Holographic Microscopy as a Technique for Recording Dynamic Microscopic Subjects

Abstract. *Holography, with submicrosecond pulsed lasers used for illumination, enables one to record an instant of time of a transient, dynamic event throughout a significant volume for later study and analysis with conventional microscopic equipment. Living marine plankton organisms are used to demonstrate one practical application of this technique.*

While the basic principles of holographic microscopy were described in 1948 by Gabor (*1*), it was not until the development of Q-switched solid-state lasers, capable of producing coherent light pulses of 100 nsec duration or less, that it became practical to make holograms of dynamic subjects. Brooks *et al.* (*2*) have reported the successful use of a pulsed laser to obtain holograms of high-speed macrophenomena. The present report extends the application of holographic photography to dynamic, microscopic objects occurring in a liquid medium. Living marine plankton organisms are used to demonstrate the ability of the hologram to record an instant of time of a complex, transient, microscopic event, throughout a significant volume for later analysis at leisure with conventional microscopic equipment.

While the example chosen relates particularly to the field of marine biology, the technique is equally applicable to the study of other microphenomena such as might be encountered in the investigation of many types of microscopic biological systems, disperse cell cultures, suspensions, small-scale hydrodynamic events, cavitation, bubble formation, sedimentation, and similar subjects.

Holography circumvents the limited depth of focus, the off-axis aberration, and the relatively short working distance of the classical microscope. Theoretically, the hologram records a large volume of object space without loss of resolution. The ultimate practical volume, in the case of the marine plankton investigations, would appear to be determined not only by physical limitations but by a number of factors which affect the quality and resolution of the reconstructed image obtainable from the hologram. These factors include such considerations as the energy of the illuminating source, the longitudinal and transverse coherence of the laser beam, the flatness of the photographic plate, the effective numerical aperture of the recording system, and the "creep" of the photographic emulsion.

The photographs shown in this report were made with a conventional microscope, to magnify the reconstructed real image from a hologram, and with a standard Polaroid camera, to record the picture. The subject is living marine plankton collected on 19 May 1966 from Pacific waters near Santa Monica, California. Organisms identifiable from the hologram include numerous fish eggs, fish larva, copepods of the genus *Acartia, Ceratium* sp., and diatoms (*3*).

Figure 1 is a series of photomicrographs all taken from the same holographic reconstruction, illustrating the ability of the hologram to record dynamic events throughout a sizeable volume. Examination of the series shows that as the plane of focus of the viewing optical system is changed, objects which were previously out of focus become sharply imaged. The range of depth in the Fig. 1 series is approximately 30 mm.

Figure 2 is another photomicrograph from the same hologram as used for Fig. 1, but showing an organism which was swimming at a distance of about 11 cm from the photographic plate at the time the hologram was made. Considering the surface area of the photographic plate (~ 100 cm^2), the total volume recorded by the holo-

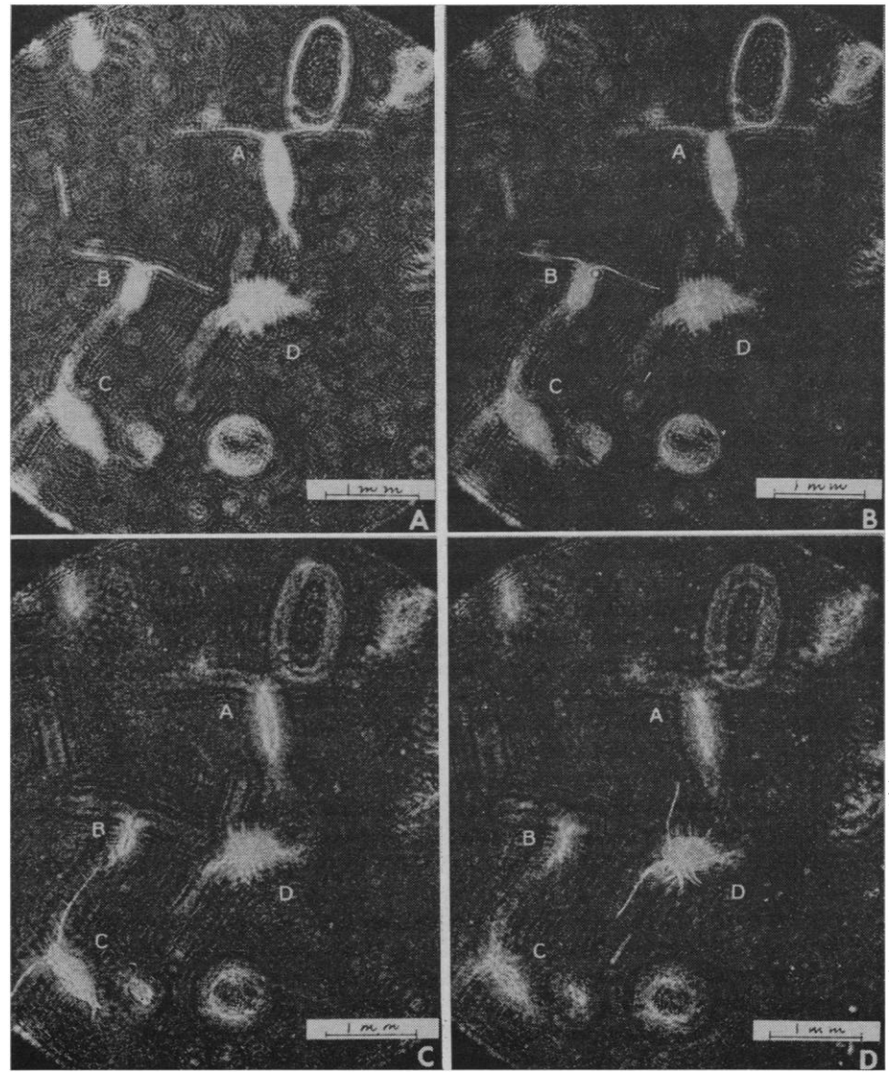

Fig. 1. Four photomicrographs from the same hologram, showing the same field of view at different planes of focus. (*A*) Microscope focussed on organism "A," 3 mm from plane of hologram plate. (*B*) Organism "B," 5 mm from plate. (*C*) Organism "C," 19 mm from plate. (*D*) Organism "D," 33 mm from plate.

Fig. 2. Photomicrograph from the same hologram used for Fig. 1, showing an organism swimming 11 cm from the hologram plate.

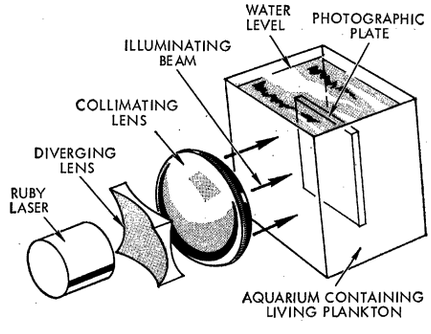

Fig. 3. Schematic diagram of apparatus used in making holograms of living plankton organisms.

gram used in Figs. 1 and 2 is in excess of 1000 cm^3. By using a conventional microscope to view the reconstructed real image from the hologram, any point in this volume may be brought sharply into focus.

The arrangement of apparatus for taking the hologram is shown schematically in Fig. 3. The distance from the front of the aquarium to the photographic plate was 14.5 cm. The aquarium was filled with sea water containing the living plankton organisms.

The "coherent" laser illuminator consisted of a ruby oscillator-amplifier with a Kerr cell Q-switch. The laser typically emitted an optical pulse of 60 nsec duration and 3 joules total energy. The coherence length of the pulse was approximately 1 cm.

In recording the hologram, high-resolution photographic plates (Eastman Kodak type 649-F, 0.04 by 4 by 5 inches in size) were used. The emulsion of the photographic plate was in direct contact with the sea water during the recording of the hologram. After exposure by the beam from the pulsed laser, the plate was removed, rinsed in fresh water, and developed with Kodak HRP developer by standard techniques.

Reconstruction of the real image was accomplished by illuminating the hologram with a collimated beam from a helium-neon continuous gas laser. In contrast to other work in the field of holographic microscopy (*1*, *4*), there was no attempt to achieve magnification with relay lenses during the recording process. Since collimated beams were used for both recording and reconstruction, the size of the reconstructed real image was essentially the same as that of the original subject (*5*).

The application of holographic microscopy to the study of plankton provides a means of viewing the organisms *in situ*. By the use of properly designed equipment it is possible to produce holograms at any depth in the ocean. The reconstructed image, representing an appreciable volume of object space, may then be searched in three-dimensional detail by conventional microscopic techniques. Data so obtained will provide accurate statistics on such matters as population density, distribution of species, and orientation of organisms with respect to physical environmental factors such as gravity, light, and movement of currents. By using a viewing microscope with a mount calibrated for x, y, and z axis movement, it is possible to plot the spatial relationships of the organisms. Such information should be useful in determining the existence of living domains and in investigating other spatially related ecological and environmental patterns.

In summary, holographic microscopy provides a new technique by which the investigator may obtain useful data, not readily available by other means, on dynamic microscopic systems, both living and nonliving.

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References and Notes

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3. I am indebted to Dr. Thomas L. Hopkins of the University of Southern California for his assistance in identifying the plankton organisms, and to my colleagues at TRW Systems, Drs. A. V. Haeff, L. O. Heflinger, R. F. Wuerker, and R. E. Brooks, for their helpful suggestions and review of the manuscript.
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5. Theoretically, some aberration is caused by the difference in the wavelengths of the recording (6943 Å) and reconstruction (6328 Å) beams and by the difference in the indices of refraction of the media in which the recording and reconstruction take place (water and air). However, there was no apparent effect at the levels of magnification used in this study.

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Stick-Slip as a Mechanism for Earthquakes

Abstract. *Stick-slip often accompanies frictional sliding in laboratory experiments with geologic materials. Shallow-focus earthquakes may represent stick-slip during sliding along old or newly formed faults in the earth. In such a situation, observed stress drops represent release of a small fraction of the stress supported by the rock surrounding the earthquake focus.*

Theories of the earthquake mechanism are based on two types of indirect observations, (i) the movements of surface rocks above the actual focal region and (ii) the behavior of rocks stressed in the laboratory under conditions similar to those found in the Earth. For some time following the great 1906 earthquake in California, Reid's elastic rebound theory held prominence. According to this theory (*1*), an earthquake is the result of strain release caused by sudden shearing motion along a fault. Reid's theory is reasonable in terms of the patterns of elastic radiation observed in many earthquakes (*2*), and is also reasonable when compared with laboratory experience. For example, a sample of granite or diabase stressed at several kilobars will fracture. Usually a fault is formed and stress is suddenly released.

Although the Reid theory seems to provide a plausible explanation for earthquakes, it has, in recent years, been challenged. Jeffreys (*3*) and then later Orowan (*4*) and Griggs and Handin (*5*) suggested that, particularly for deep-focus earthquakes, energy release due to fracture is unlikely. They argued that fracture must be accompanied by sliding in order to release energy, and that sliding with dry friction on fracture surfaces is ruled out because of the high stress required. Another difficulty