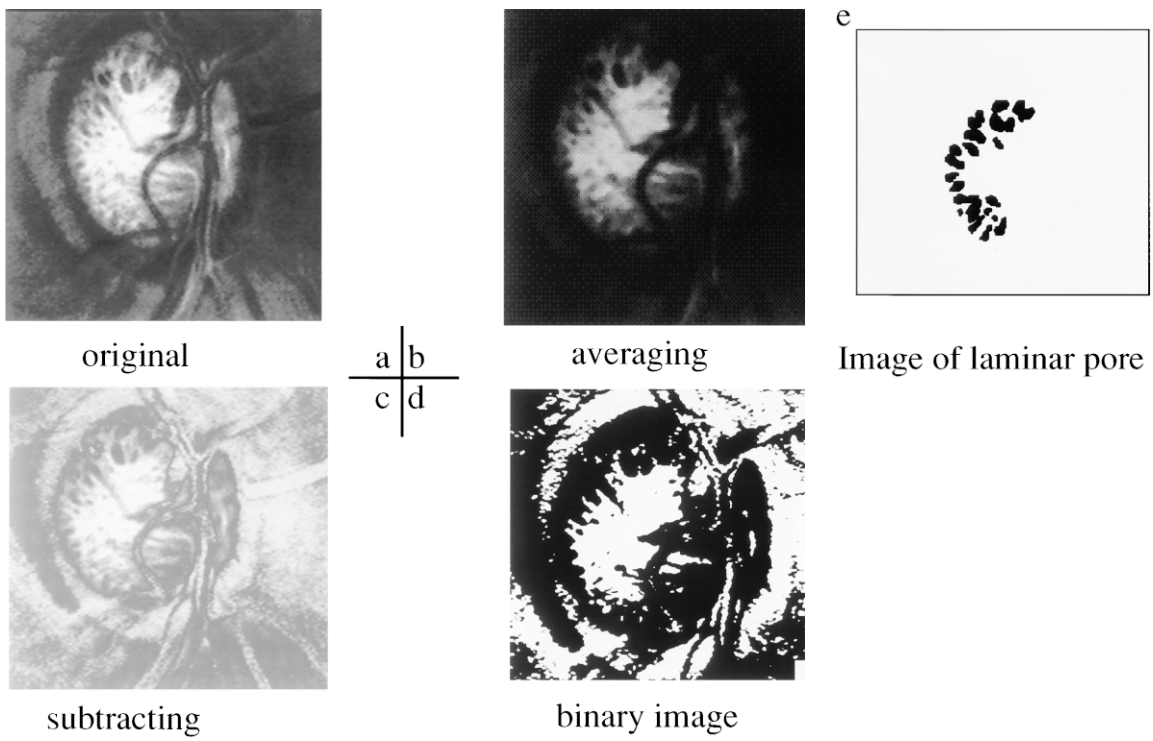

Figure 2. Steps in image processing. (**a**) Original image obtained using scanning laser ophthalmoscope (original image). (**b**) Image obtained by averaging (a) (averaged image). (**c**) Image obtained by subtraction of original image from image obtained by addition of (b) (subtracted image). (**d**) Binarization of image after above processing (binary image). (**e**) Image obtained by elimination of noise and tracing of pore outline (image of laminar pore).

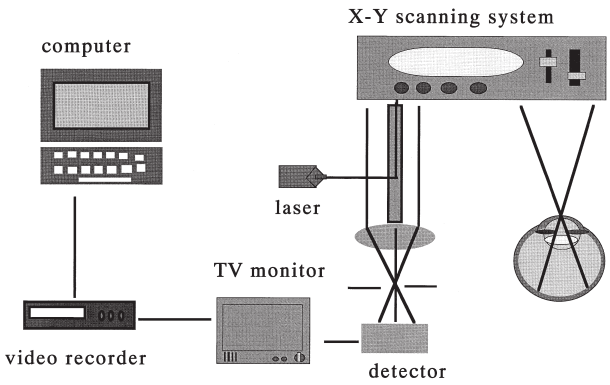

Figure 3. Schema of measurement system.

method, a model eye was used. Two holes (diameter = 3 mm) were made on opposite sides of a ping-pong ball (diameter = 30 mm). A 3-mm-diameter piece of surgical sponge for hemostasis (Spongel) was used to simulate the lamina cribrosa of the optic nerve. Its surface was colored with fluorescein fluorochrome. The sponge was then irradiated with an argon laser with a spot size of 50 μm (4 × 4 rows at equal intervals) at an output of 200 mW for 1.0 second to create circular pores by coagulation-shrinkage of the sponge. In this model eye, the optic disc was attached to the posterior area of the ping-pong ball. The morphology of the pores was then observed from the anterior hole of the ping-pong ball using an SLO, and images of the pores were obtained by the same method as described above.

Subjects

The subjects were 3 patients with large physiological cupping (PLC), 4 with primary open-angle glaucoma (POAG), and 1 with anterior ischemic optic neuropathy (AION), whose laminar pores could be observed by an SLO. Informed consent was obtained from all patients. The background of each patient is shown in Table 1. An eye with PLC was defined as one with a maximum intraocular pressure of more than 21 mm Hg accompanied by concentric enlargement of the cup. The cup/disc (C:D) ratio was 0.6 or greater; there were no optic nerve fiber layer defects or abnormal perimetric test points.

In all patients with POAG, the intraocular pressure was controlled at 18 mm Hg or lower by the use of a single drug. Patients with severe myopia (−6.00 D or more), peripapillary chorioretinal atrophy, and history of other optic nerve disease, trauma, surgery, or intracranial disease were excluded.

Evaluation of Reproducibility

Independently, two examiners performed image processing of the optic disc by the same method for each patient. The total number of pores and the pore/disc area ratio were determined by the two examiners and compared for reproducibility. The pore/disc area ratio was defined as the ratio of the total area of the pores to the area of the optic disc cup as measured on the computer.

Results

Evaluation of Validity and Reproducibility

The number of pores measured by the two examiners after image processing is shown in Figure 4A and the pore/disc area ratio, in Figure 4B. No significant difference was observed in the number of pores measured by the two examiners (Pearson's correlation coefficient $r = 0.92$, $R^2 = 0.85$). The correlation coefficient for the pore/disc ratio for the two examiners was 0.97, also indicating a high degree of reproducibility.

Table 1. Patient Backgrounds

Patient	Diagnosis	Refraction (D)	Visual Acuity	C/D Ratio	Pore Morphology
A	PLC	−2.5	1.0	0.6	Nearly round
B	PLC	E	1.0	0.8	Nearly round
C	PLC	−0.75	0.9	0.8	Round, nearly round
D	POAG	−1.25	1.0	0.7	Partially deformed
E	POAG	+0.5	1.0	0.8	Partially deformed
F	POAG	−3.5	0.8	0.9	Irregular in size, deformed
G	POAG	−1.5	1.0	0.7	Nearly round
H	AION	+1.25	0.4	0.8	Nearly round

D: diopter, C/D: cup/disc, PLC: physiological large cupping, POAG: primary open-angle glaucoma, AION: anterior ischemic optic neuropathy.

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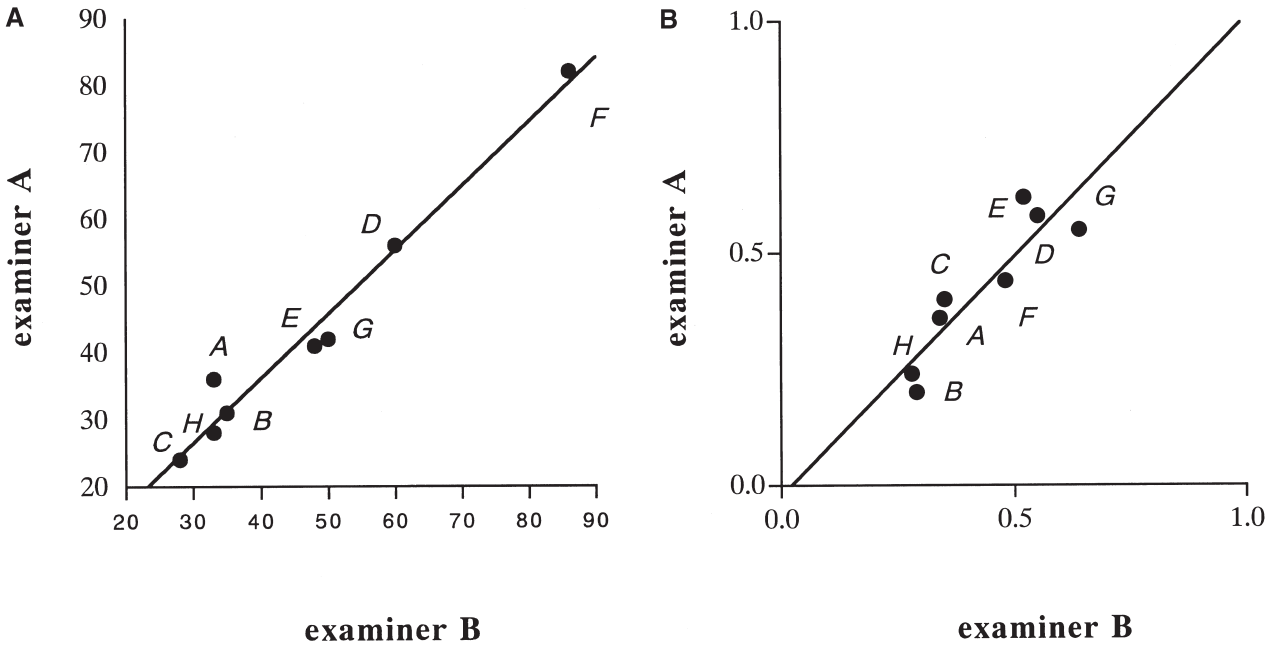

Figure 4. Inter-examiner reproducibility of measurement results. (**A**) Number of pores measured by two image processors; y = 0.92x + 3.31. (**B**) Ratio of total pore area to disc area; y = 0.97x + 0.02. Number of pores did not differ significantly for two examiners. High correlation was also observed in pore/disc ratio.

Results of Image Analysis

Evaluation in a model eye. A diagram of the model eye used to evaluate the procedures is shown in Figure 5a, and a photomicrograph of the model lamina cribrosa is shown in Figure 5b. The original image obtained using an SLO is shown in Figure 5c, and the processed image of laminar pores obtained by tracing the pore outline by our method (image of laminar pores) is shown in Figure 5d. The morphology of the pores obtained after image processing was simi-

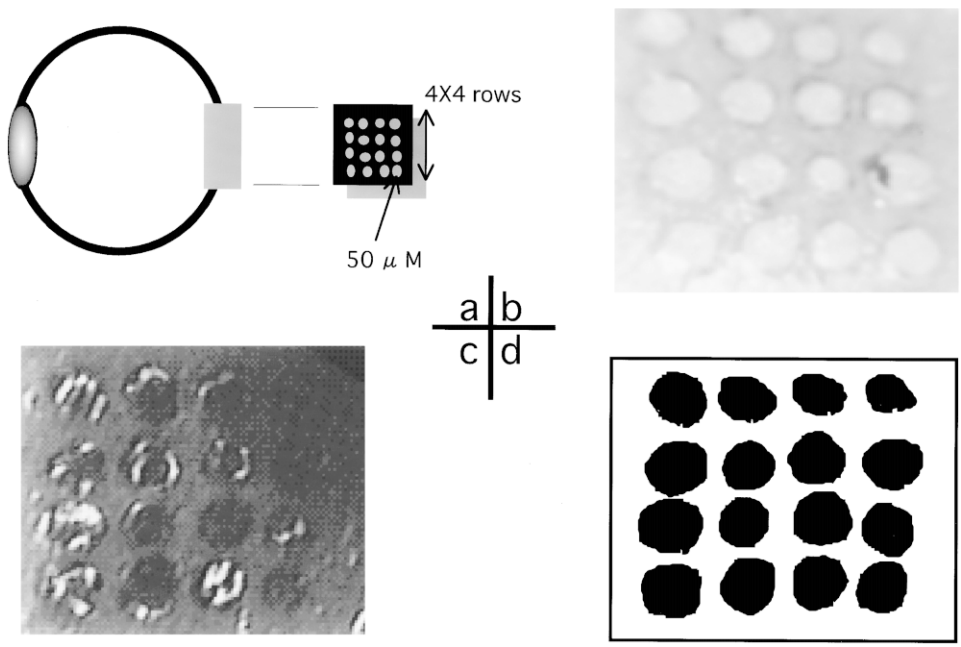

Figure 5. Results using this method in model eye. (**a**) Schema of model eye and simulated lamina cribrosa. (**b**) Simulated lamina cribrosa under microscope. (**c**) Original image obtained using scanning laser ophthalmoscope. (**d**) Images of laminar pores obtained by tracing pore outline by our method (images of laminar pores).

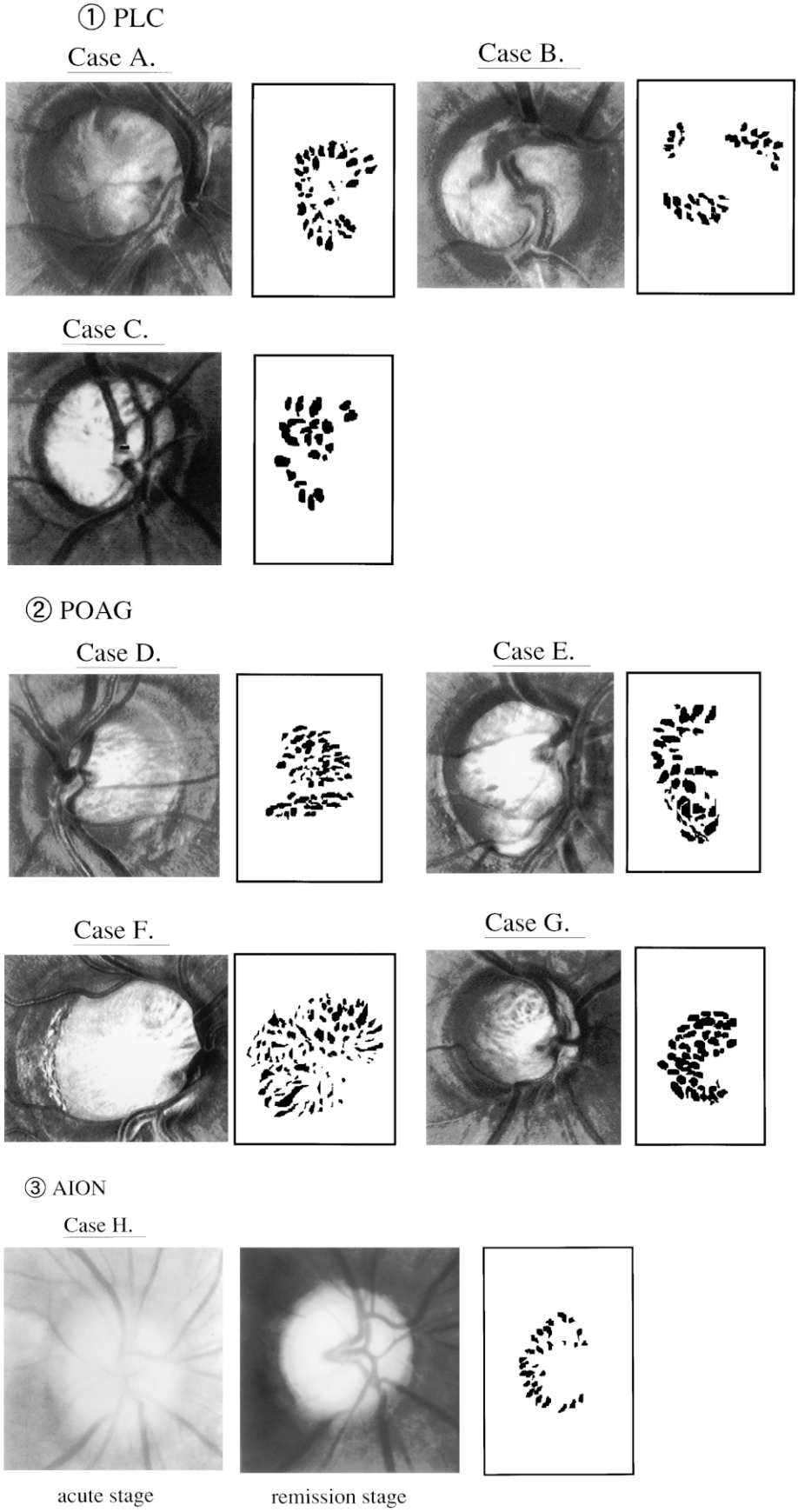

Figure 6. Original image and images of laminar pores in 8 patients (**A–H**) denote subjects in Table 1.

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lar to that on the micrograph, suggesting that the morphology of the pores can be clearly visualized by this method.

Evaluation of laminar pores in subjects. Figure 6 shows the images of the laminar pores in all 8 patients. Figures 6A–6C represent eyes with PLC; Figures 6D, 6E, and 6G, represent eyes with POAG. Case H shows large disc cup after AION. Among POAG patients, cases D and G represent early glaucoma (Aulhorn's Stage II) , case E represents intermediate (State III), and case F represents advanced (Stage V) glaucoma. In relation to the morphology of the pores, in some cases the pores were linear, boat- or star-shaped locally, whereas other cases showed round or oval configurations. In PLC, the size of the pores was nearly uniform, and many pores were round or nearly round; and a few pores were linear or compressed. In POAG, the size of pores varied, and the pores were locally or generally compressed into a linear or boat-shaped pattern in some patients. When various types of pores existed in one sample, we usually selected the laminar pores in the vicinity of the notch formation, nerve fiber layer defect, or vessel undermining.

Discussion

In glaucomatous optic neuropathy, a gap develops in the laminar structure of the lamina cribrosa at an early stage.[7] The strangulation of optic nerve fiber bundles in the lamina cribrosa causes impairment of axonal flow in the nerve fibers resulting in the atrophic disappearance of optic nerve fibers.[8] In other words, regardless of the cause of glaucoma, the atrophic cupping of the disc in glaucomatous eyes is due to the disappearance of nerve fibers and the glial tissue supporting these fibers. In addition to these changes, fibrous changes in the posterior area of the lamina cribrosa occur, and the resultant posterior shift and bending of the lamina cribrosa deepen the cup, exposing the lamina cribrosa.[9] This state is clinically referred to as the lamina dot sign.

Histologically, the pore diameter is 10–100 μm, and the pore number is 550–650 in normal eyes.[10] The pore diameter decreases toward the posterior region of the lamina cribrosa and is shorter in the central area than in the peripheral area.[11] However, in many normal eyes, the anterior region of the lamina cribrosa is present on the optic disc surface, and the laminar pores cannot be observed by ophthalmoscopy. Therefore, development of the positive dot sign requires a certain degree of cupping and exposure of the lamina cribrosa on the vitreous surface. The dot sign is positive not only in glaucoma but also in PLC, some cases of myopia, AION,[12] empty sella,[13] and some cases of intracranial disease.[14] However, the morphology of the pores may differ among the diseases. Therefore, determining the change in the morphology of the pores over time may provide some parameters for differentiating glaucoma from other diseases, and may contribute to studies on the mechanism of glaucoma.

Ophthalmoscopically, determining the tone of the optic disc and the degree of its cupping depend on the subjective judgment of the examiner, and this judgment varies even among experienced specialists.[12] In glaucomatous eyes, the depth of cupping varies, and its three-dimensional evaluation is difficult. In this study, we used an SLO in vivo and analyzed the differences in the morphology of the laminar pores in normal eyes and in eyes with various diseases. In PLC eyes with enlarged disc cupping, the pores were almost round and essentially without deformity. Therefore, we believe that the normal lamina cribrosa and regular pore arrangement are preserved in PLC eyes.

In glaucomatous eyes, pore deformation was observed in many cases at sites showing nerve fiber defects or notch formations. The local changes near the laminar cribrosa occur at the initial stage, resulting in atrophy of optic nerve fibers,[15] leading to gaps in the extracellular matrix and their supportive tissue, which is composed primarily of collagen fibers. The individual pores are asymmetrically compressed, or the round pores are compressed into a linear pattern, causing a distortion. Another possibility in glaucomatous eyes is that a slope develops in the cup resulting from atrophy of not only the extracellular matrix but also of the optic nerve fibers, and the pores are observed as if they were produced by measurement artifact. This is a major area to consider when the pathology of glaucoma is evaluated. Further studies using more cases are necessary.

Bhandari et al[16] succeeded in recording the laminar pores by a similar method using an SLO. However, their study did not evaluate the validity of the image processing method and did not include nonglaucomatous eyes. In the present study, the validity of this method was evaluated using a model eye. The results indicated that the morphology of the pores could be accurately visualized even though the size of the pores in the model was unclear.

The advantages of this method are that clear images from actual eyes can be obtained from serial photographs of the optic disc at different levels in small diopter steps within a very small confocal area (Cl) be-

cause of the confocal constitution of the SLO. This procedure was performed on all serial slices of the optic disc, and the three-dimensional structure of the pores could be visualized by a summation of the two-dimensional images. In addition, because the confocal laser was used, observation was possible with a low light level that was less than 1/1000 of the intensity of the indirect ophthalmoscope. The depth of focus of the SLO is very broad, and clear images can be obtained without mydriasis. This method fulfills the criteria for observation of three-dimensional disc images.

The problems with this method are that in normal eyes, the morphology of the pores cannot be directly observed with an ophthalmoscope because the anterior lamina cribrosa area is present. Second, in the normal eye without deep disc cupping, the morphology of the pores cannot be observed by this apparatus. Third, image processing requires some time. Fourth, the inter-examiner reproducibility has not been established (other than in this study). Fifth, it is still unclear whether the morphology of the pores located on the slope of the cup is accurately visualized by this method, in which observations are performed in a vertical direction. And sixth, the size of the pores cannot be measured accurately. We must consider how to overcome these problems.

The results of this study suggested differences in pore morphology in different diseases and according to the stage of a disease. Further investigation employing many cases and the recording of serial changes will provide useful data for the clarification of the pathogenesis of glaucoma.

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