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Applications of Holography to X-ray Imaging

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Abstract

In this paper we consider various applications of holographic techniques to the problem of soft x-ray imaging. We give special attention to imaging biological material using x-rays in the wavelength range 24-45 Å. We describe some experiments on formation and reconstruction of x-ray holograms and propose some ways in which holographic techniques might contribute to the difficult problem of fabricating optical elements for use in the soft x-ray region.

Introduction

In the spirit of this meeting we offer a collection of applications of holographic techniques to the problem of soft x-ray imaging of biological material. Some are things we have done; others are plans for the future. Some utilize x-rays only; others also involve the use of lower energy photons. We make no attempt to give a complete coverage. Indeed, we will not describe the most important present day example of our title subject which is direct x-ray imaging with holographically generated zone plate lenses.

The reason we are interested in imaging biological material with soft x-rays is that there exists a wavelength region (24-45 Å) where there is a contrast mechanism for natural biological material whilst water is effectively transparent. The contrast comes from photoelectric absorption. This wavelength "window" arises because of the positions of the oxygen K absorption edge (23.6 Å) and the carbon K absorption edge (44.8 Å). Taken together with the 1/e absorption length for soft x-rays in biological material which is about 0.5-1.0 mm, these circumstances lead us to believe that we can make soft x-ray images of biological cells in something near to their natural state, i.e. without dehydration, sectioning, fixing or staining.

We consider holography applications under the following headings:

- Direct hologram formation with soft x-rays
- Reconstruction technique for holograms recorded with soft x-rays
- High resolution zone plates by spatial frequency multiplication
- Soft x-ray beam splitters
- Grazing incidence linear zone plates

Direct hologram formation with soft x-rays

The concept of making holograms with x-rays is a very old one and various reviewers have described it. The present authors have been active in the field since 1982 and have recorded a variety of holograms using synchrotron radiation soft x-rays from the U15 beamline at the 750 MeV storage ring at Brookhaven. Initially these were mainly of demonstration objects such as spheres and cylinders for which calculated results are obtainable. We have just begun to attempt biological samples. Our first choice for these (like workers in electron microscopy and x-ray microscopy before us) is diatoms. These are easily obtained, possess sharp features to show spatial resolution and give no problems of motion or radiation damage. We show two holograms of these and one of asbestos fibres (figs 1-3). All three were made using Gabor geometry as shown in figure 4 which describes the notation. We see a rich array of image information similar to a highly magnified visible light hologram. However, we have found considerable difficulties in reconstructing these holograms, and to these questions we now turn.

Reconstruction technique for holograms recorded with soft x-rays

Since we are engaged in developing a form of microscopy we naturally feel that we should achieve a useful degree of magnification. For reconstruction by a point source distant \( z_c \) from the hologram, and of wavelength \( \lambda_2 \) the magnification of the real image is

\[
M_R = m \left[ 1 - \frac{m^2 \lambda_1^2 z_1 + z_1}{\lambda_2^2 z_2 + z_2} \right]^{-1}
\]

(1)
Figure 1. Gabor Hologram of a diatom recorded on Agfa 8E56HD film with 32Å x-rays. Magnification of the 5x4 in² print = 350, working distance = 14 mm, sample was illuminated by a 12.5 μm pinhole at 600 mm.

Figure 2. Same as figure 1 except that magnification of the 5x4 in² print = 570.
Figure 3. Gabor Hologram of 0.5-2.0μm diameter asbestos fibers recorded on Agfa 8E56HD film with 31Å x-rays. Magnification of the 5x4 in² print = 250, working distance = 29 mm, sample was illuminated by a 1μm pinhole at 115 mm.

Figure 4. Geometry and notation for formation of a Gabor Hologram.

where m is the factor by which the recorded hologram is enlarged. When \( z_c = \infty \), and \( m = 1 \) we see that \( M_R = (1 - z_1/ z_R)^{-1} = 1 \) which is not desirable. However, even for \( m = 1 \), we can get a large magnification by choosing \( z_c \) near to the value for which the denominator of (1) vanishes; i.e. when

\[
\frac{1}{z_c} = \frac{1}{\lambda_2} \left[ \frac{1}{\lambda_1} \left( \frac{1}{z_1} - \frac{1}{z_R} \right) \right]
\]

(2)
Figure 5. Calculated behavior of the magnification (continuous curves) of the reconstructed image of the Gabor Holograms shown in figs 1 and 2 for reconstruction with He: Cd laser light ($\lambda_2 = 4416$ Å, $m=1$). The real image position is also shown (dashed curve, right hand scale). These figures illustrated the practical difficulty of achieving a high magnification with visible light reconstruction.

Figure 6. Successful reconstruction of the hologram shown in fig 3 using $\lambda_2 = 4416$ Å, $Z_c = 0.9$ mm, holographic magnification = 1.7, magnification of 5x4 in² print = 360. Note that the narrowest needle is about 1/2 mm on the print or 1.4 μm in object space. On this basis we estimate the resolution as 1-2 μm.
This occurs when the reconstruction source is at the focus of the zone plate patterns in the hologram and the image distance approaches infinity. The result of choosing \( Z_c \) in this region is shown in fig 5 which refers to the holograms in figs 1 and 2. We see that \( Z_c \) needs to be extremely small—0.1 mm in this case. If the reconstruction source is provided by a diffraction limited pinhole and \( \lambda_2 \) is in the visible we find that for obtainable pinholes we can illuminate only a very small area, for our case less than the full hologram. Worse still, as one might expect the holographic aberrations show singular behavior similar to the magnification near the singular point defined by (2). In other words we are obliged to use a larger value for \( Z_c \) and therefore to achieve very little holographic magnification. At the present essentially all the magnification in our reconstructions is provided by the light microscope. This is not a great limitation to the resolution while we are using photographic film, but if we include aberration arguments as well, then we can see that the only way to achieve resolutions much less than a micron is to magnify the hologram. For the present we seem to be achieving resolutions near the limit of our approach of visible light reconstruction of an original Gabor hologram recorded on photographic film. A good demonstration of this is the reconstruction of the hologram of fig 3 which is shown in fig 6. This shows a resolution of 1-2 \( \mu m \).

**High resolution zone plates by spatial frequency multiplication**

One of the chief problems of x-ray optics has always been the provision of x-ray lenses of adequate resolution. The normal solution is to make transmission Fresnel Zone Plates either by holography or electron beam writing. The resolution of such a lens in first order is limited to the width of the smallest (outer) zone or, in other words, to the resolution of the available microfabrication technology. At the present time, the very best fabricators of zone plates achieve zone widths of 500-1000 Å. These certainly allow many interesting experiments but it is agreed that the goal is zone widths of 100-200 Å. The only way to achieve resolution better than the zone width is to utilise a higher order focus. This is generally not practical on efficiency grounds, but it provides a clue to a strategy for circumventing the first order diffraction limit. Consider the zone plate shown in figure 7. The first and third order diffracted beams are shown for plane wave illumination. This leads to diffraction limited focal spots of half width 1.22\( \lambda \) and 0.41\( \lambda \) at distances \( f \) and \( f/3 \) respectively from the zone plate. The notation is explained in fig 7. Now, suppose we place a recording medium at distance \( f/2 \) as shown. The recording would be a hologram of the third order focus with the converging first order beam as reference wave. This recording is apparently a zone plate of focal length \( f/8 \) and numerical aperture (and outer zone spacing) four times less than the original zone plate. We have thus made a fourfold gain in first order resolution.

**Figure 7.** Geometry for recording of a high resolution zone plate by spatial frequency multiplication. \( \lambda \) is the width of the narrowest zone. The original zone plate has alternating transparent and opaque zones only in the region shown dashed. The rest is all opaque. Illumination is assumed to be by an axial plane wave. \( F_1, F_2 \) and \( F_3 \) are the positive first, second and third order foci of the zone plate.

We may legitimately ask what are the conditions for the above scheme to work? The following are certainly necessary:
A technology must exist for converting the recording into a zone plate with suitable fidelity. This would presumably involve high resolution x-ray resist.

In view of the desired values of the minimum zone width it will be necessary to use a soft x-ray source for the exposure. This source must have very high coherent power to make the exposure time reasonable. We believe that even a storage ring bending magnet is marginal and that a soft x-ray undulator may be needed.

The illuminating beam must be sufficiently monochromatic for the third order focus to be diffraction limited; i.e. \( \lambda / \Delta \lambda > \frac{3n}{0.61} \)

The original zone plate must be sufficiently well made that the diffraction limited third order spot size is achieved. The Rayleigh quarter wave criterion would require placement of each ring with an error less than 1/6 \( (\lambda \text{ numerical aperture}) \). Although this sounds challenging it is probably achievable using holographic methods.

**Soft x-ray beam splitters**

Holographic and interferometric experiments in the soft x-ray region, especially the region between 24 and 45Å, have always been hampered by the lack of efficient optical elements. Even the recent advances of multilayer optics have not impinged much on this spectral region, due to absorption problems in available spacer materials. For the same reason, organic crystals are rather inefficient, and normal incidence optical elements at these short wavelengths tend to have prohibitively difficult fabrication tolerances. One hopeful step is the relatively recent demonstration of the use of reflection gratings in the so called "conical diffraction" mode. For the grazing incidence cases of interest to us, this means using the grating with the incoming rays almost parallel to the grooves. It is a characteristic of conical diffraction that diffraction efficiencies are much higher than for normal (nearly in plane) diffraction.

A novel possibility achievable using conical diffraction is an efficient soft x-ray beam splitter. This is shown in fig 8.

\[
\theta = 2 \sin^{-1} \left( \frac{\lambda}{d} \right) \tag{3}
\]

For a 3600 line/mm grating, for example, this would give a separation of 1.2 degrees at \( \lambda = 30 \text{Å} \). Of course, one could make a classical interferometer much more easily if this angle were larger as it is in some existing interferometer schemes using transmission gratings, multilayers or perfect crystals. However, none of these schemes is very efficient in the XUV region so we believe the grazing incidence grating has a place on grounds of efficiency and that interesting interferometer schemes do exist which are suited to its geometry.

The efficiency of the present system would be related to the reflectivity of the material at the angle of incidence used. For example, with nickel at 2° incidence angle, the reflectivity at 30Å is 80% giving about 35% each in the ±1 orders and 18% in zero order.
The beam splitters could be used to build a soft x-ray interferometer and hence a phase contrast microscope and one could even envisage soft x-ray fourier transform spectroscopy.

**Grazing incidence linear zone plates**

We turn now to another strategy for achieving spatial resolution values which are smaller than those of available microfabrication techniques. Consider the arrangement of fig 9. A collimated soft x-ray beam is incident from the left on a plane, grazing incidence, reflection grating of variable spacing. At each location the groove spacing is chosen so that an incident ray is focussed to the line focus shown in the figure. The groove frequency \( n(x) \) needed to achieve this is given by

\[
\lambda n(x) = \cos\alpha - \frac{x}{\sqrt{h^2 + x^2}}
\]

(4)

Where the notation is given in figure 9. The diffraction limited resolution \( \Delta_D \) of this arrangement is given by

\[
\Delta_D = \frac{\lambda}{(\theta_2 - \theta_1)}
\]

(5)

The approximation of the numerical aperture by \( (\theta_2 - \theta_1)^{-1} \) is justified by the small values of \( \theta_1 \) and \( \theta_2 \). For \( \lambda = 30\text{Å} \) the largest deflection angle that can be achieved with specular reflection is about 11° (incidence angle 5 1/2°). If we set \(\alpha = 1°, \theta_1 = 2°\) and \(\theta_2 = 10°\) equation (4) gives \( \Delta_D = 214\text{ Å} \) : an extremely interesting value; and this is achieved with a maximum line density (corresponding to \( \theta_2 \)) of about 5000 lines per mm.

![Figure 9](image_url)

**Figure 9.** (a) Principle of a diffractive "cylindrical lens" using a plane grating with variable groove spacing. (b) notation for discussion of (a). \( x \) is the coordinate of a general point on the grating.

The conditions for achieving this in practice are as follows:

- The incoming beam must have a spectral purity \( (\lambda/\Delta\lambda) \) greater than the number of grooves of the grating. The latter can be found by integration of equation (4). It is proportional to \( f \) which acts as a scale factor of the arrangement. For example, for \( f = 5 \) mm, and the angles given above the number of grooves is about 6000: a difficult but not impossible value.
- The grooves must be positioned at their ideal positions with a maximum error of 1/4 of a period (Rayleigh Criterion).
At 1° grazing incidence, for 30Å x-rays, the flatness tolerance of the substrate (Rayleigh Criterion) is about 1/30 of a wavelength of visible light. This is straightforward for the small areas involved.

We note the following advantages of making an optic in this way:

The zero order beam is not mingled with the focussed beam

With a rigid substrate more freedom in choosing the groove shape is possible than for self-supporting structures. This includes the possibility of phase gratings. For example one might manufacture the device by holography19 (sine wave profile), microcircuit methods11 (square wave profile) or ruling engine20 (blazed profile).

A rigid substrate is more robust in hostile environments and allows freedom to make optimum materials choices. For example one could use water cooling to deal with high power applications.

So far all of this is applicable only to one dimensional focussing. We naturally enquire whether two such devices can be crossed to give two dimensional focussing in the style of the Kirkpatrick-Baez reflection microscope. This is certainly possible in principle, but for the second device we are no longer treating with a collimated beam. The convergence angle leads to an additional aberration which turns out to be a form of coma. This would have the effect of limiting ($\theta_1$-$\theta_2$) to values less than about five degrees. Nevertheless we see from equation (5) that the values of $\Delta$ are still attractive.

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