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**Progress in high-resolution x-ray holographic microscopy**

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Among the various types of x-ray microscopes that have been demonstrated, the holographic microscope has had the largest gap between promise and performance. The difficulties of fabricating x-ray optical elements have led some to view holography as the most attractive method for obtaining the ultimate in high resolution x-ray micrographs; however, we know of no investigations prior to 1987 that clearly demonstrated submicron resolution in reconstructed images. Previous efforts [1] suffered from problems such as limited resolution and dynamic range in the recording media, low coherent x-ray flux, and aberrations and diffraction limits in visible light reconstruction. We have addressed the recording limitations through the use of an undulator x-ray source and high-resolution photoresist recording media. For improved results in the readout and reconstruction steps, we have employed metal shadowing and transmission electron microscopy, along with numerical reconstruction techniques. We believe that this approach will allow holography to emerge as a practical method of high-resolution x-ray microscopy.

All earlier work in x-ray holography in which reconstructions were obtained made use of x-ray film as the recording medium. It has been known since the earliest days of thinking about x-ray holographic microscopy that the resolution in the Gabor geometry can be no better than the film grain size [2], which precludes sub-100 nm resolution at soft x-ray wavelengths [3]. This has led some [4,5] to turn to turn to x-ray photoresists for recording holograms. The ultimate resolution of these photoresists is said to approach 5 nm, and they have good detective quantum efficiency [6]. However, they also suffer from low sensitivity (a necessary concomitant of small "pixel" size), and making full use of their high resolution for holography has proven to be challenging. Recently, we [7] and others [8] have taken separate approaches to overcoming these challenges, and have obtained submicron resolution in reconstructed resist holograms.

The geometry used for the recording of the holograms has been described elsewhere [7]. We used the NSLS mini-undulator beamline X17t [9] as a source of 2.5 nm x-rays, with a toroidal grating monochromator providing temporal coherence and a pinhole to provide spatial coherence. Our resulting coherent flux of typically  $10^8$  photons/sec [7,9] was more than 100 times larger than that obtained at the NSLS bending

magnet beamline U15 [10]. Besides greatly improving both exposure time and quality, this dramatically increased illumination greatly simplified the photometry (the coherent flux at X17t was sufficient to produce a photoyield of typically  $10^{-10}$  Amps on an absolutely calibrated aluminum photodiode [11]) and alignment (the coherent x-ray spot was visible when viewed on a phosphor, even with room illumination) of the experiment. The holography chamber and collimating pinhole sat on a  $10 \times 3 \times 1$  foot<sup>3</sup> granite table, supported by vibration-isolation air pistons; consequently, any vibrations between the pinhole and the specimen-recorder package (shown schematically in Fig. 1 of Ref. [7]) were negligible compared to the 50  $\mu$ m pinhole size.

Most of the holograms are of fixed and dried zymogen granules from rat pancreas acinar cells. Not only is there considerable interest in the ultrastructure of zymogen granules [12], but their small size (roughly 1  $\mu$ m across) allowed us to satisfy the desirable condition of having a largely empty object plane to minimize corruption of the reference beam. The use of thin silicon nitride windows (~60% transmittive) and thin layers of resist (~80% transmittive) means that each hologram recording layer removes slightly more than half of the 2.5 nm photons from the beam [13], so that the downstream holograms still have quite good illumination. This permits us to record several holograms simultaneously; each hologram contains essentially the same information on the specimen, and this redundancy may prove useful for averaging out noise and speckles in the reconstructed image. The thin resists and windows also allow for direct examination of the developed and coated resist images in a transmission electron microscope.

We had previously attempted to record holograms in this manner at the bending magnet beamline U15, and had obtained no better results than the hologram of a diatom fragment shown in Fig. 1A. We now believe that the poor fringe count and contrast on the hologram were due to inadequate flux (which we estimate was in the range of  $10^4$  photons/ $\mu$ m<sup>2</sup> at 3.2 nm, acquired over 10 hours) and non-optimal hologram read-out. There are indications that wet development side-cutting of step-function exposure variations on polymethyl methacrylate (PMMA) resists is quite severe for absorbed doses less than roughly 100 megarads, or  $10^7$  photons/ $\mu$ m<sup>2</sup> at 2.5 nm wavelength [14]. This implies that extremely high photon fluxes are required to record high spatial frequency information in photoresists. (Others have come to a similar conclusion by considering the shot noise of photons illuminating  $(5 \text{ nm})^2$  "pixels" of PMMA [15]. Finally, the U15 holograms were examined by the standard method of SEM imaging of the developed and normal-incidence-metallized resist surface, which we now feel is inappropriate for detecting the shallow height modulations of a few tens of nanometers expected for hologram fringes. Considerable improvements in the imaging of fine height variations on resists have been demonstrated by specialized SEM techniques [16] and by various replica methods for TEM examination [17, 18], and we feel that this problem can benefit greatly from further study.

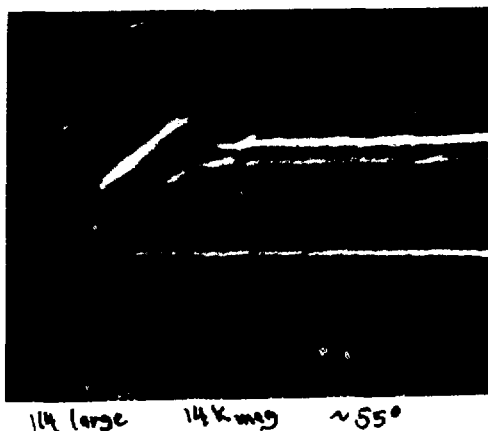


Fig. 1A

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Fig. 1B

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Figure 1. A: Hologram of a diatom fragment taken at the NSLS bending magnet beamline U15 and examined with an SEM. B: Hologram of a zymogen granule taken at the NSLS undulator beamline X17t and examined with a TEM. Both holograms were taken at a working distance of about 400  $\mu\text{m}$ , and are sub-fields of  $(200 \mu\text{m})^2$  total hologram areas.

Because of the increased coherent flux of the X17t undulator, we were able to increase the exposure of our more recent holograms to typically  $10^7$  photons/ $\mu\text{m}^2$  at 2.5 nm, acquired in about an hour. This exposure was high enough to see a light image of the specimen support grid bars on the undeveloped resist, and the resist required only light development (immersion for 0.5-3 minutes in 17% methyl isobutyl ketone-83% isopropyl alcohol was typical). In order to avoid the lateral distortions of the hologram that could potentially arise in replica methods, we evaporated a metal coating of  $\sim 20$  nm of 60% Pd-40% Au onto the developed resist surface at typically  $7^\circ$  grazing angle for direct TEM examination. When processed in this manner, developed resists of nominally 200 nm initial thickness on silicon nitride substrates remained stable in a low-dose 80-100 KeV TEM beam, although resist mass loss effects were invariably observed [18]. As can be seen in Fig. 1B, these holograms show a much greater information content than the lower flux, SEM-examined hologram of Fig. 1A.

A known drawback of using photoresists as a holographic recording material is that the resist thickness after development in the appropriate solvent is a non-linear function of incident illumination [19]. We have attempted to correct for this with an approximate model of the resist imaging process [20]. X-ray photoresists have been shown to respond to the absorbed x-ray dose independent of photon energy [21], so the first step is to calculate the dose absorbed by the resist as a function of incident x-ray intensity. Using published data on resist dissolution rate as a function of absorbed dose [8, 14, 22], one can then estimate the thickness variations of the developed resist. Finally, the TEM image contrast of thick, low-Z specimens has been modeled [23], and the response of electron microscope image films to incident electron illumination is well understood [24]. An example calculation of normalized electron film density as a function of incident x-ray intensity for typical resist parameters is shown in Fig. 2. The model indicates that an incident x-ray flux of  $3 \times 10^7$  photons/ $\mu\text{m}^2$  yields maximum electron film density (the resist has been fully developed away); this agrees nicely with the patches in Fig. 1B where the resist has been completely developed away with a measured peak incident flux of  $2.7 \times 10^7$  photons/ $\mu\text{m}^2$ . The model as it now stands is certainly incomplete, however, since it estimates the response of the photoresist to uniform illumination, while what in fact is desired is an estimate of the resist modulation transfer function (MTF) [25]. Once the resist MTF is estimated and we are able more accurately calculate developed resist surface relief, we will be able to follow a previously outlined method [19] for correcting for the effects of metal shadowing.

When holograms are to be reconstructed at a wavelength significantly different from the recording wavelength, aberrations will severely degrade the image resolution unless corrective optics are used [8] or the hologram is appropriately scaled. Because of the need to correct for resist non-linearities as well as the desire to implement hologram processing methods that are not available optically, we have instead chosen to pursue numerical reconstructions of the x-ray holograms. Towards that end, we have had several TEM negatives of holograms digitized with a microdensitometer, a step that also makes

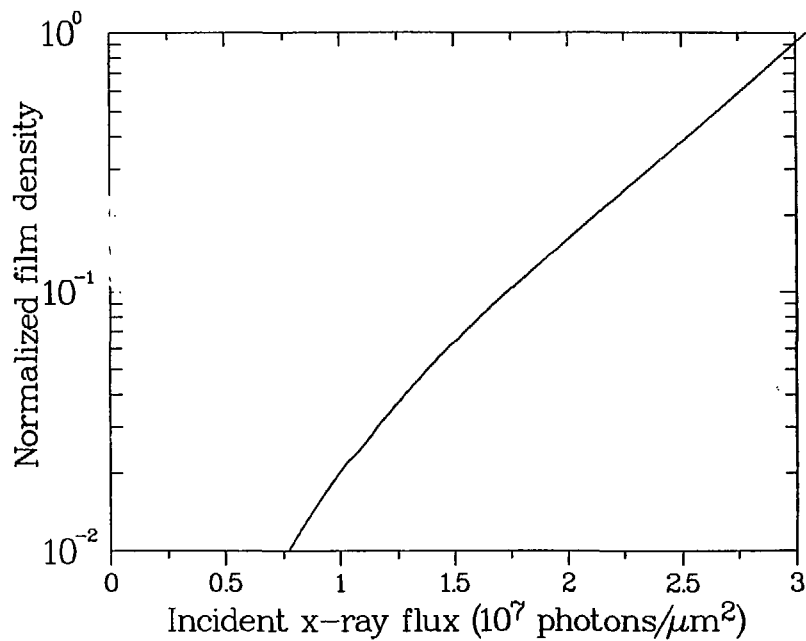


Figure 2. Model calculation of normalized TEM film density as a function of incident x-ray intensity for a 300 nm thick PMMA resist.

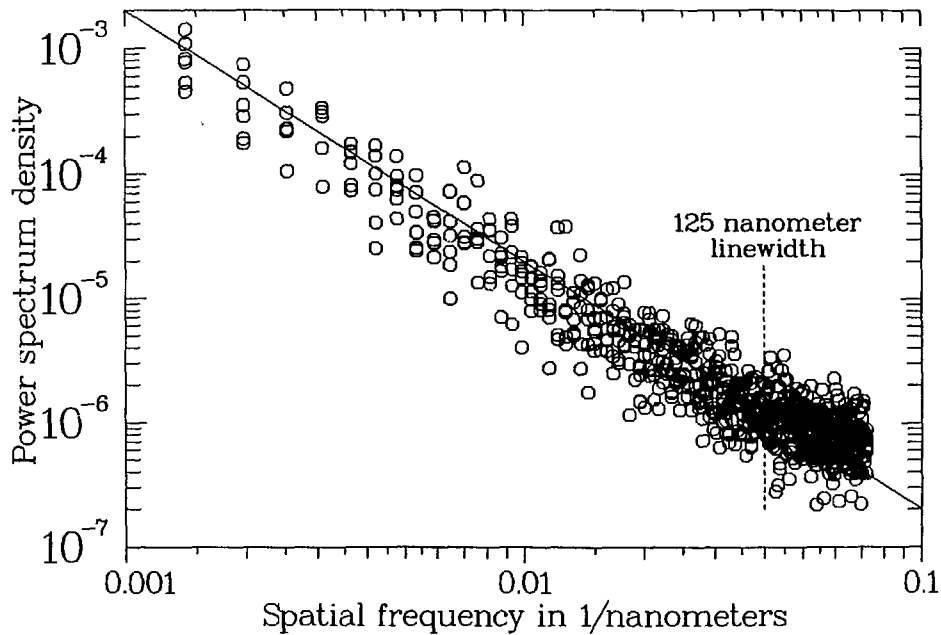


Figure 3. Power spectra of several scan lines taken across the hologram shown in Fig. 1B. Scan lines taken both roughly parallel and perpendicular to the direction of shadowing yield similar results.

possible quantitative evaluation of the quality of the recorded holographic data. After correcting for resist non-linearity using the model sketched above, we took a random series of line scans across several holograms and calculated their power spectra. The results for one hologram (which are typical for the others so far examined) are shown in Fig. 3. As can be seen, the power spectral density falls off roughly as the inverse of spatial frequency up to approximately  $0.04 \text{ nm}^{-1}$ , after which it appears to roll off to white noise. (Fringes are visible by eye on the TEM negative to a spatial frequency of about  $0.01 \text{ nm}^{-1}$ ). This suggests a finest recorded spatial period of 25 nm, or a minimum Fresnel zone width of 13 nm. A similar result is obtained if resist non-linearities are not corrected for.

Numerical reconstructions of Gabor holograms have been studied by many [26,27]. By considering the hologram to be a transparency that modulates the illuminating wave amplitude, one can use the Fresnel-Kirchoff diffraction integral to propagate the wavefield a distance  $z$  from the hologram plane  $(\xi, \eta)$  to the reconstruction plane  $(x, y)$ , at which point the reconstructed image intensity is obtained. In the Fresnel approximation, this can be written as

$$I(x, y) = \left| F \left\{ r(\xi, \eta) \exp \left( i \pi \frac{\xi^2 + \eta^2}{\lambda z} \right) \right\} \right|^2. \quad (1)$$

Thus, multiplying the two-dimensional hologram transmittance  $r(\xi, \eta)$  by the quadratic phase factor

$$\exp \left( i \pi \frac{\xi^2 + \eta^2}{\lambda z} \right) \quad (2)$$

and then performing a Fourier transform  $F\{\}$  (implemented digitally with an FFT algorithm) will produce a Fresnel transform.

We have implemented this numerical reconstruction scheme, and have used it to reconstruct the hologram shown in Fig. 1B and thus obtain the reconstructed image shown in Fig. 4. Because the hologram is at a few far-fields from the specimen, and because the "shadow" of the specimen has been apodized [28], the twin image noise inherent in Gabor holography has been eliminated (although diffraction around the Gaussian-smoothed edges of the apodizing mask itself corrupts the image to some degree). Consideration of the sampling theorem dictates that the Gabor hologram pixel size  $\Delta_\xi$  be set to the diffraction-limited spot size

$$\Delta_\xi = \frac{\lambda}{2 \text{N.A.}} = \sqrt{\frac{\lambda z}{N}} \quad (3)$$

(where  $N^2$  is the number of hologram pixels), while the condition

$$(\Delta_\xi \Delta_\eta N / \lambda z) = 1 \quad (4)$$



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Figure 4. Reconstruction of a hologram similar to that shown in Fig. 1B. Refer to text for discussion.



must be satisfied in order to have a discrete Fourier transform relationship in the reconstruction integral (1). This leads to the conclusion that the reconstruction pixel size  $\Delta_s$  will also be equal to the diffraction-limited spot size of the numerical aperture of the hologram. For the case of a  $512 \times 512$  pixel sampling of the hologram reconstructed in Fig. 4, this leads to a pixel size of 42 nm; the amount of computer time needed to perform such a reconstruction is roughly five minutes on a MicroVAX II minicomputer.

We would expect that the image quality of the reconstruction would be somewhat degraded by complications such as speckle and recording non-linearities that were not completely corrected for. The spot size of any speckle pattern should be on the same order as the diffraction-limited spot size, and since we do not see large pixel-to-pixel noise fluctuations, we conclude that the observed variations in image intensity in Fig. 4 are not due to speckle. Non-linearities in the recording of hologram intensity are more problematic. If one imagines a sine wave fringe pattern distorted into a square wave, the "extra" high spatial frequencies will manifest themselves as higher-order images (analogous to higher-order zone plate foci) and an artificial enhancement of high-spatial-frequency information on the specimen. This may be the explanation behind the bright edges and dim center of the grid bar shown in the upper right hand corner of Fig. 4. While this artifact leads us to regard the reconstructed image shown as a preliminary one, the image is reminiscent of scanning transmission x-ray micrographs taken of hydrated specimens of the same type of sample [12]. Finally, line scans taken across the grid bar edge (which may not be perfectly sharp when viewed with soft x-rays) demonstrate a knife edge transition occurring over one to two pixels, indicating that our current reconstructions have sub-100 nm resolution [29].

There are a variety of ways in which we hope to improve upon these preliminary investigations [20]. With the NSLS X1 undulator that is to be commissioned in 1988, we hope to reduce the exposure time from about one hour to a few minutes. We have used a wet cell to record holograms of hydrated specimens; examination of some of these holograms suggests that x-ray absorption in water may have reduced the exposure below the level needed to record high resolution information. Transmission electron micrographs of a carbon replica of a crossed grating have shown us that the TEM used did not suffer from image field distortions at the micron level, although such distortions may become an issue at finer scales. We feel that there is much to be learned about the handling of photoresists, both in terms of finding ways to decrease the exposure (perhaps by adding dopants to increase the x-ray absorption of the resist) and in obtaining linear resist image readout without having to resort to metal shadowing. We have only begun to explore numerical hologram reconstruction and techniques such as phase retrieval [27] for further improving the image quality, and we are, of course, keenly interested in developments in flash sources like x-ray lasers [30]. It is our hope and expectation that x-ray holography will soon have advanced from the point of demonstration to become a useful x-ray imaging technique.

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