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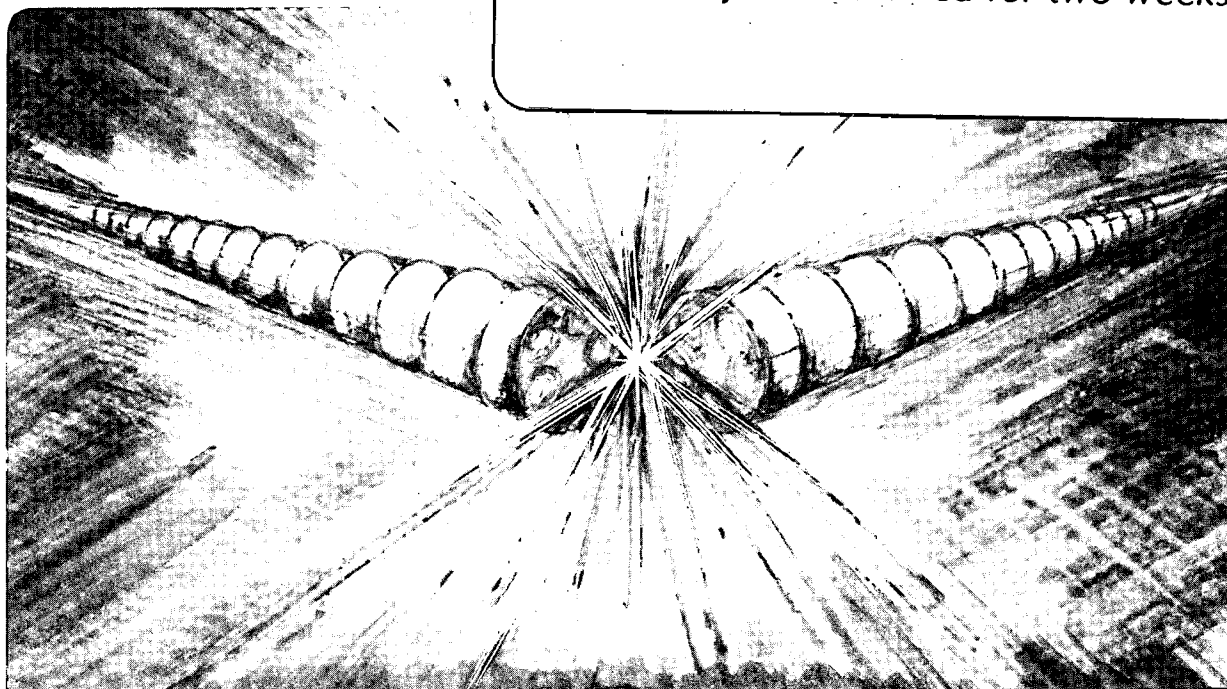
### X-Ray Holography: A History

C. Jacobsen

April 1989

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**X-ray holography: a history\***

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X-ray holographic microscopy has recently reached a milestone in its development by producing images of biological specimens at sub-micron resolution. However, x-ray holography is by no means a new subject, and it is the purpose of this article to review the history of experimental activity in this field (theoretical activities will also be mentioned).

## BEGINNINGS

Holography was invented in 1948 by the Hungarian-born British scientist Denis Gabor as a method for overcoming the limitations imposed by spherical aberration in electron microscopes of that time (1). [Gabor was, in turn, inspired by Sir Lawrence Bragg's crystallographic "x-ray microscope" (2)—which is nicely discussed by Collier *et al.* (3)—although Pluta (4) argues that this principle was first described by Wolfke (5)]. Gabor's concept of "microscopy by reconstructed wavefronts" involved recording the interference pattern between a uniform 'reference' wave and the wave scattered off of a specimen, and reilluminating the processed hologram to recreate the specimen wave with the specimen no longer present. He hoped to correct for electron lens aberrations by introducing an optical lens with identical aberrations into the hologram reconstruction step; in re-illuminating the hologram from the opposite side of the electron exposure direction, the light would retrace a path optically equivalent to what had been followed by the scattered electron beam, and thus produce an aberration-free, magnified copy of the optical wavefield from the specimen (plus other unwanted waves) for subsequent examination. Holography has since been advanced considerably with the advent of the laser and the introduction of other geometries such as the off-axis geometry of Leith and Upatnieks (6). Reviews of applications in electron (7) and optical (8) holography have been presented elsewhere.

## X-RAY HOLOGRAPHY

The possibilities of doing holography with x-rays were first considered in 1952. In that year, El-Sum and Kirkpatrick obtained a visible light reconstruction (9) of an Al  $K$  ( $8.3 \text{ \AA}$ ) x-ray interference image recorded by Kellström two decades earlier (10) that was, in essence, the hologram of a wire. Baez considered the possibilities of x-ray holography as a microscopic technique (11), drawing upon the interpretation of Gabor holograms as superpositions of zone plates that had been developed by Rogers (12). Baez concluded that film grain would limit the resolution, that the size of the illumination source would have to be kept small, and that Fresnel zone plates might be used instead in an imaging x-ray microscope. Baez and El-Sum later succeeded in recording the x-ray diffraction pattern of a wire (13), and Baez also constructed zone plates for preliminary studies in x-ray microscopy (14).

Hopes for obtaining high resolution in x-ray holography were boosted by the introduction of the Fourier transform geometry (15), where the resolution is limited not by film grain but by the size (or, in principle, knowledge of the spatial frequency distribution) of a reference point source of radiation. Several authors subsequently presented theoretical discussions of x-ray microscopes based upon Fourier transform holography (16–25). These studies all came to a similar conclusion that Fourier transform x-ray holography should be able to produce high resolution images as x-ray sources and optics improved.

Unfortunately, experimental demonstrations of high resolution x-ray holography were slow in coming. In 1967, Saccocio made an attempt to record a plane-grating hologram

using Cu *K* x-rays (1.54 Å), which failed because of film resolution and sensitivity problems (26). Two years later, Giles used an electron microprobe to generate Be *K* (114 Å) x-rays and form a Gabor hologram of a 6 μm diameter wire (27). He was able to obtain a reconstruction of sorts by re-illuminating his photographic film hologram with a visible light laser. Much more encouraging results were obtained in Japan in the early 1970s by Aoki, Kikuta, and collaborators. They began by using a C *K* (44.8 Å) microfocus x-ray tube to record a one-dimensional Fourier transform hologram of a series of slits. The hologram was recorded on photographic film, and a visible light laser was used to obtain a somewhat noisy reconstruction with resolution better than 10 μm (28). They then repeated the identical experiment using 60 Å x-rays from a synchrotron source plus monochromator, and obtained a slightly cleaner hologram and reconstructed image (29). Finally, they used an Al *K* (8.34 Å) microfocus x-ray tube to obtain several impressive Gabor holograms (30). In one example, they obtained images (again using photographic film for hologram recording and visible light laser reconstruction) of several 1.8–6 μm chemical fibers in which they could focus through and differentiate between fibers located ~4 mm apart in depth. In another example, they recorded the first x-ray holograms of biological specimens (red blood cells); however, the quoted resolution of about 4 μm was too poor to allow anything but the outline of the cells to be observed. Motivated by Aoki's results, Kondratenko and Skrinisky in 1977 considered the coherence of synchrotron radiation in further detail, and concluded that storage rings of that time provided about 10<sup>3</sup> more coherent flux than x-ray tubes (31). From 1983 to 1986, Howells and collaborators performed a series of experiments using 32 Å x-rays from a storage ring and monochromator for recording Gabor holograms of wires, glass beads, and diatoms (32–35). These studies produced images at 1–2 μm resolution, with the resolution again limited by film grain size and aberrations due to the wavelength change from recording the holograms at 32 Å to reconstructing the image at 4416 Å.

Seeking to move beyond the film grain limitations that had held Aoki and Kikuta to μm-range resolution, Reuter and Mahr experimented in 1976 with the use of a commercially available Fresnel zone plate as the reference-wave-forming optic for recording Fourier transform x-ray holograms (36). By focussing the beam from a scanning electron microscope onto a graphite target to obtain C *K* (44.8 Å) x-rays, they were able to record two-dimensional holograms of other zone plates as test objects and obtain reconstructed images using a visible light laser. However, their results were limited by a variety of factors to a resolution of several μm, and the images exhibited poor signal-to-noise ratio. A decade later, Aoki and Kikuta used the quasi-monochromatic radiation ( $\lambda \approx 15.5$  Å) of an undulator without a monochromator to record a Fourier transform hologram of a series of holes in a foil (37). The improved illumination provided by the undulator greatly reduced the appearance of noise on the hologram as compared with the results of Reuter and Mahr, but the visible light reconstructed image was limited by aberrations and the size of the reference pinhole to ~10 μm resolution.

In 1956, Ladd, Hess, and Ladd demonstrated a novel approach for bypassing the limitations of film grain size and of optical microscope resolution in observing sub-micron detail in contact x-ray microradiography. Their technique involved replacing the photographic film with ammonium dichromate crystals as a "grainless" recording medium, and enlarging the

image thus recorded using a transmitted electron microscope (38). The low sensitivity of this and other non-silver halide recording media made such work difficult, however, and only limited use of the technique was made at that time (39). In the early 1970s, the photoresist polymethyl methacrylate (PMMA), which had been developed as a resist for electron beam lithography (40), was found to be sensitive to soft x-rays (41); by 1976, Feder and collaborators had used PMMA as a recording medium, C K x-rays and synchrotron sources, and a scanning electron microscope (SEM) for sub-optical resist readout to obtain contact microradiographs with greatly improved image quality and resolution (42). The intrinsic resolution of these resists as soft x-ray image recorders appears to be around 100 Å, and considerable effort is now devoted to understanding and improving x-ray resists because of the commercial lure of x-ray lithography for fine linewidth integrated circuit manufacture (43).

Recognizing in 1974 that such photoresists could make it possible for Gabor x-ray holography to achieve high resolutions, Bjorklund and collaborators explored their use for holography with an 1182 Å harmonic line from a Nd:YAG laser (44). By using a Lloyd's mirror arrangement to produce the desired interference pattern, and SEM enlargement of the developed resist relief pattern, they were able to record a holographic grating spacing of 836 Å. They then went on to record VUV holograms of 1.3–0.37 μm latex spheres. They chose their specimen-hologram working distance to be only 25 μm, recognizing that this minimized the hologram area to be coherently illuminated while simultaneously allowing them to record Fraunhofer (far-field) holograms. The holograms thus recorded showed fringes reaching out to a radius of 5–10 μm, suggesting that a reconstructed image resolution of 0.2–0.4 μm might have been attainable. However, SEM imaging of the resist relief pattern was suitable only for qualitative examination of the hologram information, so no reconstructions were obtained. Bjorklund did present a possible solution to this problem involving the production of a metal-shadowed acetylcellulose relief pattern replica (44). He reported only limited success with transmission electron microscope (TEM) enlargement of such hologram replicas, and pointed out that the non-linearities of such recordings of hologram intensities would degrade the quality of the reconstructed image. A plan for numerically correcting for resist non-linearities and metal shadowing was presented, and digital computer reconstruction of the numerical data set was discussed.

Two years after Bjorklund's work appeared, Mueller and Jorna considered the recording of Gabor x-ray holograms with an eye on attaining 1 Å resolution; they concluded that a variety of problems, including radiation damage, would limit resolution to perhaps 20–100 Å, and that  $\lambda=10\text{--}20$  Å x-rays should be used (45). They also suggested numerical reconstruction of such holograms with the reference wave mathematically removed from the reconstructed image. Gur briefly considered the problem, and suggested that holograms could be recorded at 2 μm distance from a 1 μm specimen using 100 Å x-rays, again with TEM hologram readout and numerical reconstruction (46). Sayre and Feder proposed making multiple recordings of the near-field diffraction pattern using photoresists, and using them to numerically reconstruct an image of the object (47). Aristov *et al.* suggested using photoresists, SEM hologram readout, and numerical reconstruction in Gabor holography (they appear not to have been aware of Bjorklund's work) (48). More recently, Spiller has

considered the problems posed by speckle noise in x-ray holography (49). Howells (50) and Jacobsen (51) have considered x-ray scattering by resolution elements and the consequences for hologram formation, and London *et al.* (52) have studied scattering by smaller, spherical objects in an aqueous environment. These latter studies have concluded that  $\sim 200 \text{ \AA}$  resolution holograms will require that biological specimens be exposed to a radiation dose of  $10^8$ – $10^9$  rad; though high, such a dose is smaller than that usually required for electron microscopy.

X-ray lasers have come to figure prominently in discussions of x-ray holography. In 1975, Chapline and Wood wrote an article in *Physics Today* on x-ray lasers which suggested that one possible application of  $\lambda = 1 \text{ \AA}$  lasers would be three-dimensional, atom-scale resolution imaging of DNA replication in living cells (53). In a series of letters to *Physics Today* (53), Trammel on the one hand and Chapline and Wood on the other debated this suggestion. Trammel's arguments were based on an earlier paper written with Breedlove (54) which concluded that atomic resolution x-ray microscopy was impossible due to the fact that for every photon coherently scattered by an atom at  $\lambda \sim 1 \text{ \AA}$ , about 10 would be absorbed and would thus ionize the atom and dramatically damage its bond with a molecule. X-ray lasers again entered into discussions of x-ray holography when, in early 1981, rumors surfaced that researchers at Lawrence Livermore National Laboratory had developed a nuclear bomb pumped x-ray laser with a wavelength of  $14 \text{ \AA}$  (55). Scientists there began "sounding out the interest of non-Livermore investigators in a holography experiment" (56); however, no results have appeared, at least in the open literature. Solem and collaborators considered x-ray holography with an emphasis on understanding the benefits and limitations of several recording geometries, and x-ray interactions and their implications for "freezing" motion in living cells using picosecond exposures such as might be produced by an x-ray laser (22, 23, 25, 57). In 1984, laboratory x-ray lasers were announced with lines at  $206$  and  $209 \text{ \AA}$  (58) and at  $182 \text{ \AA}$  (59). While recognizing that these lasers were not yet capable of recording high-resolution holograms of hydrated biological specimens (60), Trebes *et al.* recorded and reconstructed holograms of wire structures at a resolution of a few  $\mu\text{m}$  (61); this was one of the first experimental applications of x-ray lasers, and the holograms were notable for the fast ( $\sim 200$  picoseconds!) exposure time involved. More recently, laser gain has been reported at wavelengths as short as  $50.3 \text{ \AA}$  (62), and London *et al.* have suggested that x-ray wavelengths slightly *longer* than that of the Carbon *K*-edge will lead to minimal dose imaging of hydrated biological specimens with  $\leq 50$  psec exposure times (52). Haddad *et al.* have proposed using a spherical reference scatterer for forming x-ray laser holograms in the Fourier Transform geometry (63), and XUV free-electron lasers have been considered for x-ray holography (24, 64).

While x-ray lasers offer promise for the future, undulators on storage rings have demonstrated a soft x-ray brightness three orders of magnitude greater than that produced by bending magnet sources, and thus at least six orders of magnitude greater than that available from x-ray tubes (65). Undulators have been used by several groups for x-ray holography. In 1985, Aoki and Kikuta used the quasi-monochromatic radiation ( $\lambda \approx 15.5 \text{ \AA}$ ) of the Photon Factory PMU-2 undulator without a monochromator to record a Gabor hologram of chemical fibers on PMMA with a specimen-hologram separation distance of  $22.3 \text{ mm}$  (37).



The resist was deposited on an opaque substrate, so that only an optical micrograph of the hologram was presented, and no reconstructed image has been reported.

In 1987, Joyeux *et al.* recorded Gabor holograms using 100 Å radiation from an undulator and monochromator on the storage ring ACO (66). Their holograms of diatoms were recorded on the resist Olin-Hunt 320F with a specimen-hologram separation distance of ~ 5 mm. Drawing upon experience gained in visible light holography, they preexposed their resists to UV radiation so as to work in the approximately linear part of the resist exposure response curve. Using the developed resists as phase holograms for 6320 Å laser reconstruction, they have achieved 0.5–0.6 μm resolution in the imaging of silica skeletons of diatoms (see Fig. 1). More recently, they have proposed a scheme in which the developed photoresist hologram would be metallized so as to serve as a reflection hologram for reconstruction with UV radiation. By using aberration cancelling optics and scanning the hologram so that the image would be built up pixel-by-pixel along the optical axis, they believe that an image resolution of 1000 Å or better could ultimately be obtained (67).

In 1986, Howells *et al.* used 25 Å radiation from an undulator and monochromator to record Gabor holograms at the NSLS X-ray storage ring (68, 51). The holograms were of rat pancreatic zymogen granules, and were recorded at a specimen-hologram separation distance of 400 μm using the photoresist PMMA deposited on a Si<sub>3</sub>N<sub>4</sub> window. Jacobsen *et al.* enlarged the metal-shadowed resist holograms using a transmission electron microscope (TEM), digitized the magnified hologram using a scanning microdensitometer, and obtained numerically reconstructed images with sub-1000 Å resolution (51, 69, 70) (see Fig. 2). Fringe widths as small as 500 Å can be observed in these holograms, and examination of their power spectra has suggested that they contain information at the sub-200 Å level. This group has also explored readout of the developed holograms with an atomic force microscope.

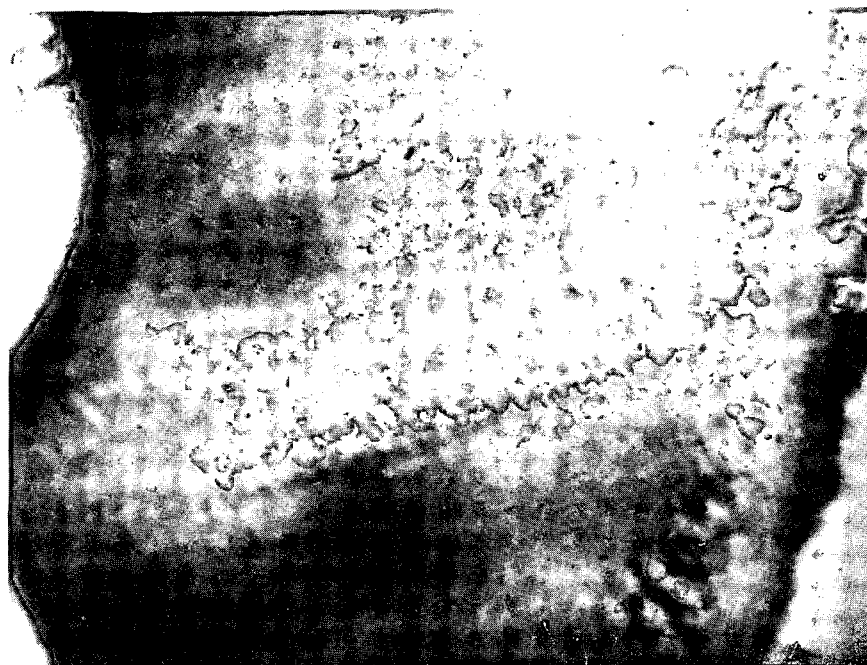
## SUMMARY

X-ray holography has received renewed attention as a method for soft x-ray microscopy of biological specimens. The promise of the technique has been recognized for many years, but progress was long frustrated by the limitations of x-ray sources and detectors. Soft x-ray undulators have recently served as dramatically improved x-ray sources, and x-ray lasers are beginning to demonstrate their potential as well. Photoresists are now being used to record holograms which do not suffer from the resolution limitations of photographic film grain. As a consequence, x-ray holography has reached a milestone in its development by producing images of biological specimens at sub-micron resolution. Further progress is to be expected; in particular, several groups are preparing Fourier Transform x-ray holography experiments which will exploit other advances in x-ray optics (e.g., Fresnel zone plates) and detectors (e.g., charge coupled devices). It is reasonable to expect that future developments will include improved image quality and resolution, and the *application* of x-ray holography to structural studies of both dry and hydrated biological and materials specimens.

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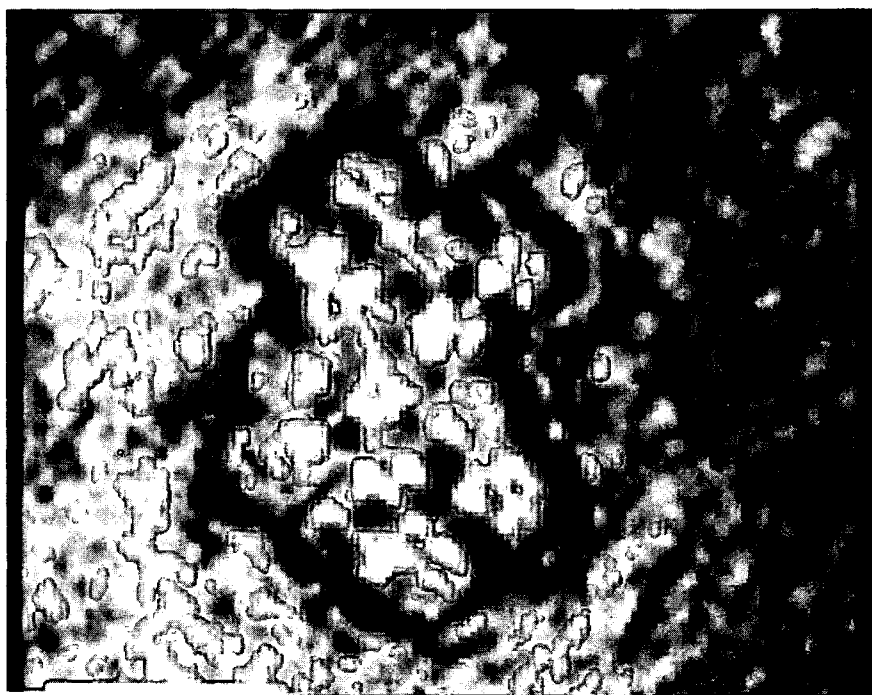
Figure 1 of C. Jacobsen, "X-ray holography: a history."



ZBB 893-2425

**Figure 1.** Reconstructed image of the silica skeleton of a diatom obtained by Joyeux *et al.* using 100 Å radiation from an undulator and 6320 Å laser reconstruction of the developed photoresist hologram. A  $48 \times 63 \mu\text{m}^2$  field is shown. From Ref. 67; used with permission.

Figure 2 of C. Jacobsen, "X-ray holography: a history."



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Figure 2. Reconstructed image of rat pancreatic zymogen granules obtained by Jacobsen *et al.* using 25 Å radiation from an undulator and numerical reconstruction of the TEM-enlarged and digitized hologram. A  $3.0 \times 3.0 \mu\text{m}^2$  field is shown. From Ref. 70; used with permission.

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