Simultaneous Indocyanine Green and Fluorescein Angiography Using a Confocal Scanning Laser Ophthalmoscope

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Background: Fluorescein and indocyanine green (ICG) angiography are both useful in the diagnosis and treatment of many retinal diseases. In some cases, both tests must be performed for diagnosis and treatment; however, performing both is time-consuming and may require multiple injections.

Methods: We designed a compact digital confocal scanning laser ophthalmoscope to perform true simultaneous fluorescein and ICG angiography. We report our experience using the instrument to perform 169 angiograms in 117 patients.

Results: There were no unexpected adverse effects from mixing the dyes and administering them in 1 injection. An entire examination, including fundus photography, fluorescein angiography, and ICG angiography, could be performed in 45 minutes. It was possible to study differences in fluorescein patterns by comparing identically timed frames and to find cases in which ICG or fluorescein was optimal in visualizing retinal and subretinal structures. Confocal optical sections in the depth (z) dimension allowed viewing in different planes. It was possible to overlay ICG and fluorescein images or compare them side-by-side using a linked cursor. Digital transmission of the images was also performed.

Conclusions: Simultaneous ICG and fluorescein angiography can be performed rapidly, safely, and conveniently. The availability of simultaneous angiography will allow critical determination of the relative advantages and disadvantages of both types of angiography.


Fluorescein angiography is a useful tool for the diagnosis of many retinal diseases. It provides diagnostic as well as treatment information by allowing visualization of the retinal and choroidal vasculature. Angiography allows identification of leakage of the small fluorescein molecule in pathological states. More recently, indocyanine green (ICG) angiography has been developed. This molecule is larger (molecular weight, 775 d vs 332 d for fluorescein) and more protein-bound in plasma than is fluorescein and fluoresces in the infrared spectrum. Initially, ICG angiography was performed with infrared photographic film. However, the poor sensitivity of film coupled with the relatively weak fluorescence properties of the dye caused this method to be abandoned. The strong binding of ICG dye to plasma proteins results in slow leakage as compared with fluorescein and reduces the amount of extravascular fluorescence available for imaging. Digital video cameras have been used to capture images for ICG angiography. This has made ICG angiography a useful clinical diagnostic tool, particularly for imaging of subretinal neovascular membranes in cases where such membranes can not be adequately imaged with fluorescein angiography.

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Another approach to fluorescence angiography (using either fluorescein or ICG) is the scanning laser ophthalmoscope. This instrument has come into common clinical practice because of its commercialization in recent years. Advantages of the scanning laser ophthalmoscope include the ability to use an excitation light that scans the retina, allowing more intense excitation (thus providing a stronger emission signal) while still using safe levels of illumination. This is possible because the scanning beam illuminates each area point of the retina for only 0.1 to 0.7 microseconds. Scanning laser ophthalmoscope angiography gives temporal information that

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MATERIALS AND METHODS

We used a confocal scanning laser ophthalmoscope with 2 laser light sources to illuminate the retina (Heidelberg Retina Angiograph, Heidelberg Engineering Inc, Carlsbad, Calif). The instrument uses 2 lasers with 3 wavelengths as light sources for scanning fundus illumination. An argon-ion laser (488 nm and 514 nm wavelength) was used to provide red-free photographs (green, 514 nm) and blue light was used for excitation during fluorescein angiograms (blue, 488 nm). The second laser was a diode laser (795 nm wavelength) that provided illumination to excite the ICG dye, which fluoresces at 835 nm. The instrument was operated in a tight confocal imaging mode and acquired up to 12 simultaneous frame pairs per second. For each video line of the angiogram, the forward scan was used for one angiogram and the backward scan was used for the other angiogram. Thus, the time separation between corresponding lines of the 2 angiograms was on the order of 0.1 milliseconds; each frame pair was acquired in 0.08 seconds. The instrument allowed acquisition of 12 frame pairs per second with 256 × 256 pixel and 8 bit per pixel intensity quantitation.

The images were recorded in the confocal mode while the photographer moved the plane of focus in the depth (z) dimension during the angiogram to optimally image the structures of interest. The chromatic aberration of the human eye caused a 1-diopter (D) focus shift between the blue-green fluorescein angiogram image plane and the infrared ICG angiogram image plane. The photographer selected a focal plane that allowed in-focus imaging of the retinal vasculature in the fluorescein angiogram, which placed the ICG image plane 300 µm deeper in the retina and choroid.

is richer than still (“digital”) imaging systems because true video allows imaging at rates of 20 to 30 frames per second. This allows more detail to be seen in the transit phases and may give better information about patterns of leaking vessels. Furthermore, the illuminating beam is monochromatic and the intensity of fluorescence may be higher because of the scanning laser ophthalmoscope beam and the fact that point-by-point illumination is used. Stereoscopic imaging is also possible using scanning laser ophthalmoscope techniques.

We recently described the use of a confocal scanning laser ophthalmoscope to perform tomographic imaging of macular diseases. By optically isolating the image plane to a narrow plane in the depth (z) dimension, depth information and measurements could be obtained. Subsequently, we adapted the scanning laser tomograph to perform ICG angiography. The instrument allowed better discrimination against out-of-focus objects (tomography) and provided higher contrast than other systems. The simple optical path and optimized excitation and emission detection systems improved the sensitivity of the instrument so that 70% of the photons exiting the eye could be detected. This allowed excellent visualization of vessels in the late stages (45 minutes) of the ICG angiogram without the use of a second (“landmark”) injection of ICG dye. This instrument was the first completely digital instrument to allow scanning laser videoangiography. Image capture and processing is done digitally so that no information is lost at any stage of processing, image manipulation, or transmission.

Although ICG angiography may be advantageous in certain cases of subretinal neovascularization and in diagnosing other disorders, one of its drawbacks is the long period of time required for angiography (45 minutes) and the need to obtain a second angiogram after a fluorescein angiogram is obtained. The process of obtaining a fluorescein and ICG angiogram sequentially is time consuming and requires 2 or 3 (if a landmark injection is used) injections for each patient for completion of the set of studies. Moreover, the angiograms are performed at different times, making it difficult to know if differences in fluorescence leakage patterns are due to differences in properties of the dyes or in the quality of the photographs. This may be one reason why different investigators vary in their assessment of the additional incremental benefit of ICG over fluorescein angiography. To determine if high-quality simultaneous images from fluorescein and ICG angiograms could be obtained, we modified a small, digital, personal computer–based confocal scanning laser ophthalmoscope to allow

PATIENT EXAMINATIONS

We performed 169 angiograms in 117 patients with age-related macular degeneration, ocular melanoma, and other retinal diseases. The patients were studied after a complete ophthalmic examination that included fundus photography, slitlamp examination, and indirect ophthalmoscopy. We injected 2 mL of a mixture of 25 mg of ICG and 500 mg of sodium fluorescein intravenously. This was prepared using sterile commercially available dyes suitable for intravenous injection. The liquid sodium fluorescein (25% solution in 2 mL; Fluorescein, Alcon, Fort Worth, Tex) was placed into a vial containing sterile ICG powder (CardioGreen, Becton Dickinson, Cockeysville, Md) and the ICG was dissolved in the liquid fluorescein. No precipitates were seen. This resulted in a sterile solution containing 25 mg of ICG and 500 mg of sodium fluorescein. The patients underwent imaging in the early phase (0-2 minutes after injection), the mid phase (3-5 minutes for sodium fluorescein, 3-15 minutes for ICG), and late phase (10-12 minutes for sodium fluorescein, 40-45 minutes for ICG). All patients were informed as to the risk and benefit factors of all procedures. The images were analyzed by 2 ophthalmologists (W.R.F. and A.J.M.).

IMAGE STORAGE AND RETRIEVAL

The images were stored digitally in the RAM of the computer during acquisition and subsequently transferred onto the hard disk. The still frames of a typical angiogram sequence (n=50 frames) required 3.2 megabytes of hard disk memory. Video sequences (20 frames per second) required 13 megabytes for each 10-second sequence of video. Using 40 megabytes of RAM, we allowed 30 seconds of live video storage before moving the data to permanent hard disk storage.
simultaneous imaging after a single intravenous injection of both ICG and fluorescein. We do not advocate performing simultaneous angiography on every patient; rather, our goal was to determine whether such angiography could be performed and to evaluate the characteristics of confocal simultaneous angiography.

RESULTS

CLINICAL ADVANTAGES

One hundred sixty-nine angiograms were performed with no serious adverse reactions. Preparation of the dye mixture from the 2 commercially available sterile preparations was performed without problems. A saline solution “flush” injection was not necessary to obtain good image quality. The time necessary for an entire study was 45 minutes and only 1 injection per patient was needed. Red-free color images were also obtained using the green wavelength of the argon laser. Infared fundus photographs were obtained using the diode laser. It was not necessary to reinject ICG dye to image retinal vessels in the late phase of the study. The retinal vessels were visible at the 40-minute time point owing to the sensitivity of the detector, which allowed visualization of the background choroidal ICG dye fluorescence. This allowed imaging of the retinal vessels as dark linear structures silhouetted by this background fluorescence13 (Figure 1).

![Figure 1. Confocal fluorescein–indocyanine green (ICG) angiogram showing hyperfluorescence and early leakage due to subretinal neovascular membrane at 56.03 seconds. The fluorescein angiogram shows early leakage (A) but the vascular membrane is not well defined. The ICG frame (B) shows a vascular plexus (top of cursor). The cursors in A and B frames are at the same location on the fundus. Late frames on the fluorescein (C) and ICG (D) angiograms show leakage at 42 minutes 15 seconds. Note that the dark outlines of retinal vessels are clearly seen in the late ICG frame, allowing localization of retinal vascular landmarks without a second (“landmark”) injection.](https://archopht.jamanetwork.com/)
TIME COURSE OF APPEARANCE OF BOTH DYES

Analysis of patients in whom rapid sequences of early frames were performed showed that the transit of ICG and fluorescein to the eye appeared to be simultaneous. This was best seen in the early laminar flow or arterial filling phases where arterial filling or localized venous laminar flow patterns could be seen. In these cases, the patterns of vascular filling over time appeared to be the same with both dyes (Figure 2). We found that it was possible to artificially make either dye appear to fluoresce earlier by changing the gain settings on the instrument. We did not perform absolute calibration of the emitted light from each point on the fundus. We found that it was easy to study the differences in fluorescence patterns, lesion localization, and leakage patterns using ICG and fluorescein with the simultaneously acquired images (Figure 2).

CLINICAL UTILITY IN SUBRETINAL NEOVASCULARIZATION AND OTHER DISEASES

The production of true simultaneous images negated the possibility that one of the angiograms was of higher quality than the other and that this allowed better visualization of pathological states. The potential reasons that one study might be of better quality include better centering of the camera image pathway in the pupil, less patient movement, better focus, more correct exposure, less blinking, and other factors. Using the simultaneous angiogram, in some cases ICG showed a subretinal neovascular membrane better than fluorescein and in other cases fluorescein showed it better than ICG (Figure 3). Only a larger study can determine the relative sensitivity and specificity of the 2 techniques. Our review of the images suggests that both studies are needed in difficult cases. It is interesting that in some cases, feeder vessels can be seen in ICG and not in fluorescein angiogram frames (Figure 4).

We found that the ability to view multiple images or video-rate images provided more information than single frames. A pseudostereo image was possible by slowly moving the instrument in the horizontal meridian. The information density included horizontal resolution and a temporal component. During the study, frames were selectively focused at different planes because of the confocal nature of the detection system. The ability to procure images at rapid rates allowed us to select the most optimal frame for determining and guiding treatment.

CONFOCALITY

The use of confocal optics had 2 effects. It allowed imaging in a relatively narrow plane of focus and it also increased the contrast considerably. This improved the quality of the images. In addition, the ability to move the plane of focus in depth allowed the physician to discern the optimal plane of focus for visualizing the pathological structures of interest. As previously described, the higher contrast of this system allowed visualization of the retinal vasculature superimposed on the background ICG fluorescence in 40-minute late frames of the ICG angiograms (Figure 1). In addition, we were able to show the depth location of subretinal neovascular membranes by scanning at different planes of focus (Figure 5).

REGISTRATION AND COMPARISON OF SIMULTANEOUS IMAGES

We studied 2 methods for comparing the simultaneous ICG and fluorescein images. Color-coding the images (fluorescein in green and ICG in red) allowed overlay of
the 2 images (Figure 6). We also were able to study the 2 monochromatic gray-scaled images side by side, electronically linked with a cursor. The cursor location in the 2 images always corresponded to the identical location in the fundus. This allowed simultaneous viewing of a given point on the fundus in both ICG and fluorescein images (Figure 7). We found this less confusing and more precise than the dual-color method. This method was possible only because each line of the angiogram was scanned both for ICG and fluorescein images in a quasi-simultaneous fashion and corresponding points could be determined. There was no image manipulation involved in performing this electronic linking between the 2 images.

**Figure 3.** Confocal fluorescein–indocyanine green (ICG) angiogram showing an organizing pigment epithelial detachment with underlying subretinal neovascular membrane seen better on fluorescein than ICG images. Hyperfluorescence and early leakage of dye are seen more impressively at 1 minute 5 seconds on the fluorescein (A) than ICG (B) frames. Later frames of the fluorescein (C) and ICG (D) studies also show more leakage and dye pooling in the fluorescein study (6 minutes 47 seconds).

**IMAGE STORAGE, TRANSFER, AND RETRIEVAL**

The output of the instrument was digital. Thus each image entailed 256 x 256 bytes plus 8-bit gray-scale information at each location. Each location of the fundus was imaged twice so that each image pair (ICG and fluorescein) was approximately 130 kilobytes. The completely digital nature of the output allowed storage of all of the acquired information electronically without any loss of detail. The images could be stored using the software designed for the instrument or could be loaded into any commercially available software on any platform, either PC or Macintosh. Several selected pairs of images could be easily transported on a single floppy disk to a second com-
Figure 4. A, Confocal fluorescein–indocyanine green (ICG) angiogram showing hyperfluorescence and early fluorescein leakage in the foveal area at 53.04 seconds. B, Simultaneous ICG frame shows a feeder vessel perfusing a round subretinal neovascular membrane. C, ×2 magnification of (B) shows details of feeder vessel and subretinal neovascular membrane. In all 3 images the cursors are in precisely the same location because the images are digitally aligned pixel by pixel. The cursors are linked and correspond to the same location for each simultaneous pair.

Figure 5. Frames from an indocyanine green (ICG) image series showing a subretinal neovascular membrane. The confocal series was taken at varying depths. A, −6.0 diopters (D); B, −4.0 D; C, −2.5 D; and D, +2.0 D. The subretinal neovascular membrane is seen at the −2.25 D plane as are other choroidal details.
puter in a laser treatment room. We also were able to transport images via Internet connections (approximately 5 seconds per image pair) to remote sites anywhere in the world. Video information was stored on portable hard disk drives and a variety of other media at low cost. At our institution, we chose to use Ethernet cable to transport image pairs at times of approximately 1 second per ICG/fluorescein pair from the angiography suite to the laser suite.

Red-free photographs were also obtained with the green 514.5-nm channel of the argon laser and infrared photographs were procured with the diode laser wavelength. These showed subretinal structures such as drusen more clearly then color or red-free fundus photographs.19

These photographic images were not simultaneous but were taken at the same magnification and were suitable for performing overlays on angiographic images, as are used in posttreatment assessment in eyes treated for subretinal neovascularization.9,20 Infrared fundus photographs could also be performed with the instrument.

**COMMENT**

Simultaneous confocal ICG and fluorescein angiography using a confocal scanning laser ophthalmoscope has several advantages. Clinically, we noted no serious adverse reactions in a series of 169 simultaneous ICG/fluorescein injections. The time required to perform the entire study was considerably shorter than first performing a fluorescein study, reviewing it, and subsequently performing the ICG study. In addition, the confocal nature of the instrument allowed for extremely high contrast in the later images, which allowed visualization of retinal vessels at the late time points and obviated the need for a landmark injection of fluorescein. Thus, simultaneous angiography is beneficial both from the patient's perspective as well as that of the ophthalmologist's office staff; only 1 injection is needed and in 40 minutes fundus photography, ICG angiography, and fluorescein angiography can be completed and reviewed to diagnose and guide treatment. In general, we performed simultaneous angiography in cases in which prior fluorescein angiography did not or was not expected to reveal sufficient information for treatment or diagnosis. A saline solution flush injection was not used as high-quality images were obtained without it.

The ability to perform simultaneous angiography was demonstrated by Bischoff et al,21 who described a prototype simultaneous ICG and fluorescein angiogram based on a scanning laser ophthalmoscope. Their instrument differed from the instrument we describe. Their instrument acquired true simultaneous images of the fundus with 2 continuous laser light sources and 2 light detectors. It was a prototype and required the presence of a complicated computer setup, 2 video recorders, and 2 monitors, filling half a room. The set-up of the instrument in our study switches laser light sources during the acquisition of a single video line to capture quasi-simultaneous images (time separation, 0.1 milliseconds). The light exposure is limited as sources are never simultaneously active during the scan. In addition, the present instrument allows acquisition of red-free and infrared fundus photographs, as well as storing all images digitally so that any image can be recalled or transported at any time or location without any loss of image quality. The use of a PC computer platform allows the use of any software system and great flexibility in image storage and processing. It is important to realize that information density not only includes horizontal resolution but also a time component. By shifting planes of focus and angle of imaging, one can often see better details of the structure of interest and can also choose the individual frame that best shows the pathological feature of interest or the one that must be projected to guide laser therapy. Other advantages of video imaging include the ability to see moving particles in the fluorescein images; this may enhance the visualization of subretinal neovascular membranes.22

There has been some controversy regarding the issue of transit times of the 2 fluorescent dyes.21,22 We found...
both dyes arrived simultaneously in the early phase of our study. There is no reason for the dyes to arrive at different time points. When the relative gains of our detectors were equal, there was no evidence that one of the dyes transited faster than the other.

We found that it was easy to study the differences in fluorescence patterns and lesion localization using ICG and fluorescein with the simultaneously acquired images. We preferred the linked cursor software mode to critically and simultaneously compare locations of the
ICG and fluorescein images. Color-coding of the 2 images with display of both images simultaneously could also be performed. We recognize that treatment based on ICG fluorescence has not been studied in randomized clinical trials. Clearly this is necessary, but the ability to obtain both studies simultaneously should be advantageous in designing such a trial. Using simultaneous angiography is the only way to determine the relative value of each type of angiography. Only through a large study of patients with a variety of retinal diseases using simultaneous ICG and fluorescein angiography will it be possible to critically determine the differences in vascular patterns and their clinical significance. We do not advocate the use of simultaneous angiography for all patients. However, in those patients in whom conventional fluorescein angiography does not allow a conclusive analysis, simultaneous angiography seems to offer additional insight.

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