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X-RAY HOLOGRAPHIC MICROSCOPY USING THE ATOMIC-FORCE MICROSCOPE*°

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X-ray holographic microscopy using the atomic-force microscope

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1. INTRODUCTION

Although x-ray holography has a long history (as reviewed for example by [Jacobsen 1990]), it is only in recent years that the technologies needed for it to be successful have begun to be available. The advent of undulators and x-ray lasers and the prospect of even more advanced pulsed sources such as high-gain free-electron lasers [Winick 1993] have led to an increase in the level of interest and activity in x-ray holography at several centers around the world. Some recent activities have concentrated on developing the three-dimensional potential of the technique [Trebes, 1990; McNulty, 1992] while others have focused on more accurate and user-friendly methods of image reconstruction [Joyeux, 1989; Koren, 1993]. A new development is the use of the x-ray lasers at Osaka for soft x-ray holography [Shultz 1993]. Most of the activity has been in in-line techniques but high resolution Fourier Transform holography has also been demonstrated [McNulty, 1992] and used.

The present authors have been seeking for some time to improve the resolution of holographic microscopy and have engaged in a continuing series of experiments [Jacobsen, 1990; Howells 1986] using the X1A soft x-ray undulator beam line at Brookhaven. The principle strategy for pushing the resolution lower in these experiments has been the use of polymer resists as x-ray detectors and the primary goal has been to develop the technique to become useful for examining wet biological material. In the present paper we report on progress in the use of resist for high-spatial-resolution x-ray detection. This is the key step in in-line holography and the one which sets the ultimate limit to the image resolution. The actual recording has always been quite easy, given a high-brightness undulator source, but the difficult step was the readout of the recorded pattern. We describe in what follows how we have built a special instrument: an atomic force microscope (AFM) [Lindaas 1992] to read holograms recorded in resist. The AFM was introduced in 1986 by Binnig, Quate and Gerber [Binnig, 1986] and is now available commercially from several vendors. We report our technical reasons for building, rather than buying, such an instrument and we give details of the design and performance of our device. We also describe our first attempts to use the system for real holography and we show results of both recorded holograms and the corresponding reconstructed images. Finally, we try to analyze the effect that these advances are likely to have on the future prospects for success in applications of x-ray holography and the degree to which the other technical systems that are needed for such success are available or within reach.

2. OVERVIEW OF THE STATUS OF X-RAY HOLOGRAPHIC MICROSCOPY

The production of high-resolution holographic images of two-dimensional objects was demonstrated several years ago [Joyeux 1988, Jacobsen 1990] and we may ask why the technique has not been used in a more routine way since then. The reasons are (i) the difficulty in reading the hologram, which we address in this paper, (ii) the fact that soft-x-ray undulators are scarce and, for the moment, only they can give holograms with good signal-to-noise ratio and (iii) because the radiation dose to the sample needed for high-resolution hologram recording is high ($\gtrsim 10^2$ Mrad). The question of dose is the most fundamental. We know that a true three-dimensional reconstruction will require a diffraction tomography experiment [Spiller, 1987; Devaney, 1986] and although it appears that x-ray holography is the right way to record each view in such an experiment [Howells, 1990], many views, probably dozens, will be required. Moreover, it appears as though such a sequence will not be possible for unfrozen biological material without alteration of the sample during data collection. The traditional response to the damage problem in x-ray imaging is to propose a flash experiment [Solem, 1982, 1984]. One of the advantages of holographic imaging is compatibility with this type of exposure. However, there is still a problem in acquiring a *sequence* of flash images because the first one or first few may still change the sample. There are two ways out of the dilemma. One is to argue that there is no dilemma. Given that the data from each view will be combined in forming the final three-dimensional image, we might argue that the total counts needed (and hence the total dose) may not be much greater than for a single high-quality two-dimensional image [Nugent 1993]. The second way is to perform a flash experiment where *all* the views are taken simultaneously [Trebes 1990]. We consider these two possibilities in the sections that follow.

2.1 The Question of the Dose per View in Diffraction Tomography

Nugent and Trebes [Nugent 1993] have speculated that the following rule might apply for calculating the dose per view in a diffraction tomography experiment. Suppose that N absorbed x-rays per pixel are needed to acquire a two-dimensional image with a given resolution and signal-to-noise ratio. The proposed rule then states that if m views are used then the number of absorbed x-rays per pixel for each view required to reconstruct a three-dimensional image with the same resolution and signal-to-noise ratio as before, would need to be N/m. If this were true then the dose needed for the three-dimensional image would be about the same as the two-dimensional. The reasoning behind the idea is based on an image reconstructed by unfiltered back propagation. That means simple addition of the waves backpropagated from the planes where their amplitudes and phases were recorded (as holograms) for each view.

This is a complex subject and the above proposition is a preliminary though very interesting result. For the moment there is no final answer regarding its validity. However, a recent calculation performed at Lawrence Berkeley Laboratory [Llacer 1993] provides useful insight and some evidence of the correctness of the theorem within the limitation of reconstruction by unfiltered back propagation. The calculation consisted of image reconstructions based on model data from a soft x-ray absorption experiment with conventional (geometrical optical) tomographic geometry. The reconstructions were made by two methods: unfiltered (simple) backprojection and filtered back projection. The backprojection methods in conventional tomography are the direct analogs of the corresponding *backpropagation* methods in diffraction tomography. The conclusions were roughly as follows. For a given dose for a particular test case, the filtered back projection method gave a signal-to-noise ratio of 3.6 which was within a factor 1.5 of the expected value according to two separate theoretical methods which are known to predict the behavior of tomographic reconstruction algorithms. For the same dose and test case the simple backprojection method gave a signal-to-noise ratio of 481. This was in good agreement with the value predicted by the argument of Nugent and Trebes, namely, the signal-to-noise ratio of an individual projection multiplied by the square root of the number of projections. However, the result is not a cause for celebration. The spatial resolution of images reconstructed by unfiltered backprojection is so bad that they are useless for almost all practical purposes and are never used by workers in the tomography community. This follows as a consequence of the fact that optical density measured by integrating along a certain "tube" of specimen space is simply assumed to be *distributed uniformly* throughout the whole length of the tube.

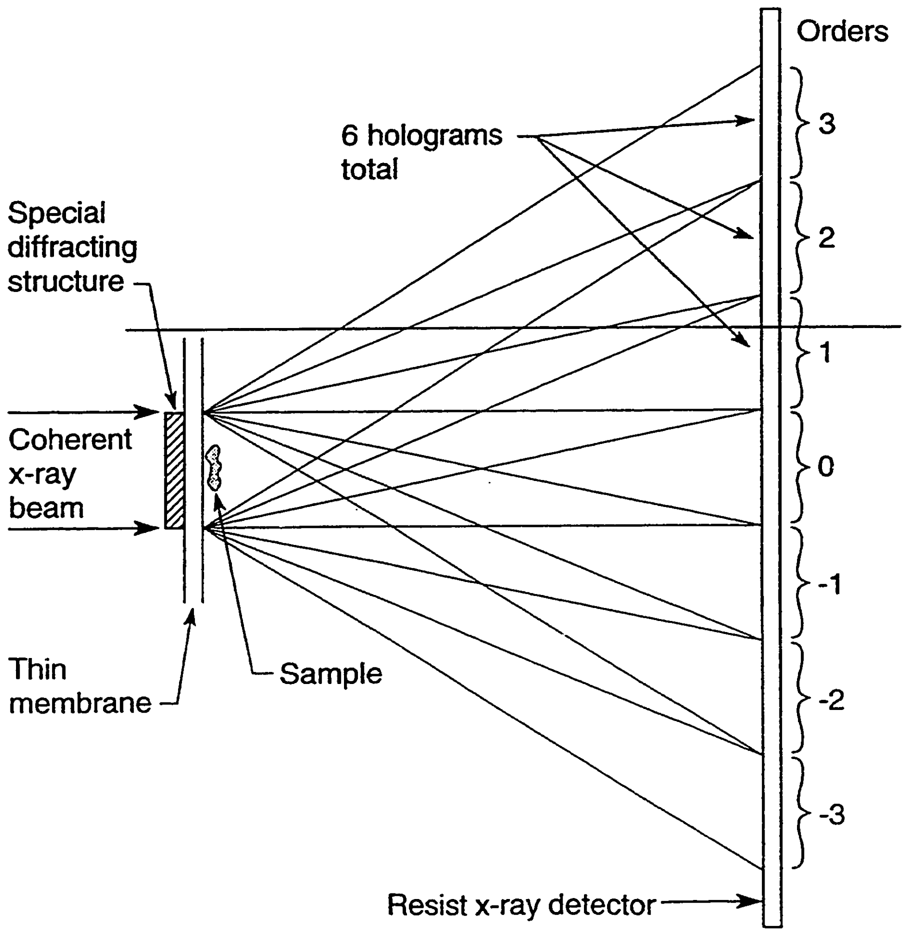

Fig. 1. Principles of a possible method of one-shot flash tomography. A total of six holograms are shown but they are part of a two-dimensional array of 7x7-1=48

The indications from this are, therefore, that the speculation of Nugent and Trebes is probably correct but is limited to reconstructions by simple backpropagation or backprojection which are generally of too poor a quality to be useful. Evidently a good signal-to-noise ratio alone is not enough for an image to be useful.

2.2 Possibility for One-shot Flash Tomography

In order to illuminate a sample with a considerable number of beams with different directions at the same time we need a device that can receive a single coherent beam as input and deliver a multiplicity of similarly coherent beams in a variety of directions as output. The setup could be as shown in Fig. 1 with the "beam splitter" mounted on the upstream side of a thin membrane and the sample on the downstream. The beam splitter sounds similar to a crystal in a crystallography experiment but we need to investigate further. If we have a monochromatic beam, then for a given orientation of a crystal like silicon the number of diffracted beams may be very few or even zero. We are trying to find cases where a significant number of reciprocal lattice points lie on or within a rocking curve width of being on the Ewald reflection sphere. To increase the chance of a "hit" we could try increasing the density of points in the reciprocal lattice by using a crystal with a large unit cell. However, to do holography we would need to work in a longer wavelength range than conventional crystallography and that reduces the size of the Ewald sphere which again reduces the chance of a substantial number of hits. Protein crystals would be quite inconvenient as optical elements although they would only need to last a few nanoseconds in the beam. Mica suggests itself as having a fairly high d-spacing and some favorable convenience factors. Another variable is the incoming wavelength distribution which could be polychromatic. One would probably not want to use the "white-beam-Laue" geometry because an undulator would be preferable for coherence reasons but the undulator could have several harmonics of substantial strength. Including all these possibilities it may be that crystal diffraction does offer a solution to this problem along the lines suggested but it is not the only possibility.

For the beam splitter idea to work it would have to be thin enough to transmit a major fraction of a soft x-ray beam. This suggests a two-dimensional crystal. The advantage of this would be that the spots in the reciprocal lattice of a three-dimensional crystal become rods for a two-dimensional one. This means that they would be *certain* to make a hit with the Ewald sphere. In other words we would switch from the Bragg equation to the grating equation. Ordinarily, we think of a transmission grating as having a square-wave transmission function with a bar-to-period ratio of about 1:2. This does what we usually want which is to concentrate as much light as possible into the +1 and -1 orders. However we can produce the opposite effect by making the bar-to-period ratio either close to zero or to one. This distributes the diffracted intensity over many orders. We can do the same in two dimensions in which case we would have an array of either towers or holes. The diffraction pattern is the same (by Babinet's principle) for the two cases anywhere outside of the footprint of the incident beam. As an example, an array of 10nm holes on a 50nm grid would give 4% transmission which would be divided among 49 beams on a square grid with the orders m, n such that $-3 < m < 3, -3 < n < 3$ having significant relative strength. If such a system were used to disperse 3nm radiation then a set of 48 holograms with numerical aperture of 1/20 (resolution 30nm) and a maximum angle of over 30° between illumination directions, could in principle be recorded. The low transmission of such a system could be solved by using π-phase-shifting towers instead of holes. The chief practical challenge apart from building the x-ray source would be shielding the detector from debris.

3.0 THE NEED FOR A "DIRECT READ" TECHNIQUE FOR RESIST HOLOGRAMS

Exposure of a polymer resist layer in a holography experiment plus wet development results in a relief pattern which contains the hologram information. It is the relief pattern that we want to read into the computer to allow calculation of the reconstructed image. Until now we have accomplished this by glancing incidence shadowing with heavy metal, transmission electron microscopy with photographic

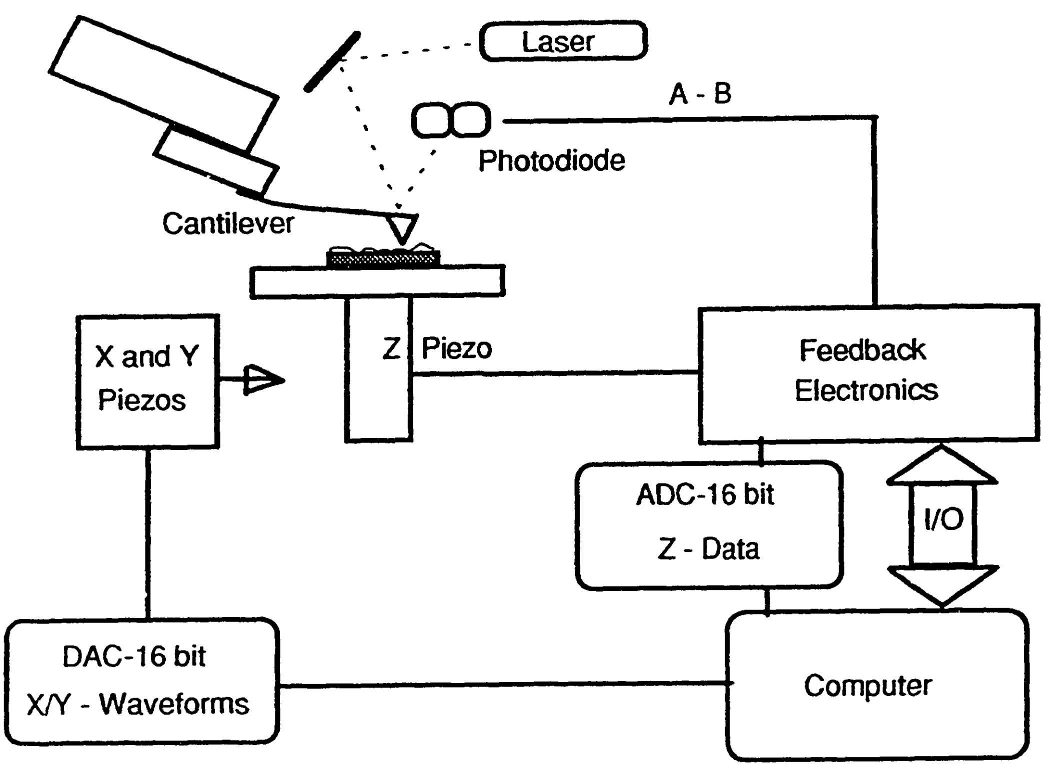

Fig. 2. Schematic of the main components of the atomic force microscope.

recording followed by microdensitometry. This procedure suffers from a number of drawbacks (i) the shadowing is intrinsically inaccurate and nonlinear, (ii) the electron microscope (when set for low damage) suffers from spiral distortion over the large object fields being imaged, (iii) the metallization of the resist prevents further development (an important consideration since it limits monitoring of the development process to light microscope examination which is blind to the finest, resolution-determining fringes, (iv) electron microscopy requires the recordings to be made on thin membranes.

We have therefore developed a "direct read" method based on the atomic force microscope (AFM) [Lindaas, 1992]. The measurement is done in air and is simple, rapid and places a digitized form of the hologram in the computer in a single step. It thus overcomes all of the difficulties of the earlier procedure. The use of an AFM to read resist recordings is not new [Cotton, 1992, Tomie, 1991] but our particular application of it generates new problems. Hologram information is mainly encoded as the position, rather than the depth, of the fringes. Therefore, we would like to measure the position of the hologram fringes with an accuracy of (say) a quarter of a fringe or half of a resolution element. Now we know that we have already achieved a resolution of 50-60nm and that PMMA could support a resolution of around 10nm. Thus, taking the 10nm as a goal, we need to measure fringes with a positioning accuracy of about 5nm over a field of at least 2048x2048 10nm pixels. In round numbers, we could set a goal of one part in 10^4

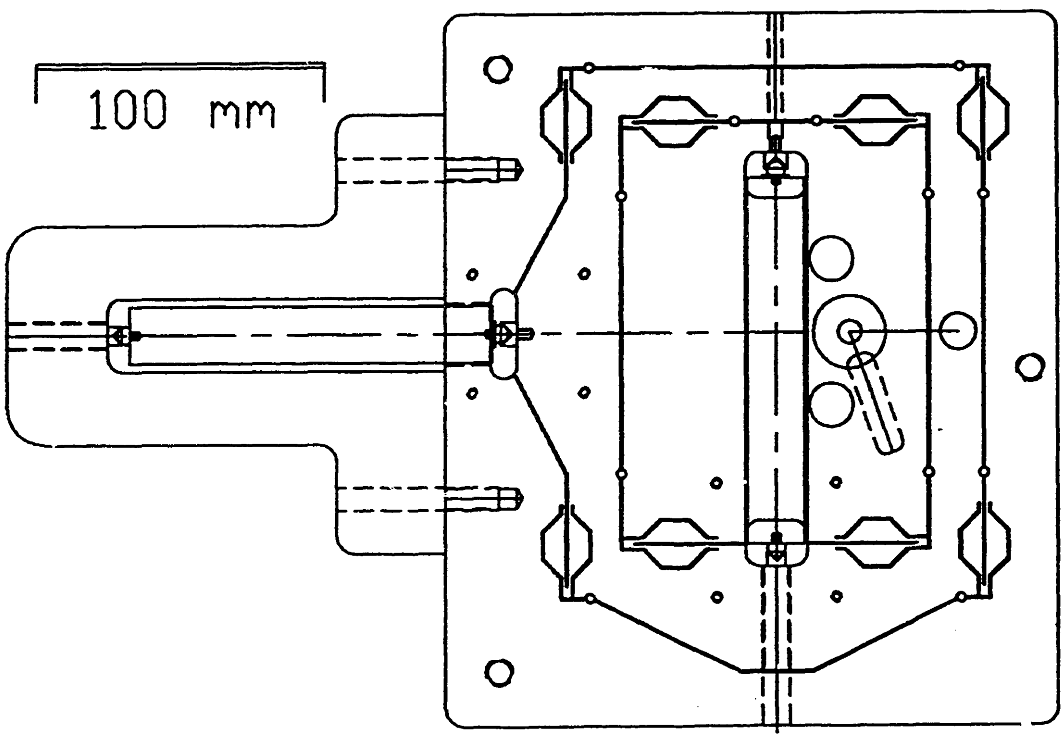

Fig. 3. Mechanical layout of the x - y scanning stage of the atomic force microscope

linearity. Such linearity is neither attempted nor achieved by any of the commercially available AFM's so we were forced to develop a system which was at least partly custom built. A second aspect of most of the commercial systems that we wanted to avoid was their lack of flexibility in choosing large numbers of pixels per image and in software changes in general.

4.0 ATOMIC FORCE MICROSCOPE: TECHNICAL DESIGN

Our design consists essentially of a Park Scientific [Park, 1993] AFM force sensing head (containing the AFM lever and the laser-split-detector system which senses the lever deflections) plus the standard Park electronics for such a head. The *x-y* scanning stage and its indexing system and the *z* transducer system which moves the sample at constant lever deflection are of our own design. The operation of the system is explained in more detail in Fig. 2.

The instrument is controlled by an IBM RS6000 model 220H UNIX workstation via IEEE 488.2 interfaces. This system drives the AFM in real time and also performs off-line image processing tasks such as hologram reconstructions. Two 16-bit digital-to-analog converters provide the wave forms for the *x* and *y* axes of the stage and an 8-channel 16-bit analog-to-digital converter reads various useful signals. Among these are the image gray level, the temperature of the stage and of the room, the humidity, the output of an accelerometer which can sense unwanted vibrations and the difference signal from the split detector.

The subsystem determining the linearity which is our chief concern is the *x-y* stage and the design of this is shown in Fig. 3. The stage has 8 identical flexural arms (Fig. 4) per axis which are essentially composite beams with dimensions given in Table 1. The system is designed to make the *x* and *y* drives independent of each other to very high accuracy. The material at present is Aluminum 6061-T6.

Table 1: Flexural Hinge Parameters	
w (thickness)	1.42mm
d (depth)	28.7mm
L	22.25mm
l/2	2.85mm

By applying beam theory to the individual beams, and recognizing that there is a twofold parallel and a fourfold series combination, one calculates a spring constant of 5.5×10^5 N/m for each axis. By direct mechanical measurement we obtained a value of $6.0 \pm 0.5 \times 10^5$ N/m which is in good agreement. The field area of $75 \times 75 \mu m^2$ which the stage can cover therefore requires a maximum force of 41 N. Combining the above figures with the mass, 0.8 kg, of the inner moving carriage we predict a resonant frequency of 140 Hz which is also in good agreement with the value of 150 Hz measured by a directional accelerometer.

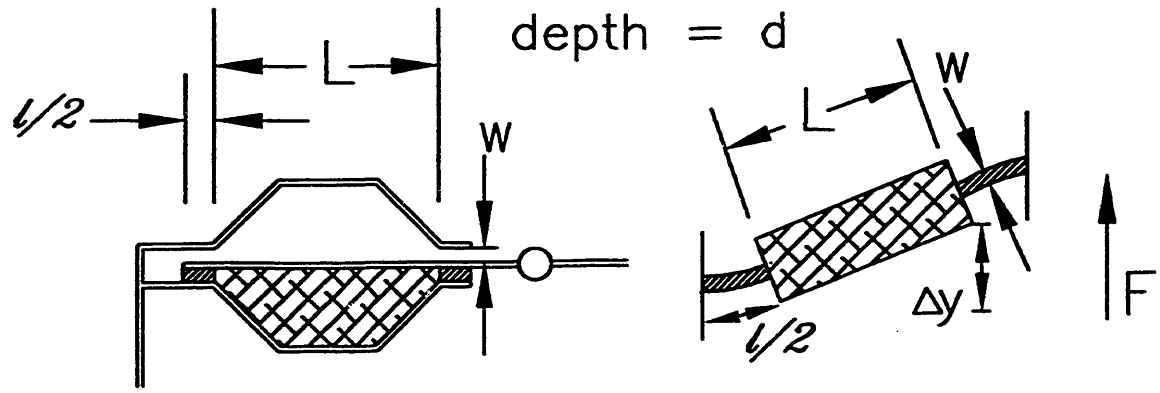

Fig. 4. Notation for analysis of the bending elements which are incorporated in pairs in the design of the x - y flexure system.

The x and y drives are by Queensgate Instruments [Queensgate 1993] piezoelectric transducers which are internally indexed by capacitative sensors so as to provide exact linear positioning via a feedback system. Apart from the flexure stage, this system is similar to the one used for scanning transmission x-ray microscopy at Brookhaven [Brown, 1992]

5.0 ATOMIC FORCE MICROSCOPE: PERFORMANCE

The performance of the Queensgate transducers has been close to expectations. Their maximum scanning speed is about 50 lines per second so they allow, for example, a 512x512 image to be scanned in about 10 seconds. The x - y noise of our present system is equivalent to about 1nm displacement. Another limit to the resolution is set by complex interactions between the tip and the sample. In the mode in which we now work which is known as "contact mode", the sample is moved to maintain constant repulsive force on the tip. This tends to mean that, for soft samples, the tip is "ploughing" at constant resistance which is not desirable. It is damaging to the sample, does not map the topography that we are interested in and can lead to changes in the tip.

A second operating method, "non-contact mode" is also available and may be preferable for soft samples. In this method the tip is kept several nm from the surface and the attractive van der Waals force is used to influence the lever. However, the attractive force is much smaller and has to be sensed indirectly via its effect on the resonant frequency of the lever. The z piezoelectric transducer is driven to maintain constant oscillation frequency of the lever in this case. We are in the process of acquiring the capability to operate in non-contact mode. This is apparently an area which will repay further research.

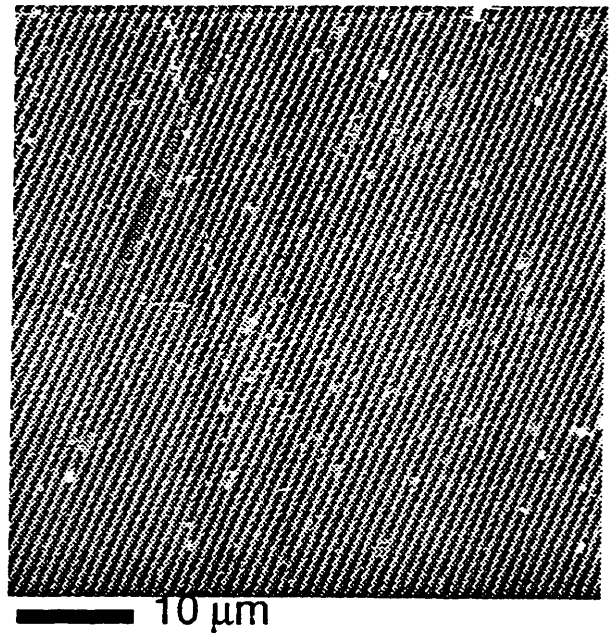

Fig. 5. AFM image of a 1200 l/mm blazed holographic grating sampled on a 1024x1024 grid of 50nm pixels.

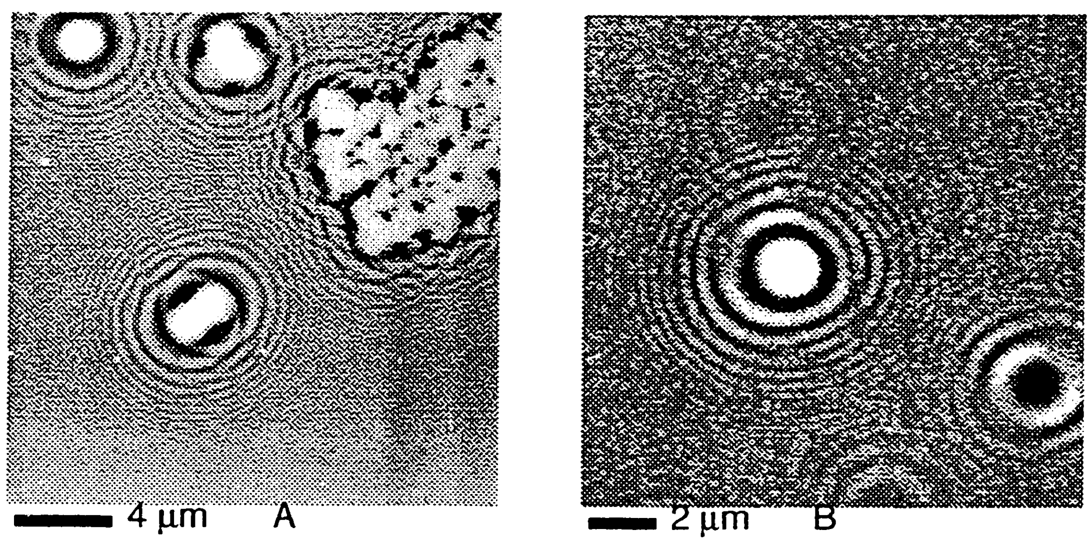

Fig. 6. (a) hologram of 1.09 µm-diameter latex spheres recorded on PMMA with 1.89 nm x-rays and read out on a 512x512 grid of 40nm pixels using the AFM, (b) similar hologram of 30 nm-diameter gold spheres read on a 600x600 grid of 25nm pixels.

The present system operates with only minimal thermal shielding and for most of its life has not run at the transducers' full speed. In view of this and the aluminum construction, it is not surprising that temperature effects can be discerned. It appears that under present conditions 0.01°C temperature stabilization would be needed to ensure full freedom from temperature effects. However, even without this, some quite good results have been obtained. In the future, as well as scanning at full speed, we plane to rebuild the flexural stage in super invar and, if necessary, enclose the microscope in an insulating box.

In order to assess the linearity of the AFM scan, we have recently taken pictures (Fig. 5) of a holographic diffraction grating. The grating groove placement errors are less than half of a period per meter. A superficial examination of the data reveals no departure from linearity. However, we plan to analyze the data more quantitatively so as to determine limits on the absolute accuracy of the measured x - y coordinates.

6.0 RESULTS

As examples of the capability of the instrument in its present form we show in Fig. 6, the direct readout of parts of test holograms of latex spheres and gold spheres. It is clear that the basic task is being performed cleanly, although the rendering of the

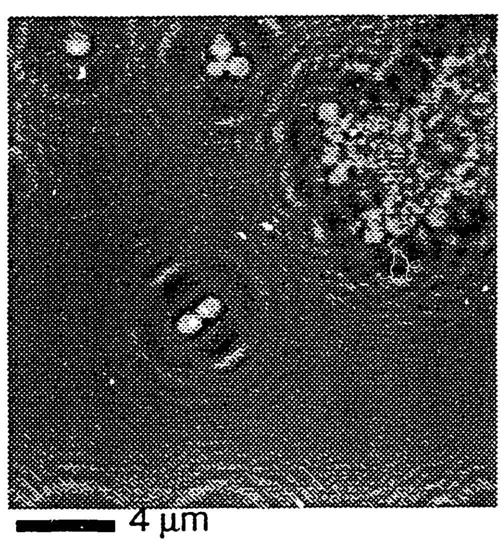

Fig. 7. The real part of the image of the hologram in Fig. 5a reconstructed at a distance of 580 µm and shown on a 512x512 grid of 40nm pixels.

measurement into a gray scale on paper is not its main purpose and does not allow the fine details of resolution and sensitivity to be evaluated. Fig. 7 shows the real part of the numerical reconstruction of the image from the hologram in Fig. 6a. This was calculated

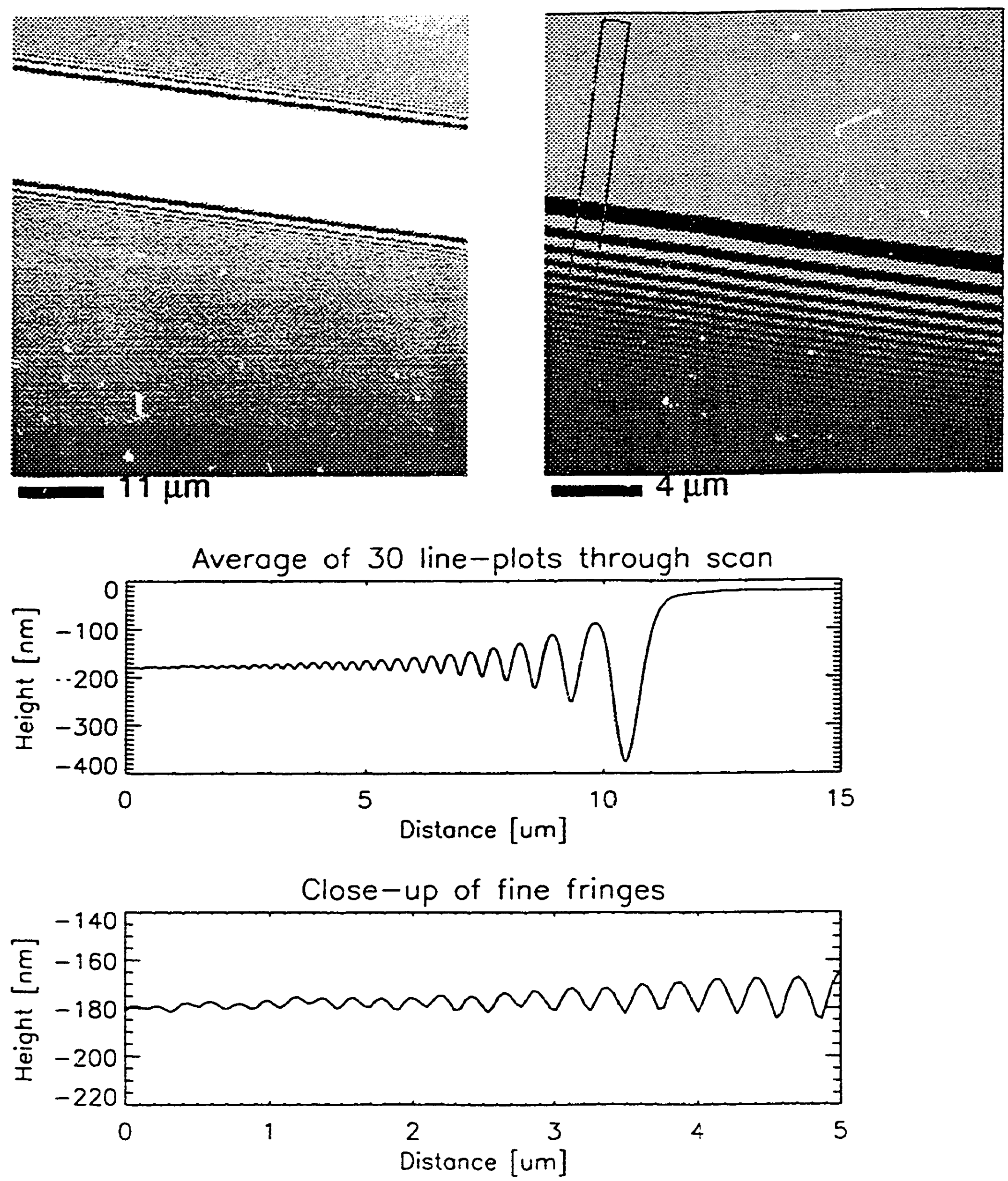

Figure. 8. Overviews and close-ups of the hologram of a 12 µm-diameter gold wire recorded on PMMA with 1.89 nm x-rays. The left-hand hologram is read on a 400x400 grid of 150nm pixels and the right hand one on a 512x512 grid of 40nm pixels. The graphs are made by integrating the two-dimensional data over the rectangular area marked on the right hand hologram. The smallest recorded oscillations have an amplitude of 2-3nm.

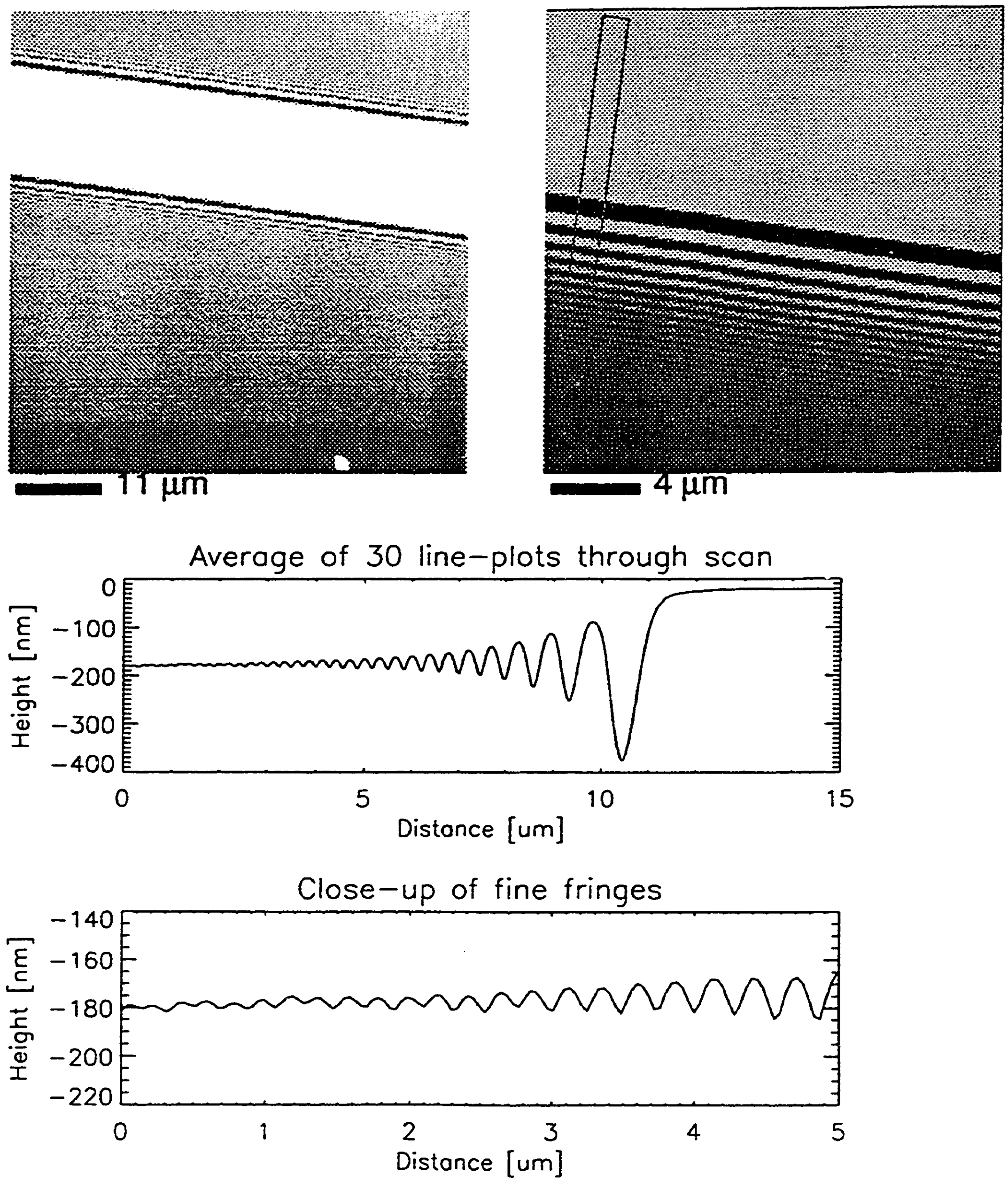

Figure. 8. Overviews and close-ups of the hologram of a 12 μm-diameter gold wire recorded on PMMA with 1.89 nm x-rays. The left-hand hologram is read on a 400x400 grid of 150nm pixels and the right hand one on a 512x512 grid of 40nm pixels. The graphs are made by integrating the two-dimensional data over the rectangular area marked on the right hand hologram. The smallest recorded oscillations have an amplitude of 2-3nm.

by a simple Fresnel transform without any attempt at twin-image suppression and one can see clearly that the twin image problem is still an important one.

Fig. 8 shows the hologram of a 12-μm-diameter gold wire. The close-up view and line scans give an idea of the effectiveness of the AFM, even without temperature stabilization

7.0 DISCUSSION AND FUTURE PROSPECTS

The preliminary conclusion of this report is that we now have a good solution to the problem of reading x-ray holograms recorded in resist layers. This enables us to proceed with other developments needed to move the technique forward. This will include work on the twin-image artifact, which has been addressed already by the Orsay group [Koren, 1993] and in a preliminary way by us [Jacobsen, 1992]. It will also include improved thermal stability of the AFM, an improved optical microscope attachment and optimization of its contact and non-contact modes, new methods of both wet and dry development of PMMA resist and new types of resist.

These are essentially details of the technique and we also need to consider our goals in a wider sense. We are presently moving to establish slow two-dimensional holography more firmly and with better performance. However, even if this were fully successful, and the technique were to become competitive with or even superior to other x-ray microscopies from a resolution standpoint, holography is still lacking the key spectroscopic and analytic capabilities of the scanning x-ray microscope. We may then ask what would be its main contribution. It is certainly attractive because of its simplicity but its chief strength as a microscopic technique is its potential for fast exposures including compatibility with flash experiments. This means that slow holography can evolve toward faster exposures as sources allow and this can be expected to become increasingly useful for imaging and also revealing about damage mechanisms as it begins to "overtake" the significant damage processes. The slow experiments are also important in developing the holographic technique in preparation for the two-dimensional flash imaging which we expect to begin soon with pulsed x-ray sources. These latter experiments will themselves be rehearsals for the three-dimensional (tomographic) flash experiments which seem as if they may be possible in the future.

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