Clinical Investigation of a True Color Scanning Laser Ophthalmoscope

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**Objective:** To show true color scanning laser ophthalmoscope (SLO) images produced by simultaneously imaging the retina with red, green, and blue lasers.

**Methods:** Low-power red, green, and blue lasers were combined using fiber optics. By rapidly pulsing the lasers, each point on the fundus is illuminated by the 3 colors in quick succession, with the total power level being similar to that from a single laser. The reflected light is then decoded to extract the red, green, and blue color information and the true color fundus image is displayed live on a computer monitor.

**Results:** Comparison was made between the color SLO images from 5 patients and their digitized fundus photographs. The background fundus and retinal vasculature showed a similar appearance. The SLO gave better quality information in patients with ocular histoplasmosis, macular dystrophy, and optic disc drusen. By operating the color SLO in the indirect mode, macular edema could be clearly seen as lines and ridges surrounding the fovea.

**Conclusion:** The color SLO offers all the advantages of the present commercially available monochromatic device, with the added advantage of true color representation of the fundus without increasing either imaging time or the level of exposure.

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**FUNDUS IMAGING**

Color fundus photography remains the “gold standard” for the documentation of fundus pathological conditions and routinely is used by ophthalmologists to record the physiological condition of the eye. Commercial fundus cameras, which have been used regularly since the early 1930s, have undergone dramatic changes in the last 60 years. Although most eye clinics record the fundus pictures on photographic film, recently color CCD cameras interfaced to fundus cameras have made it possible to digitally capture and store the pictures on a computer.1

**SCANNING LASER OPHTHALMOSCOPE**

Scanning laser ophthalmoscopy, first reported by Webb and colleagues,2 uses a narrow 1-mm laser beam to scan the fundus. The beam is focused by the eye's optics to a spot of 10 to 15 µm on the retina. The reflected light is detected and synchronously decoded. Commercial scanning laser ophthalmoscopes (SLOs) provide the output in the standard television format, enabling the fundus image to be viewed live on a television monitor and it can then be recorded on a standard video recorder. The output of the SLO can also be fed to a frame grabber that digitizes the analog image so that it can be stored on a computer, enabling, for example, further image processing to quantify any pathological information. Any laser in the visible or near infrared spectrum may be used. As lasers are monochromatic, the resulting image is represented by a monochrome black-and-white image.

We have previously reported a novel method of producing color SLO images using a custom-built instrument,3 in which patients’ eyes were imaged using low-power blue, green, and red lasers in sequence. These images were then corrected for eye movement and combined to form a color image. Although this method gave true color pictures, it could not produce live pictures of the fundus because each monochrome image was produced sequentially. It also required image-processing techniques to correct for the eye movement between image frames. Herein, we describe a technique that produces live pictures of the fundus in full color.
RESULTS

Scanning laser ophthalmoscopic images of 1 healthy volunteer and 5 patients with a variety of fundus lesions are presented. Digitized fundus camera color photographs and/or digitally acquired red-free images are shown for comparison.

The normal fundus of a healthy 33-year-old male volunteer is shown in Figure 1. In general, the background fundus and retinal vasculature are similar for the 2 imaging modalities. The optic disc in the color SLO image is dark with a well-defined edge and a bright central optic cup. This is at variance with the uniformly bright appearance of the optic disc on the digitized color fundus camera image.

Patient A is a 33-year-old woman with presumed ocular histoplasmosis and choroidal neovascularization. After an initially favorable response to immunosuppression, she underwent vitrectomy and subretinal membrane removal for recurrent choroidal neovascularization. Figure 2A-B shows color SLO pictures of the patient’s right posterior pole after surgery. Figure 2A represents the superficial retina, whereas Figure 2B focuses on deeper retinal layers and retinal pigment epithelium (RPE). Figure 2C shows a digitized color fundus photograph for comparison. It appears that by using the confocal optics it is possible to highlight the detail in the precise plane of interest. Figure 2B shows highly accurate and sharp RPE detail and provides more information about the level of RPE change than the color photograph, particularly superior to the hypertrophic scar.

SUBJECTS AND METHODS

Low-power blue (argon, 488 nm), green (diode pumped YAG, 532 nm), and red (semiconductor diode, 670 nm) lasers are combined using a fiber optic system to produce a single beam. Each laser is modulated to produce an ultrashort pulse with every point scanned on the retina exposed to the 3 lasers sequentially and each laser switching on for 22 nanoseconds. This process is repeated for 768 points on each image line with a total of 576 lines, giving the complete image of the retina. Thus, the total time per image is 40 milliseconds. As the lasers are switched sequentially, the total fundus exposure is 120 µW, which is the same as that from an individual laser. A single avalanche photodiode detects the reflected light and synchronously decodes the color information. The 3 color outputs are then fed to 3 channels of a color frame grabber that combines them to produce a live color image on the computer monitor. Images can be captured at a rate of 25 images per second on a computer or recorded onto videotape.

Patients and volunteers for color SLO imaging were selected from the Eye Outpatient Department, Aberdeen Royal Infirmary, Aberdeen, Scotland. All subjects gave informed consent and the study was approved by the local hospital ethics committee. Digital 24-bit color images were captured corresponding to an area of 24° × 18° field of view. For comparison with the SLO color images, high-quality color Kodachrome 64 PKR 135-36P slides (Eastman Kodak Co, Rochester, NY) were taken on the same day using a fundus camera (Topcon TRC 501A; Topcon [GB] Ltd, Newbury, England). The fundus slides, corresponding to a 45° field of view, were later scanned (Coolscan LS2000; Nikon UK Ltd, London, England) to obtain high-resolution digital images. For accurate comparison, only the area corresponding to the color SLO image is shown. Pupillary dilation with 0.5% tropicamide and 2.5% phenylephrine hydrochloride was used for both photographic and SLO imaging.

Figure 1. The color scanning laser ophthalmoscopic image (A) and the digitized fundus photograph (B) of a healthy male volunteer. The optic disc appears darker in the scanning laser ophthalmoscopic image due to the narrow depth of focus obtained with an 800-µm confocal aperture. C, The red-free image created from a color image shows the nerve fiber layer brighter than the macula.
Patient B is a 42-year-old man with an asymmetrical pattern macular dystrophy that was labeled as adult Best disease. He has retained an acuity of 6/9 in the left eye while vision in his right eye has deteriorated to counting fingers. The color SLO images of both eyes are shown in Figure 3A-B. In both SLO images a high degree of fundus detail is seen and the pigimentary changes are particularly evident. Figure 3C-D shows digitized color photographs of the same patient taken with the fundus camera.

Patient C is a 30-year-old man with type 1 diabetes mellitus who underwent panretinal photocoagulation in both eyes for new vessels elsewhere, which have now regressed. Figure 4A is an SLO image of his left macula. The image was acquired in indirect mode and shows subtle cystoid macular edema as lines and ridges surrounding the fovea. This is a feature never seen with color fundus photography. The digitized fundus camera image is shown for comparison (Figure 4B).

Figure 5A-B shows the optic disc drusen of patient D who attended the eye clinic with peripheral loss of visual field. There are obvious differences from the fundus camera color digital images, shown in Figure 5C-D. The SLO image shows the optic disc at a much lower intensity than in the digitized fundus camera color image but without losing information. We presume this to be due to a reduction in reflection as the SLO focuses on a thin tissue layer. The drusen can be seen individually as high-intensity objects against a dark background in the SLO image, while the digitized fundus camera disc photographs look overexposed by comparison. The pigimentary pattern surrounding the disc is clear in the SLO image, as also is the disc margin.

Patient E is a 46-year-old man with type 2 diabetes mellitus with exudative maculopathy. Figure 6A shows an SLO image of his left macula, which can be compared with the digitized fundus camera image in Figure 6C. A red-free image can be instantly produced from the color SLO image by omitting the signal from the red laser in the final image output, the result being shown in Figure 6B. A digitally acquired red-free fundus camera image is shown in Figure 6D. The hard exudates are well defined in the red-free images, allowing easy localization of the exudates relative to fovea and vessels. Scanning laser ophthalmoscopic images are acquired and stored digitally and can therefore be easily manipulated to highlight detail.

COMMENT

We have described a new method for true color laser imaging of the fundus that gives digital images with comparable quality to silver-based fundus photographs. To date, color imaging of the fundus is restricted to flash photography with a fundus camera or digital-color imaging using a 3-color CCD camera with image capture linked to the fundus camera flash.

The Kodachrome 64 PKR 135-36P film used in this study is a professional nonsubstantive high-resolution slide film that can resolve the equivalent of up to 3500 × 2300 pixels (50 lines/mm). The disadvantage is that to maintain the high process quality control required, there is only 1 laboratory in the United Kingdom that can develop the film. This results in time-consuming transactions before the film is available for viewing. Although the flash is set to obtain optimum exposure of the film, variations of pathological conditions and degree of pigmentation within the RPE can lead to localized underexposed or overexposure and cause a loss of image detail in the developed film. Also, the gray levels of film are nonlinear with respect to exposure because of the inherent property of the photographic processing. Despite these disadvantages, color fundus photography remains the gold standard for recording the pathological conditions of the eye.

Fundus cameras have higher resolution and a larger field of view than most SLOs. A good fundus camera when
used to capture a 45° field of view will give a retinal resolution of 6 µm, whereas that of the SLO is between 10 and 15 µm. Scanning laser ophthalmoscopic images have higher contrast due to the SLO imaging process, which involves illuminating only 1 point on the retina at a time, resulting in less scattered light. The relatively small depth of field of the SLO also reduces light scattered by deeper tissue layers.

Image detail obtained with the SLO is equivalent to the digitized fundus camera color images, with the benefit of increased patient comfort owing to lower light levels. Vessels, exudates, and pigmentary markings are all well visualized and colors are as expected, although exudates look a little paler compared with the digitized fundus camera pictures. It can also be seen that the optic disc is darker compared with the fundus camera digitized image. This is mainly due to the use of a confocal aperture, which gives a depth resolution of approximately 600 µm. This results in light being collected only from a thin layer, improving detail but reducing intensity. In a structure such as the optic disc, its topography can be imaged further using the confocal optics.

We have shown that additional information can be obtained from SLO images by using confocal optics and indirect mode imaging. Scanning laser ophthalmoscopic images can also show considerably more detail at the RPE level than the fundus camera photographs.

Cystoid macular edema can cause moderate to severe visual loss as a complication of several ocular conditions, including uveitis and diabetic retinopathy. Cystoid macular edema can be identified by subjective
Figure 5. Scanning laser ophthalmoscopic images show drusen as discrete, well-defined yellowish-white objects on a dark background (A and B), whereas, in the digitized color fundus camera images (C and D), they are seen against a much brighter background.

Figure 6. A patient with exudative diabetic maculopathy showing the color scanning laser ophthalmoscopic image (A), color scanning laser ophthalmoscopic red-free image (B), digitized color fundus camera image (C), and digital red-free image (D). The hard exudates are well-defined in the red-free images.
evaluation of the fundus but fluorescein angiography may be required to confirm the diagnosis. In addition, this technique provides qualitative information about the severity of the edema but cannot be used to quantify cystoid macular edema. Various other diagnostic techniques to detect and quantify cystoid macular edema have been reported in the literature.8-10 The SLO in indirect mode can visualize macular edema, and it may prove a useful adjunct to our arsenal of diagnostic tools. The use of SLO to quantify cystoid macular edema has already been described.11 We are currently investigating the use of color SLO in detecting macular edema.

The appearance of the optic disc differs between the 2 modalities, but there is no lack of detail in the color SLO image. The reduced intensity can be useful for imaging pathological conditions of the optic nerve, such as drusen, which can occasionally be difficult to diagnose clinically, particularly when buried. Although ultrasound is the diagnostic technique of choice,12 we have previously shown that monochromatic SLO can easily diagnose buried drusen13; drusen appear even more clearly on the color SLO images.

Because the images are acquired and stored digitally as 3 separate color frames, there is significant potential for data manipulation. For example, a red-free image can be produced simply by subtracting the information obtained with the red laser from the final image. In the healthy volunteer, the red-free image, Figure 1C shows the retinal nerve fiber layer clearly. The fundus camera digital red-free image shown in Figure 6D is better than the SLO red-free image (Figure 6B). This is largely because the color SLO is a prototype where all the electronics, including the amplifiers, are built using “breadboards,” which introduces electronic “noise.” Use of custom-made printed circuit boards and optimized layout will reduce this noise.

Images are also ready for automated analysis, eg, for detection of the number of microaneurysms,14 which may be of great benefit in screening programs for diabetic retinopathy.

The drawbacks of SLOs are their small field of view, low resolution, and high equipment cost. Currently, monochrome SLOs are available at a cost of £60,000 to £70,000 with a choice of lasers. Our color facility has been designed to be readily integrated into a monochrome SLO. The development costs of the color unit were about £15,000 but will decrease significantly once semiconductor diode blue and green lasers are available.

In summary, the color SLO has added value over other digital imaging techniques as a diagnostic tool, with a capability to detect and the potential to quantify a wide range of fundus pathology.

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