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Nerve fiber layer photography

A comparative study

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Abstract. Observation of the retinal nerve fiber layer with red-free light is an important means of evaluating optic nerve integrity. Many investigators have attempted to increase the usefulness of the technique by improving photographic procedures. Most reports comparing various parameters neither indicate the method of comparison nor give quantitative measure of the improvement gained with different films, filters, cameras, or processing procedures. We have compared in a masked fashion the parameters most recently recommended in the literature. Twelve trained observers compared 12 masked pairs of nerve fiber layer photos taken from the same eye in the same manner on two cameras and preferred those taken with a Canon CF-60Z camera 69% of the time and those taken with a Zeiss FF-111 17% of the time (this difference was statistically significant, $P < 0.01$). Fourteen observers compared 14 pairs of photographs and judged Panatomic-X film to be superior to Plus-X film 51% of the time and both films to be of the same quality 25% of the time (this difference was also significant $P < 0.05$). Fifteen observers compared 12 pairs and preferred the Standard SE-40 blue filter 2:1 over the green Spectrotech 540 filter. This difference was not statistically significant, and the preference was found to be dependent on fundus pigmentation and clarity of the media. The blue filter gave better contrast for the nerve fiber layer in patients with lightly pigmented fundi, and use of the green filter resulted in less light scatter in patients with ocular media opacities.

Key words: nerve fiber layer – glaucoma – fundus photography – optic neuropathy.

Red-free light ophthalmoscopy of the nerve fiber layer was reported by Vogt (1913), but it was not until 1965 that photography of the nerve fiber layer was first introduced by Behrendt & Wilson (1965). They used a Zeiss camera and reported increased visibility of the nerve fiber layer with shorter wavelength light. Similar improvement of nerve fiber layer visibility with green illumination was noted by Mizuno et al. (1968).

The fine, striated pattern of the nerve fiber layer is difficult to observe and photograph because it is essentially transparent. Many attempts have been made to improve photographic techniques since nerve fiber layer analysis gained popularity in the early 1970s following a series of papers by Hoyt et al. (1972, 1973). Initially, a Zeiss camera with a 2X converter, Plus-X film, and green (Wratten 58) filter were used, (Hoyt et al. 1972, 1973), and although results were reported to be satisfactory, many other investigators have continued to search for improved photographic techniques.

The best wavelength for illuminating the nerve fiber layer was studied first. Miller & George (1978) preferred the Spectrotech 540 narrowband filter. Gragoudas et al. (1976) found 505 nm to be the optimal wavelength for nerve fiber layer observation and photography; however, they noted that the nerve fiber layer is visible over a wide range of wavelengths (400–580 nm). Fede-

rico (1978) compared 13 filters, 5 films, and 5 developers found the Panatomic-X to be superior and recommended use of the Spectrotech 550-nm filter. No indication was given of how conclusions were derived or whether the differences between parameters were significant.

In an elaborate study, Frisèn (1980) tested three colour films and four black and white films. Film performance was evaluated by measuring high- and low-contrast resolution of optical targets using all of the films. Resolving power was significantly reduced for all films compared to manufacturers' specifications, indicating that the limiting factor in fundus photography resolution lay more with camera optics than with film capabilities. In the same study, Frisèn also evaluated fundus photographs taken with the various combinations of parameters; to date, this is the only nerve fiber layer photography study that has reported its method of evaluation (Frisèn 1980). Image quality was judged subjectively by sequentially ranking series of images until two sequential rankings produced the same result. The Kodachrome colour films used in this test were found to be superior. Improved definition with red-free illumination was more significant with Technical Pan film than with other black and white films.

A comparison of the Zeiss camera with a 30° field and the Canon CF-60Z camera was conducted in 1982 (Airaksinen et al. 1982). Although only three photographs were used, the 60° field on the Canon was reported to be superior to the smaller field on the Zeiss. An important benefit of the Canon's larger field was an increase in the depth of focus.

A new film and filter combination was suggested by Sommer et al. (1983). Thirty-six subjects were photographed with four combinations of films and filters, including Spectrotech 540-nm and Ditric short-pass 560-nm filters and Plus-X and Technical Pan films. The Zeiss camera with a 1.6X converter was used. No details were given on the method used to evaluate and compare photographs, but the authors did report that the short-pass blue filter required more light output from the flash. With additional light the Technical Pan film results were much superior to those of the Plus-X film, especially for photographing eyes with clear media.

A number of other recent publications have recommended other combinations of films and filters without specifying a method of comparison and relative benefit. Roloff (1984) recommended use of the Technical Pan film with the narrow-band green filter. Mawson & Nieminen (1985) recommended use of the Canon camera with Panatomic-X film. Airaksinen & Nieminen (1985) described the same photographic technique with the Canon CF-60Z camera and Panatomic-X film and recommended that additional high-magnification stereophotographs of peripapillary area should be used; the use of stereo prints had been recommended previously by Manor et al. (1981).

The variability in recommended photographic techniques and the lack of information regarding the relative benefit of using one film, filter, or camera instead of another has compelled us to compare a number of parameters in a masked fashion using a number of observers in order to obtain a measure of the relative benefit.

Complete comparison of all parameters would be lengthy and expensive. Therefore, we employed a successive exclusion protocol, comparing first the cameras, then using the camera that was found to be the better choice to compare the films, and using the selected camera and film to compare the filters. We chose to compare the Zeiss FF-111 and the Canon CF-60Z cameras because they were available to us and because they have been consistently used in most photographic studies to date (Hoyt et al. 1973; Airaksinen et al. 1982, 1984; Sommer et al. 1983, 1984). The combination of Plus-X film and Spectrotech 540 filter was used in the camera comparison because this was the method of choice for most initial reports on nerve fiber layer photography through the 1970s (Hoyt et al. 1972; Miller & George 1978); this was also the technique previously used in our photography service (Peli et al. 1986). Plus-X film has been compared to Panatomic-X film; the latter has been recommended in recent years because of its tighter grain and better resolution (Frisèn 1980; Airaksinen et al. 1982). The SE-40 filter, which is used for fluorescein angiography, is a blue filter centered around 495 nm (Airaksinen et al. 1982). This was compared to the Spectrotech 540, which is a green, narrow-band filter centered around 540 nm (Miller & George 1978; Federico 1978).

Material and Methods

Twenty-five patients with various optic nerve diseases (including glaucoma, optic neuritis, and

compressive optic neuropathy) and 20 healthy, young adult paid volunteers participated in the study. The purpose of the study was explained to all participants and informed consent was obtained. All patients were referred by their physicians on the glaucoma and neuroophthalmology services of the New England Medical Center; volunteers were recruited from the hospital staff by advertisement in the local newsletter. All volunteers underwent complete eye examination including ophthalmoscopic evaluation of the nerve fiber layer with pupils dilated to at least 6 mm. Octopus visual fields were recorded for patients and confrontation fields for normal volunteers. The same photographer took all clinical photographs, and a different photographer processed the images for the study. Observers included clinicians and trained researchers experienced in evaluating the retinal nerve fiber layer by ophthalmoscopy and from photographs.

Comparison of cameras

A Canon CF-60Z camera was compared to a Zeiss FF-111. To achieve equal magnification with both cameras the temporal upper quadrant of the right eye of one subject was photographed with the Zeiss camera. Similar photographs were taken immediately thereafter with the Canon camera while changing the zoom in small, marked increments from 25° to 35°. Black and white enlargements were made of each frame and compared to find the zoom setting that matched magnifications from the two cameras. Magnification was evaluated by comparison of retinal topography. One additional volunteer was photographed to verify the accuracy of the match.

All subjects were photographed first with the Zeiss camera, and 4 or 5 frames were taken of the temporal quadrant in one eye. The same quadrant was then photographed with the Canon using the zoom setting that matched the magnifications of both cameras. Kodak Plus-X film rated at ASA 125 was used. The film was developed in D-11, 1:1 at 68°F for 6 min. Both cameras were fitted with Spectrotech 540-nm green filters. Flash output was adjusted to match the negative densities for each camera: 1 on the Zeiss FF-111 and 7 on the Canon CF-60Z. Using the sharpest negatives taken with each camera, a final set of paired prints were made for masked comparison under the following conditions: the printing easel was set to produce a rectangular print taken from the center of the circular fundus image; any edge marks, halos, or code numbers that could identify the camera were cropped; each pair of prints was composed to place the disc and arcuate nerve fiber bundles in the same position within the rectangle (Fig. 1); the contrast was set at grade 5 using a colour head filtration source; and images were printed on Ilford RC multigrade paper. Slight variations (± ½ grade) in filtration were used to match print luminosity visually to reduce bias from slight contrast differences.

Seventeen mounted pairs of photographs labelled A and B were presented to 12 observers to be scored. The set of 17 pairs included 12 test comparisons of the Zeiss and Canon cameras and 5 control pairs. Two control pairs were composed of two identical prints from the same negative. A third pair consisted of two prints from the same negative in which the mask of one was rotated compared to the mask of the other to change the orientation of landmarks. A fourth set was made from one negative, but one of the two prints had more contrast (+1 grade). The fifth control pair had a Canon photo that was slightly out of focus and a clear Zeiss photo. Observers were required to decide which of the pairs was better or if they were equal in the quality of the nerve fiber layer presentation.

Comparison of films

We compared Plus-X film to Panatomic-X film, using the Canon camera in all cases. Four or five images were obtained from one temporal retinal quadrant using each of the films. A green Spectrotech 540-nm filter was used, and the photographer used a slit-prism focusing aid for all photographs. The flash settings were 7 for the Plus-X and 7+8 for the Panatomic-X exposures.

The Plus-X was developed in Kodak D 11 1:1 for 6 min at 68° F. The Pan-X film was developed in Kodak HC-110 dilution A for 12 min at 68° F. The sharpest pair of negatives selected for each comparison pair was printed on paper and mounted in a random order of test pairs marked A and B (Fig. 2). Fourteen observers scored 14 plates and selected the better representation of the nerve fiber layer or indicated if the images seemed to be of equal quality.

Comparison of filters

An SE-40 fluorescein exciter filter was compared to a Spectrotech 540-nm filter. Paired exposures

Fig. 1.
Image preparation for comparison of cameras plates. The photographs taken with the Zeiss (top) and the Canon (bottom) were masked as indicated by the white lines to remove identifying marks (such as the vignetting in the Zeiss) as compared with the sharp image to the edge on the Canon, code numbers, etc.

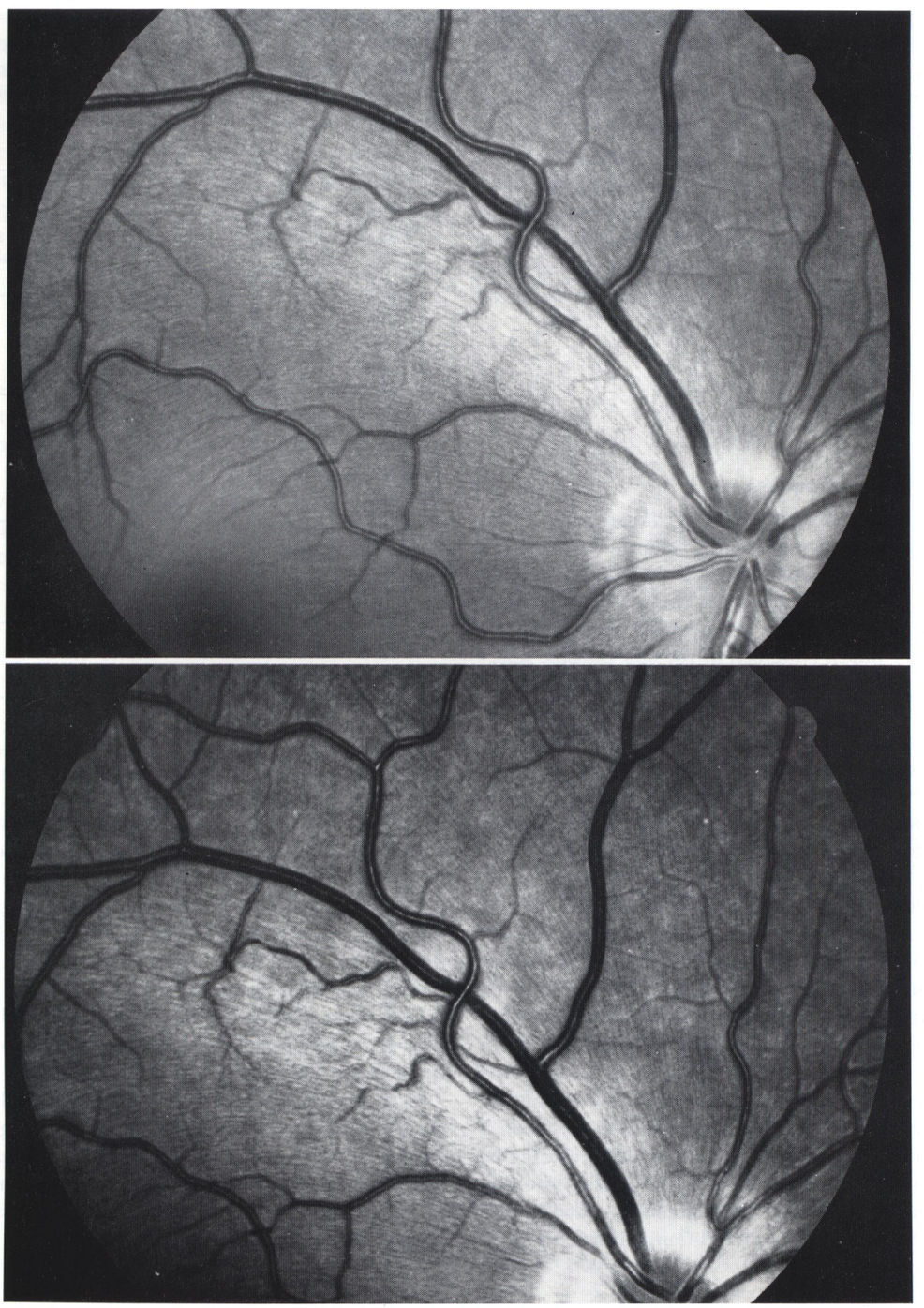

Fig. 2.
Example of a plate used in the film comparison testing. The Pan-X film (top) was compared with the Plus-X film (bottom). Note the finer grain of the Pan-X film.

using the two filters were obtained from 12 subjects, using the Canon camera with a 30° setting for 10 subjects and a 60° setting for two subjects and Panatomic-X film for all photographs. Visualization of the nerve fiber layer during photography was significantly more difficult with the darker blue filter (SE-40), and a flash power setting of 7+8 was required; a flash setting of 8 was used with the green filter. Development of film and the printing process were the same as described above. Twelve prints were reviewed by 15 observers.

Results

Comparison of cameras

The results of the 144 test comparisons of the cameras are presented in Table 1. Overall, the photographs obtained with the Canon camera were selected as better 111 times as compared to those taken with the Zeiss, which were preferred only 14 times. Nineteen of the 144 possible selections were judged to be equal. For all test pairs the Canon photographs were selected by more observers than the Zeiss images; this difference was statistically significant ($P < 0.01$, $t = 3.48$, df = 11).

Table 1.
The number of observers who preferred the photograph taken with the Zeiss FF-111 compared with the number who preferred the Canon CF-60Z, or who judged the photographs to be of equal in quality of the nerve fiber layer rendition.

Plate No.	Comparison of fundus cameras		
	Canon Better	Zeiss Better	Equal
2	12	0	0
4	12	0	0
5	10	1	1
6	8	3	1
7	12	0	0
8	11	0	1
10	6	3	3
11	8	1	3
12	7	3	2
13	10	0	2
16	10	1	1
17	5	2	5
Total	111	14	19
%	77	10	13

The results of the control panels indicate good reliability of the observations. The two equal pairs were selected as equal 7 and 8 times, respectively. Selection A was preferred by 5 observers of one pair, and selection B was chosen by 4 observers of the other pair. The pair with equal but rotated frames was found to be equal by 7 observers. Frame A was selected by two observers, and frame B was chosen by 3 observers. In plate 9, wherein the two frames were obtained from the same negative but with plate B printed to have higher contrast, 4 observers scored that pair as equal, 1 observer selected A, and 6 observers preferred B, indicating a preference for the higher contrast image. The clearer Zeiss image in plate 14 was preferred over the blurred Canon image by all 12 observers.

Comparison of films

The results of the film comparison are recorded in Table 2. Panatomic-X film was felt to produce the better image in 100 selections. Plus-X film was selected as better 50 times, and the films were found to be equal in 46 selections. Statistical analysis of the results, illustrated in Table 2, indicates that the difference is significant ($P < 0.05$, $t = 2.21$, df = 13), and evaluation of Table 2 indicates that the selection in this case was plate-dependent. For some pairs the Panatomic-X photo was chosen more consistently, and for others more observers consistently preferred the Plus-X frame; many more equal selections were made by the observers in this case.

Even with the maximum flash output available on the Canon, it was difficult to obtain good density with the Panatomic-X film. The reduced light sensitivity required a more careful alignment of the fundus camera illumination to assure proper exposure for this slower film.

Comparison of filters

The blue SE-40 filter was preferred over the Spectrotech 540 green filter 64% vs 28% of the time (Table 3), and the filters were felt to be equal 8% of the time. The difference was not statistically significant ($t = 1.74$, df = 11). In reviewing

responses to the individual plates, it was found that the blue filter eliminated the choroidal background very well in lightly pigmented eyes (Fig. 3), thus enabling better observation of the nerve fiber layer in these eyes. On the other hand, light scatter from opacities in the ocular media was less when the green filter was used, resulting in sharper images with this filter for darkly pigmented eyes.

Discussion

Frisèn's (1980) finding that the camera is the limiting factor in the resolution of fundus photography suggests that the proper camera should be the first consideration in difficult tasks such as nerve fiber layer photography. The results of this masked study indicate that the Canon camera is superior to the Zeiss camera. Our findings are in agreement with those of Airaksinen et al. (1982) who also found the Canon camera to be superior to the Zeiss camera, although it was chosen mostly because of its larger 60° field and increased depth of focus. Others (Newman et al. 1982) have suggested that proper focusing of the camera is most important in obtaining good nerve fiber layer images. This is supported by our finding that a sharp Zeiss image was preferred universally over a slightly blurred Canon image; the split-prism focusing aid device in the Canon is of great importance in this regard.

We found Panatomic-X film to be superior to Plus-X film. Federico 1978; Airaksinen & Nieminen 1985; Mawson 1985) have also recommended this film, while other investigators (Frisèn 1980; Sommer et al. 1983; Roloff 1984) have recommended Technical Pan film. Both Panatomic-X and Technical Pan films must be used at their slower ASA rating if they are to retain their higher resolving properties. This requires stronger flash output and requires greater pupillary dilation. We have not used Technical Pan film because we cannot obtain satisfactory exposures with the flash unit of the Canon camera.

Table 2.
The number of observers who preferred the photograph taken with the Panatomic-X film compared with the number who preferred the Plus X-film or judged the photographs to be equal in quality of the nerve fiber layer rendition.

Plate No.	Comparison of films		
	Plus-X Better	Panatomic-X Better	Equal
1	2	12	0
2	0	12	2
3	5	4	5
4	2	2	10
5	8	3	3
6	8	4	2
7	5	3	6
8	2	11	1
9	4	5	5
10	0	13	1
11	0	9	5
12	5	7	2
13	3	8	3
14	6	7	1
Total	50	100	46
%	26	51	23

Table 3.
The number of observers who preferred the photograph taken with the SE-40 blue filter compared with the number who preferred the Spectrotech 540 green filter.

Plate No.	Comparison of filters		
	Blue filter Better	Green filter Better	Equal
1	2	10	3
2	15	0	0
3	15	0	0
4	14	1	0
5	13	1	1
6	5	8	2
7	2	11	2
8	3	6	6
9	13	2	0
10	2	12	1
11	15	0	0
12	15	0	0
Total	114	51	15
%	64	28	8

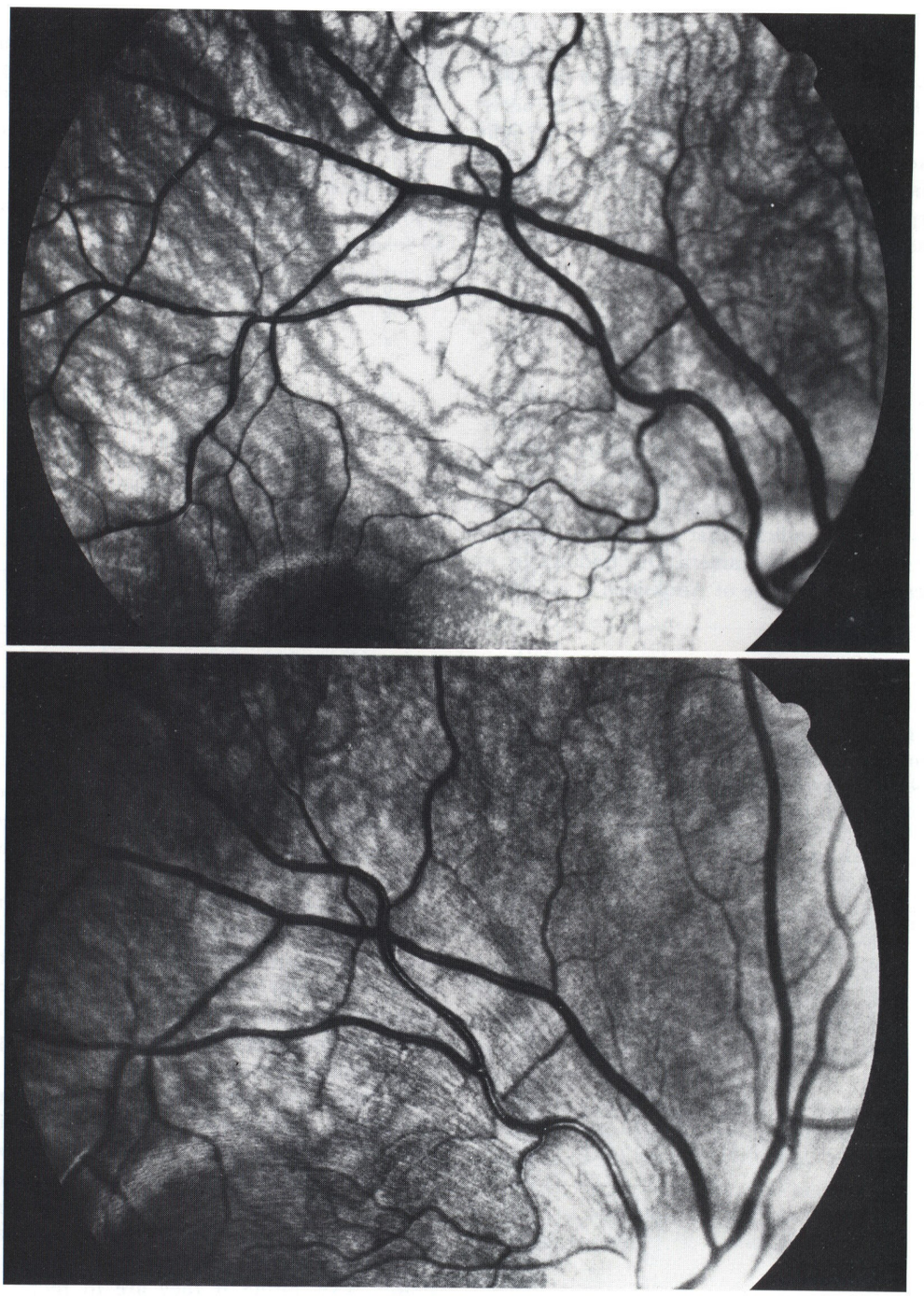

Fig. 3.
Comparison of filter plate. The image taken with the Spectrotech 540 green filter (top) clearly demonstrates the visibility of choroidal vasculature in this lightly pigmented subject. The darker appearance of the pigment epithelium with the SE-40 blue filter significantly improves the visibility of the nerve fiber layer in this case.

In Frisèn's study, (1980) Panatomic-X film was not tested at all with fundus photographs because of its low speed. Technical Pan film was superior at a 1.7X magnification but was not fast enough for higher magnifications. As a result of this study, Frisèn recommended 'optimal procedure' for nerve fiber layer photography using contact copying on Technical Pan of colour slides illuminated with green light. To our knowledge, this technique has not been duplicated.

The blue fluorescein exciter filter is only slightly better than the green Spectrotech 540 filter for overall use, according to our results. The blue filter is much better for photographing eyes with lightly pigmented fundi as long as the media are clear. Hazy media, from cataract for example, causes more scatter of blue light than green. Nerve fiber layer details are clearer in eyes with cataract if green light is used. Airaksinen (1982) found the blue SE-40 filter superior to the green filter, perhaps because his patients usually were very lightly pigmented. Others also reported that light scatter from cataracts reduced image quality more with shorter wavelength illumination than with 540 green illumination (Gragoudas et al. 1976; Sommer et al. 1984).

In addition to camera, film, and filter manipulation, other techniques have been tested. Photography with cross polarization of various degrees and various orientations was reported by Sommer et al. (1984). They compared different orientation of polarizers for enhancement of arcuate bundle visibility. Although some benefit was noted, the loss of light and the difficulty involved in controlling the two filters and the need to obtain numerous photographs of the same area render this technique somewhat impractical. Frisèn & Hoyt (1973) have applied the unsharp masking technique, which is a photographic image enhancement technique that can sharpen the image and increase the visibility of the nerve fibre layer. Peli et al. (1986) have used computerized image enhancement to improve nerve fiber layer detail and contrast.

We plan to use the following procedures for photographing the retinal nerve fiber layer in patients with a variety of optic nerve disorders: the Canon camera will be used with a 30° as well as 60° zoom and Panatomic-X film. The green Spectrotech 540 filter will be employed in darkly pigmented eyes or in those with media turbidity, and the SE-40 blue fluorescein exciter filter will be employed in all lightly pigmented eyes. We plan also to use the same technique in our continuing studies of computerized image analysis of the retinal nerve fiber layer.

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