A New Method for Noninvasive Optical Sectioning of the Chorioretinal Vasculature

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PURPOSE. To report a new method for optical sectioning of the chorioretinal vasculature to improve the visualization of vascular abnormalities due to chorioretinal eye diseases.

METHODS. An imaging system was developed for optical sectioning of the vasculature called chorioretinal optical sectioning (CROS). CROS consists of projecting a laser beam at an oblique angle on the retina after injection of a fluorescent dye and viewing the fluorescence. On the fluorescence optical section (FOS) image, the vasculature of the retina and choroid appear laterally displaced according to their depth location. The laser beam is scanned over a 2 × 2-mm area to generate 40 FOS images, each spatially separated by 50 μm on the retina. Optical section images of the vascular layers are constructed from the series of FOS images.

RESULTS. CROS permitted optical separation of vascular layers in living eyes. Optical section images of normal and laser-photocoagulated retinas had higher contrast than conventional angiography because of the separation of the fluorescence from the overlapping layers and allowed enhanced visualization of vascular abnormalities.

CONCLUSIONS. CROS enhances the visualization of the retinal and subretinal vasculature and promises to be a beneficial tool for evaluation of chorioretinal diseases. (Invest Ophthalmol Vis Sci. 1998;39:2733-2743)
appear laterally displaced on the FOS image according to their depth location. By scanning the laser beam in small horizontal steps across an area of the retina, FOS images from spatially adjacent locations are generated. Components that correspond to a given retinal depth are isolated from each of the spatially displaced FOS images and are placed next to each other consecutively. Thus, a two-dimensional optical section image of a vascular layer is constructed at a given retinal depth. For CROS, the depth resolution can be estimated by the laser width and the angle between the incident beam and the viewing axis. In a dilated pupil of 5 mm, the angle between the incident beam and the viewing axis can be adjusted to yield a depth resolution of 50 μm, using a visible laser beam. The depth resolution for ICG imaging is larger because of the longer wavelength of the infrared laser beam.

**Image Acquisition**

The system for CROS is based on the optics of a slit lamp biomicroscope (Carl Zeiss, Oberkochen, Germany) (Fig. 1). An argon laser (Coherent, Palo Alto, CA) operating at a wavelength of 488 nm and a diode laser (Power Technology, Little Rock, AR) operating at a wavelength of 790 nm are projected at an oblique angle on the retina. The wavelengths of the two lasers coincide with the peak absorption of sodium fluorescein and ICG, respectively. A combination of a spherical lens (f, 100 mm) and a cylindrical lens (f, 300 mm) focus the laser beam to a 2-mm vertical line of diffraction-limited width on the retina. An aerial image of the retina is created with a 60-diopter (D) precorneal lens (Volk Lens, Farmington Hills, MI) that eliminates the need for a contact lens during imaging. The optics of the slit lamp microscope transfer the image to a charge-coupled device (CCD) video camera (Texas Instruments, Dallas, TX, Fig. 1, CCD1). The images are recorded on a high-resolution video recorder (U-matic, Sony, Japan). The angle between the incident beam and viewing axis is adjusted optimally according to the pupil dilation. A mirror mounted on a galvanometer scanner allows scanning of the laser beam horizontally to image a 2 × 2-mm retinal area. The mirror is placed at the conjugate image plane of the iris to minimize beam movement on the cornea during scanning and to ensure uniformity of the scans. In a laser scan, a series of 40 FOS images, spatially separated by 50 μm on the retina, are acquired. A computer
FIGURE 2. The serial fluorescence optical section (FOS) images were digitized and stored in buffers of the imaging board. On the FOS image, the vasculature appeared laterally displaced according to their depth location. A dedicated computer software program generated an optical section image of a vascular layer from the series of FOS images. A slice was removed from each FOS image (black lines) and placed consecutively next to each other in the reconstruction image buffer. To construct an optical section image of a vascular layer, displaced in depth compared with the first layer, a slice laterally displaced from the first slice on the FOS image (gray lines) was removed.

equipped with an input-output board (Data Translation, Marlboro, MA) synchronizes the scan rate with the video camera to acquire FOS images at video rate (16.7 msec per image). A 2 × 2-mm retinal area is thereby imaged in 660 msec. A second CCD video camera (Watec, Kawasaki, Japan; Fig. 1, CCD2), attached to the slit lamp biomicroscope, displays a live image of the 60-D precorneal lens and the side view of the cornea on a video monitor. To ensure a constant magnification during imaging, the operator keeps constant the distance between the 60-D precorneal lens and the eye by viewing the image on a video monitor.

Optical Section Image Construction

The series of 40 FOS images generated in a single laser scan were digitized at video rate by an imaging board (Epix, Buffalo Grove, IL) and transferred to a high-capacity magneto-optical storage disc (Pinnacle Micro, Irvine, CA). For the analysis, the series of digitized FOS images were downloaded from the optical drive and temporarily stored in 40 image buffers of the imaging board. Each image buffer consisted of 1000 × 180 pixels. Optical section images of the vascular layers were constructed by extracting and combining slices from each of the series of FOS images. A dedicated computer software program removed a slice from every FOS image and placed the slices next to each other consecutively in the reconstruction image buffer to generate an optical section image of a vascular layer (Fig. 2). A box, 10 × 180 pixels in dimensions, was placed at the left edge of the first FOS image. The portion of the image within the box (slice) was extracted from the first FOS image and placed in the reconstruction image buffer. A similar slice was extracted from the second FOS image and placed in the reconstruction image buffer exactly to the right of the slice from the previous FOS image. This process was repeated for the remaining 38 FOS images to generate an optical section image of the first vascular layer. To generate an optical section image of the next vascular layer, the box was laterally displaced by 10 pixels from its initial position on the first FOS image, which corresponded to a slice from a deeper layer of the retina. The process of image reconstruction was then repeated to generate a series of optical section images of the choriotrinal vascular layers that were parallel to the surface of the retina and separated in depth. The optical section images were processed to remove the lines between the slices and create a uniform image. On the FOS image, the width of the
fluorescence generated by the incident laser was more than the width of the laser slit. To provide a continuous optical section image, the width of the slices extracted from the FOS images was matched with the 50-μm spatial separation between the FOS images. Because the FOS image represented an angled view of the retina, a slice width of 10 pixels on the FOS image corresponded to a depth of 100 μm. A composite image that displayed the serial FOS images (Fig. 3B) was created by placing in a blank image buffer 10 of the 40 FOS images, each spatially separated by 200 μm on the retina. All images were enhanced in contrast and rotated to present the same orientation as in the fluorescein angiograms.

Animals

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. In vivo optical section images of the vasculature were generated in baboon eyes. General anesthesia was administered with intubation and intravenous pentobarbital sodium (Nembutal; Abbott, North Chicago, IL) was provided during the procedure. The eye was dilated using 10% phenylephrine and 1% tropicamide. Local anesthetic drops were applied (0.5% proparacaine). During the experiment, the eye was fitted with a contact lens to prevent corneal dehydration. After injection of sodium fluorescein or ICG dyes at a dose of 7 mg/kg, an argon or diode laser was projected and scanned on the retina. The incident argon and diode laser powers were 760 μW and 900 μW, respectively. According to the American National Standard Institute (ANSI) safety standards, the maximum permissible exposure time is 0.7 seconds for the argon laser and 1.8 seconds for the diode laser, assuming the laser power is concentrated on a point. These values are well above the laser exposure time of 16.5 msec in CROS. In the same eyes, conventional fluorescein angiography was performed by a fundus photographer with the use of a 30° field fundus camera (Carl Zeiss) after the administration of the same dye dose. In one of the eyes, laser lesions were induced by applying an argon laser (power, 700 mW; spot, 100 μm; time, 100 msec) to create a model for subretinal neovascularization.

RESULTS

In Vivo Optical Sectioning of the Normal Vasculature

CROS using fluorescein was performed in a normal eye nasal to the fovea, indicated by the rectangle on the fluorescein angiogram (Fig. 5A). The composite image of FOS images showed a lateral displacement between the chorioretinal vasculature (Fig. 5B). Optical section images of the vasculature were constructed from a series of FOS images. The optical section images displayed the vascular layers at three equally spaced retinal depths, with the leftmost image corresponding to a layer closest to the vitreoretinal interface (Fig. 5C). A fluorescein angiogram of the same area was enlarged to provide the same magnification as that in the CROS images (Fig. 4A). The optical section image of the vascular layer closest to the vitreoretinal interface had high contrast because of the absence of fluorescence from the dye in the underlying choroidal vasculature. On the third optical section image that corresponded to the layer closest to the chorioretinal interface, fluorescence from the dye in the choriocapillaris was visible, whereas the overlying major retinal vessels appeared as dark entities overshadowing the vasculature.

In Vivo Optical Sectioning of the Vasculature after Laser Photocoagulation

CROS using fluorescein was performed 4 weeks after laser photocoagulation in the area indicated by the rectangle on the fluorescein angiogram (Fig. 6A). The composite image of 10 of 40 FOS images revealed irregularities in the topography and fluorescence in the chorioretinal vasculature along the FOS images (Fig. 6B). On the fourth FOS image from the right, the retinal vasculature on the lower portion appears more anterior compared with that in the upper portion, suggesting the presence of retinal elevation in the lower portion. A series of six optical section images was constructed, with the leftmost optical section image corresponding to a layer closest to the vitreoretinal interface (Fig. 6C). Elevation of the retina in the inferior of the imaged area is shown in the series of three optical section images (top row). The retinal vasculature can be viewed in the lower portion of the first and second images from the left but is barely visible in the upper portion of the images. The retinal vasculature is apparent in the upper portion of the third image from the left at the same depth as the leakage in the subretinal space is viewed in the lower portion of the image. Optical sectioning of the chorioretinal vasculature is clearly depicted in the three optical section images (bottom row). On the upper portion of the first left image, the retinal vasculature can be viewed devoid of fluorescence from the dye in the underlying subretinal scar tissues. On the upper portion of the third image, hyperfluorescence in the location of the laser scar can be appreciated without the appearance of the overlying retinal vasculature.

CROS using fluorescein was performed 16 weeks after laser photocoagulation, in the area indicated by the rectangle on the fluorescein angiogram (Fig. 7A). The composite image revealed abnormalities in the chorioretinal vasculature depicted by variations in the fluorescence within the FOS images (Fig. 7B). The series of five optical section images allowed distinct viewing of the chorioretinal vascular layers (Fig. 7C). On the leftmost optical section image, the retinal vasculature was visualized with minimal fluorescence from the dye leakage in the underlying retinal space. Similarly, on rightmost optical section image, irregularities in the pattern of dye fluorescence in the subretinal vasculature were clearly visualized. On the conventional fluorescein angiogram of the same area, visualiza-
Figure 3. (A) The vertical lines superimposed on the fluorescein angiogram depict the location of a series of 10 fluorescence optical section (FOS) images, spatially separated by 200 μm on the retina. (B) Placing FOS images from every fourth buffer next to each other created a composite of FOS images (10 of 40). The images were rotated to display the same orientation as the fluorescein angiogram. The arrowhead points to the retinal vasculature component of the FOS image, and the arrows demarcate the foveal avascular zone. (C) A series of optical section images constructed from equally displaced depths. Computer-constructed optical section images of the retinal vasculature delineate the foveal avascular zone and the adjacent microvasculature.
tion of detail in the vasculature was obscured by the dye fluorescence in the overlapping layers and by the dye leakage from the subretinal neovascularization (Fig. 4B).

CROS using ICG was performed 5 weeks after laser photocoagulation, in the area indicated by the rectangle on the fluorescein angiogram (Fig. 8A). The composite image indicated increased fluorescence from the dye at localized points within the FOS images (Fig. 8B). The leftmost optical section image, from the series of 5 optical section images, displayed the retinal structure overlying the leakage associated with the laser-induced lesion (Fig. 8C). On the following 4 optical section images of the subretinal vasculature, a local area of hyperfluorescence was clearly visible at the edge of the lesion.

DISCUSSION

Diagnosis and monitoring of many chorioretinal diseases are based on visualization of abnormalities in the retinal and sub-
Figure 5. (A) Chorioretinal optical sectioning using fluorescein was performed in a normal eye nasal to the fovea in the location indicated by the rectangle on the fluorescein angigram. (B) A composite of fluorescence optical section images showing consistent height, uniform fluorescence, and a clear lateral displacement of the chorioretinal vasculature. (C) Forty FOS images processed to construct a series of optical section images parallel to the retinal surface and from equally displaced depths. The leftmost and rightmost images correspond to layers closest to vitreoretinal and chorioretinal interface, respectively. On the image that corresponds to a layer closest to chorioretinal interface, the major retinal vessels appear as dark entities overshadowing the vasculature.
retinal vasculature. In conventional fluorescein angiography, visualization of the retinal microvasculature is obscured because of the presence of strong background dye fluorescence in the larger choroidal vessels. This is revealed by the density of the microvasculature observed in Stargardt's disease, in which the pigment epithelium reduces the fluorescence from the choroid. Similarly, with ICG angiography, visualization of CNV can be obscured by the choroidal vasculature. In addition, on the early fluorescein angiograms, the overlap of the dye fluorescence in the retinal vasculature obstructs clear visualization of the neovascular net. On the late fluorescein angiogram, the dye leakage from the neovascularization precludes identification of the borders of the subretinal neovascular membrane. Optical section imaging is advantageous because it optically separates the overlapping vascular layers to provide higher contrast images of the vasculature and thereby en-

Figure 6. (A) Chorioretinal optical sectioning using fluorescein was performed 4 weeks after laser photocoagulation in the location indicated by the rectangle on the fluorescein angiogram. (B) On the fourth fluorescence optical section image from the right, the retinal vasculature appears anterior on the lower portion compared with the upper portion (arrows). (C) Series of optical section images shows retinal elevation in the inferior of the imaged area (top row) and optical separation of the retinal vasculature and the subretinal scar tissues (bottom row).
FIGURE 7. (A) Chorioretinal optical sectioning using fluorescein was performed 16 weeks after laser photocoagulation in the location indicated by the rectangle on the fluorescein angiogram. (B) A composite of fluorescence optical section images revealing abnormalities in the fluorescence from the dye in the chorioretinal vasculature. (C) Series of optical section images allowing distinct viewing of the irregular pattern of the underlying subretinal vasculature.

hances the visualization of vascular abnormalities in chorioretinal diseases.

The technique of optical coherence tomography provides high-resolution cross-sectional images of the retinal structures, using a laser with a wavelength of 830 nm. Because the nature of positive interference in optical coherence tomography precludes recording light at a different wavelength, the technique does not allow visualization of any fluorescence information. The SLO provides better contrast images of the vasculature compared with conventional angiograms. However, the depth resolution of the SLO is limited to 300 μm, approximately the thickness of the retina. Thus, high-contrast imaging of the retinal microvasculature is limited to the perifoveal region, where the vasculature is single layered. Furthermore, because of the three-dimensional structure of the choroid, discrimination of the normal vessels and neovascular membrane and localization of the feeding vessels of the neovascular membrane may be difficult on images provided by the SLO. A confocal infrared SLO has been used to provide confocal images of the retinal and choroidal vasculature with ICG angiography. A depth discrimination of 330 μm can be achieved with a confocal aperture of 200 μm. The use of smaller apertures requires a higher incident light power and prohibits visualization of the retina and choroid in one frame. Recently, simultaneous fluorescein and ICG angiography has been demonstrated using the confocal SLO.

We have shown the capability of CROS, a new method for optical section imaging, to provide enhanced images of the vascular layers and document retinal elevations in living eyes. CROS generated images of the chorioretinal vascular layers that were superior in contrast by eliminating fluorescence from the dye in the overlapping or underlying vasculature. Optical section images obtained in the fovea of a normal eye clearly depicted the foveal avascular zone and the adjacent microvascularity. Optical section images provided a high-contrast view of the retinal microvasculature, because there was no fluorescence from dye in the deeper layers. The validity of CROS for separating the chorioretinal vasculature was further confirmed by the appearance of the major retinal vessels as dark entities overshadowing the subretinal vasculature. In the eye with laser-induced lesions, images obtained by CROS by use of fluorescein displayed vascular abnormalities and fluorescein leakage in the subretinal vasculature better than conventional angiograms because of the improved contrast. CROS using ICG allowed visualization of the various vascular layers and discrimination of details. A local area of hyperfluorescence was noted at the edge of the laser lesion, which is referred to as a “hot spot” and has been observed on conventional ICG angiograms approximately 1 hour after injection of the dye.

CROS provides the capability of optically separating the overlapping vascular layers and thereby produces higher contrast images of the vasculature compared with those obtained with conventional angiography. The ability to perform optical sectioning of the vascular layers with CROS is determined by the angle between the incident laser and viewing axis and thus is limited by the size of the pupil. In addition, because the optical section images are constructed from the FOS images, the depth separation is determined by the width of the slices and the spatial displacement of the FOS images. CROS has a higher depth discrimination and is capable of providing ICG
and fluorescein angiograms. Similar to the laser scanning technique, with CROS ICG angiography, the imaging of choroidal vascular layers is hampered because of the attenuation of the emitted infrared light by the choroid, pigment epithelium, and retina. With CROS, images are acquired in only 660 msec; therefore, eye motion artifacts occur rarely. Overall, CROS enhances the visualization of the retinal and subretinal vasculature and promises to be a beneficial tool for evaluation of chorioretinal diseases.

References


