Holographic recording of a retina using a continuous wave laser

Joseph L. Calkins and Carl D. Leonard

A new method for examining and recording features of the eye has been developed. Using holography rather than photography, we obtained, in a single exposure of a cat's eye, a three-dimensional image containing information about the retina as well as all layers along the optical path. Recording a hologram of a living object posed special problems of physical stability necessitating short exposure intervals. With shorter exposure intervals one needs higher levels of retinal illumination to adequately expose the photographic emulsion, and, unless care is exercised, these high levels of light energy may damage the retina. To optimize conditions for the exposure, the emulsion sensitivity was increased and the anesthesized cat was kept motionless. Agfa 10E70 emulsion was employed and special processing used to increase its speed. The light source was a continuous-wave helium-neon laser with an output of 58 mw. Light energy incident on the retina was only 1,200 erg/cm², whereas Department of Health, Education, and Welfare (HEW) guidelines place safe exposures between 50,000 and 100,000 erg/cm².

Key words: fundus photography, holography, helium neon laser, retina, iris, cats, laser, coherent light, coherent imagery, contact lens.

Holography, or three-dimensional photography, can now be used for recording the living retina using safe levels of coherent light energy. In ordinary photography, only the intensity of the light is recorded, resulting in a two-dimensional image. With holography, both the amplitude and phase of the light are recorded. In holography, it is no longer meaningful to speak about an out-of-focus image: Each object point comes to focus at a certain distance from the hologram. One has the impression of looking through a window into a truly three-dimensional setting when properly viewing a hologram.

A hologram is recorded by letting two beams of laser light fall simultaneously on a photographic plate. The plate, which is subsequently developed, records the interference pattern between the two beams. One beam, designated the reference beam, consists of a spherical wavefront emanating from a pinhole. The other beam, designated the signal beam, consists of some complex wavefront reflected off the object. Both beams must come from the same laser. The mathematical principles involved in this process and further descriptive explanations of it are presented elsewhere.1 2

From the Department of Ophthalmology, The University of Michigan Medical Center, and the Radar and Optics Laboratory, Institute of Science and Technology, The University of Michigan, Ann Arbor, Mich.

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The first recorded hologram was reported by Gabor in 1948. Two problems that hindered further development were immediately apparent. A much more coherent source of light than the mercury vapor lamp then available was needed to record all but the simplest of objects. Also, some means had to be devised to get rid of a secondary, or twin, image that arose in the process. The problem of the coherent source was resolved in the early 1960's with the invention of the laser. The first successful means for overcoming the twin-image problem was implemented by Leith and Upatnieks in 1962. Since that time, much research has gone into developing holography and exploring a wide variety of technological applications for it. Its application to investigations of the eye was first suggested in 1966, but the problems involved in making a holographic recording of a living eye were not solved at that time.

Materials and methods

The animal selected for this effort was a cat, which was anesthetized with sodium pentobarbital, 40 mg. per kilogram, injected intraperitoneally. Two drops of cyclopentolate hydrochloride were instilled in each eye prior to induction. To insure an absolute minimum of motion during exposure of the photographic emulsion, the cat's head was held stable by an aluminum jig (Fig. 1). A planoconcave contact lens was placed on the cornea using methylcellulose as a wetting agent. This neutralized the refractive power of the cornea and permitted a larger area of the retina to be recorded. Without a contact lens on the anesthetized animal, phase changes due to corneal drying became a problem.

The experiment was arranged on an optical bench as depicted schematically in Fig. 2. The laser was a continuous-wave helium-neon Spectra-Physics model 125 with an output of 58 mw. at 6,328 A. The laser beam was passed through a beam splitter which divided it into a reference and an object beam. The reference beam was formed by passing the collimated laser beam through a lens of short focal length so that its rays came to focus on a pinhole and then diverged to produce a spherical wavefront incident on the holographic plate. Because of the extended position of the cat's head, a mirror was necessary to reflect the reference beam onto the plate. The object beam was similarly passed through a lens of short focal length, and the diverging rays were projected onto the retina via a tiny mirror. Emerging light waves from the retina reached the holographic plate by passing about the illuminating beam mirror.

The laser emitted a vertically polarized beam, so the electric field component of the reference beam was vertically polarized. The object beam

![Fig. 1. Anesthetized cat held in a supporting structure for stability. Illuminating lens and reference mirror are visible. The photographic plate is removed from the foreground for clarity.](image-url)
Fig. 2. Basic holographic recording arrangement. The beam splitter (B.S.) taps off a small portion of the laser beam to be used for the reference beam. The two beams are then reflected by mirrors (M), diverged by lenses (L), and again reflected by mirrors (M). Because of the plano-concave contact lens and the proximity of the illuminating lens, a large area of the retina is illuminated.

was rotated so that the illumination reaching the eye of the cat was horizontally polarized. Undesired specular reflection from the contact lens surface and the illuminating mirror retained the horizontal polarization, while the diffuse reflection produced by the retina emerged depolarized. Since interference takes place only between fields that have the same polarization, the specular reflections were minimized. Yet, the vertically polarized half of the retinal reflection and the reference beam created a satisfactory interference pattern.

The hologram was recorded on a glass plate with an Agfa 10E70 emulsion. Special processing was employed to achieve high recording speed. Before exposure, the film had been hypersensitized in 2 per cent triethanolamine. After exposure, it was developed 12 minutes in Agfa Metinol U and bleached. The reference beam was adjusted to give an exposure of 6 erg/cm² at the photographic plate. The exposure time was 1/250 second, with the energy density at the retina being 1,200 erg/cm². To reconstruct and photograph the image from the developed hologram, the hologram was illuminated with a converging wavefront of laser light. This projected a real image onto the film plane and eliminated the need for a lens.

Results

Figs. 3 and 4 are photographs of the holographically reconstructed image; they illustrate the enormous depth of field contained in a single hologram. Fig. 3 shows a good deal of retinal detail, with the optic disc and retinal vessels clearly visible. Fig. 4 is a view of the mydriatic iris which was photographed simply by moving the camera film plane closer to the hologram.

Discussion

Perhaps the most significant finding here is that retinal holography is indeed possible using safe levels of coherent light energy. Recent HEW Department guidelines governing this type and level of laser irradiation, i.e., the pulsed, non-Q-switched mode in the millisecond range, place the threshold for detectable retinal damage in human subjects between $0.5 \times 10^7$ and $1.0 \times 10^7$ erg/cm². Recommended safe levels are chosen two orders of magnitude lower than the damage threshold level, which is still more than 50 times greater than the light energy used here. Of course, for the human retina, a slightly higher level of illumination than that used on the cat would be required, because man lacks a reflecting tapetum. However, a satisfactory margin of safety is still possible.

It is also noteworthy that the relatively imperfect optical medium, consisting of aqueous and vitreous humor, a biological lens, and a cornea, will permit holographic recording, since small phase or refractive changes during exposure can greatly alter image quality. Moreover, it is significant that a live animal could be rendered sufficiently stable during the 1/250 second exposure. For a good hologram, one normally assumes that the object (retina) must remain stationary to less than one eighth wavelength of the illuminating light.

The three-dimensional aspect of retinal holography may well justify its eventual use as a clinical tool. One advantage afforded by the hologram is in the time factor. A single holographic exposure, conceivably involving a fraction of a second of the patient's time, may be examined at will or perhaps used in large-scale screening studies where a single photograph would not permit evaluation of the entire eye. Other holographic techniques such as interferometry, depth contouring and non-
Fig. 3. Reconstructed holographic image of a cat's retina made using a planoconcave contact lens. Retinal vessels and optic disc are clearly visible. The reconstructed image was photographed by illuminating the hologram with convergent laser light and placing film in the plane of the real image.

Fig. 4. Reconstructed holographic image of the iris. This view was photographed from the same hologram as Fig. 3 by placing the film in a different plane.
destructive interferometric testing, and pattern recognition which are actively being investigated in industry and other areas of technology could also have valuable applications in ophthalmology.

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REFERENCES