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TITLE HOLOGRAPHY AT X-RAY WAVELENGTHS

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HOLOGRAPHY AT X-RAY WAVELENGTHS

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Abstract

We discuss alternative holographic techniques for imaging microscopic structures with a short-pulse, high-intensity, high-quantum-energy laser. We find that Fresnel transform holography using a photoresist for registration of the hologram is most likely to be within the scope of near term technology. Although it has advantages in time gating, using an in-line electron microscope for hologram registration has an unacceptable tradeoff between quantum efficiency and resolution. Fourier transform holography using a reflector to generate the reference beam might be a reasonable alternative using low resolution film, but is necessarily more complicated. We discuss the dependence of the required laser intensity on the resolution sought and on the elastic and absorption cross sections. We conclude that resonant scattering must be used to obtain holograms at reasonable intensities.

Introduction

In this paper we discuss holography with very intense, short-pulse, high-quantum-energy lasers. The possibilities for holography with such a laser are: (1) in-vivo high-resolution holograms of cells in three dimensions; (2) the ability to distinguish individual atomic species; and (3) the ability to freeze mechanical action within a cell on the time scale of picoseconds.

We first review techniques for making holograms of microscopic objects with special reference to the problem of obtaining high resolutions. We then discuss recording methods for X-ray holography and, in particular, discuss the problems with a photoelectric recording system. Finally we discuss the physics of the interaction of intense monochromatic X-ray beams with biological materials, and derive intensity requirements for X-ray holography of biological structures. Our principal conclusion is that one must make use of resonance lines to obtain holograms with interesting resolutions using reasonable intensities.

Holographic Techniques

We have investigated numerous holographic techniques and have settled on two possibilities for making holograms of microscopic specimens with a high-intensity short pulse high quantum energy laser. These are: (1) Fresnel transform holography, otherwise known as in-line holography; and (2) Fourier transform holography, sometimes known as "lensless" Fourier transform holography.

Figure 1a shows a typical setup for a Fresnel transform holograph. The principal advantage of the technique is simplicity. It requires only one laser beam. The object to be holographed is placed in the laser beam itself. The same beam provides both the reference and illumination for the object. When the object size divided by the wavelength is small compared to the distance from the object to the recording medium divided by the object size, this technic is called Fraunhofer holography. The basic distinction is a certain simplification that can be made in the equations for the fringe pattern at the recording medium.

The difficulty of Fresnel transform holography is that it requires a very high resolution recording medium. A feeling for this can be derived from an argument given by Stroke,[1] illustrated in Fig. 1b. If our object consists of a series of point scatterers separated by distance c, it can be thought of as a diffraction grating. The angle at which the first maximum in the diffraction pattern will occur is $\sin^{-1}(\lambda/c)$. This maximum must interfere with the plane waves of the reference beam that are parallel to the recording medium. In order to record the interference pattern between the diffracted beam and the reference beam, the spacing between the grains, g, must be at least large enough for alternating grains to record the maxima and minima of the resulting fringes. Consequently, $g \tan(\sin^{-1}(\lambda/c)) \geq \lambda/2$. For small angles this means $g(\lambda/c) \geq \lambda/2$ or that we must have $c \geq 2g$. The minimum spacing that can be resolved is greater than twice the grain spacing. As long as the angles are small, the result is independent of wavelength. The essence of this argument plus extensions to include the effects of finite bandwidth and penetration of the specimen has been given by Baez and El-Sum.[2]

At large angles, which occur when the wavelength becomes on the order of the size of the grid spacing, this result become dependent on the wavelength. This is because the first maximum of the diffraction pattern occurs at a very large angle. This limits the effectiveness of Fresnel transform holography: frequently the area of reference illumination will be too small to encompass the peak of the diffraction pattern. If $\lambda = c$ the diffraction peak will occur at 90°. The recording medium would have to be infinite in extent to record

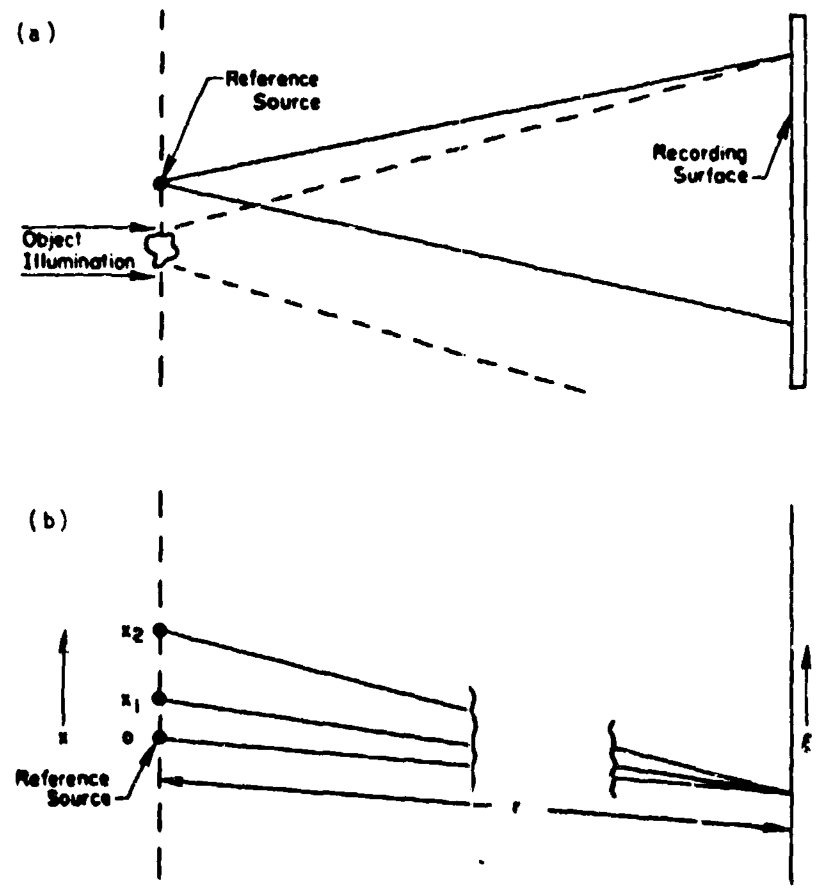

Figure 1. Fresnel transform holography: (a) typical setup of holographic apparatus; (b) diagram demonstrating that Fresnel transform holography is limited by resolution of recording medium.

the first diffraction peak. As pointed by out by Mueller or Jorna,[3] at large diffraction angles and short wavelengths we would run into difficulty with roughness of the surface of the recording medium let alone its intrinsic graininess.

It is also interesting to note that if the structure of the object and the wavelength were appropriate for the object to scatter plane waves, then the interference pattern at the recording medium would have a spatial frequency of $(\sin\alpha)/\lambda$ where α is the half-angle of interference.

Figure 2a shows an idealized configuration for lensless Fourier transform holography.[4,5] The reference source emits spherical waves, which interfere with waves from the object at the recording surface. The object is separately illuminated by a plane wave source. Figure 2b shows a simplified arrangement with a reference source, o, and two object point scatterers, x_1 and x_2, all emitting spherical waves originally at the same phase. The intensity pattern at the screen will be given by

$$I \doteq \frac{A^2}{r^2}\left[1 + \frac{4a}{A} \cdot \cos\frac{\pi(x_1+x_2)\xi}{2\lambda r} \cdot \cos\frac{\pi(x_1-x_2)\xi}{2\lambda r}\right] \qquad (1)$$

where a and A are amplitudes of the reference and scattered waves, respectively, and $A >> a$. This pattern consists of a dc level plus a rapidly oscillating function with frequency proportional to the mean distance of the two object scatterers from the reference source modulating a slowly oscillating function with frequency proportional to the difference in distance between the two object sources. It is called Fourier transform holography because every distance from the reference source maps to unique spatial frequency at the recording surface. It is lensless because the usual Fourier transforming technique requires a lens. The maximum spatial frequency of the interference pattern can be adjusted arbitrarily by placing the object at various distances from the reference source. Note that difficulty occurs again when spacing of points in the object is smaller than the wavelength. For example, if $(x_1 - x_2) < \lambda$, the modulation pattern never reaches a maximum.

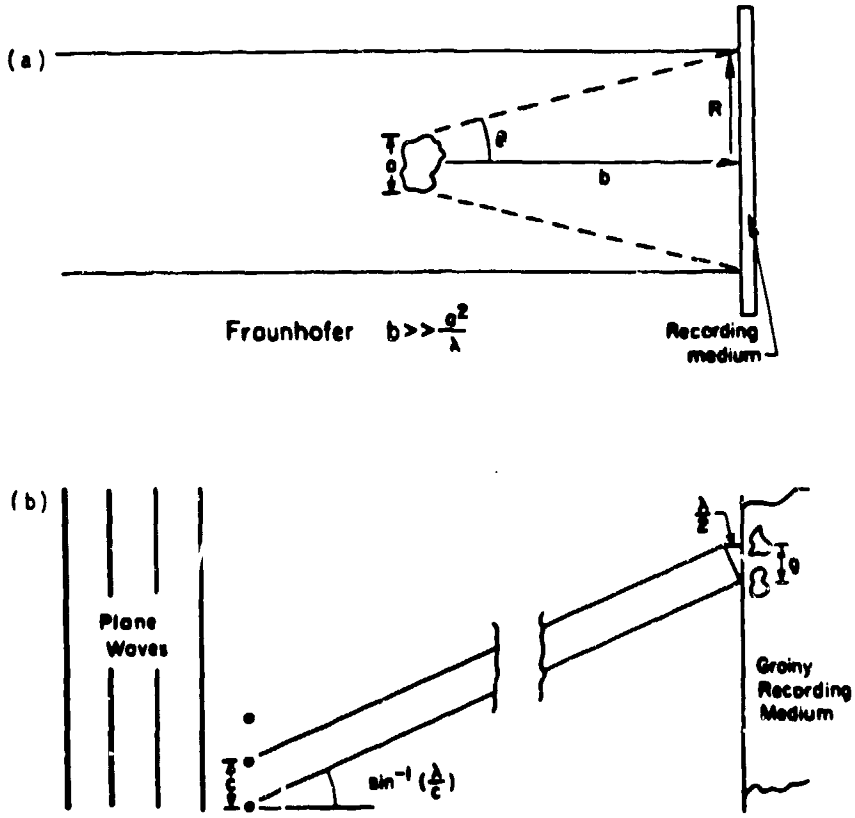

Figure 2: Lensless Fourier transform holography: (a) idealized configuration of apparatus; (b) simplified arrangement for calculation consisting of reference source and two object scatterers.

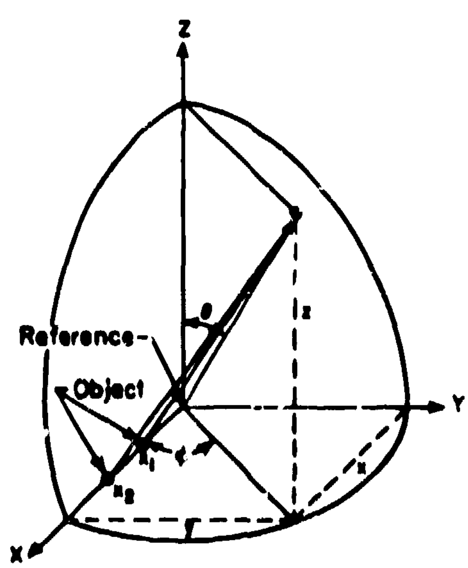

Figure 3: Geometry of spherical Fourier transform holography on a sphere of unit radius

We can mitigate the large angle problem by making a spherical recording surface. The geometry on a unit sphere for such a Fourier transform technique is shown in Fig. 3. If we consider the reference source of spherical waves to be at the origin and the object to again consist of two point scatterers at x_1 and x_2, then we find the following intensity pattern:

$$I \doteq A^2 \left[1 - \frac{4a}{A} \cdot \cos \frac{\pi(x_1+x_2)\sin\theta\cos\phi}{\lambda} \cdot \cos \frac{\pi(x_1-x_2)\sin\theta\cos\phi}{\lambda}\right] \quad (2)$$

where again A >> a. The intensity pattern consists of bands parallel to the y-z plane and centered on the x-axis. As in the planar case, the spatial (or angular) frequency consists of a rapid oscillatory function, proportional to the mean distance of the object points from the reference, modulated by a slowly varying function depending on the distance between the object points. Again, if the point spacing is less than the wavelength, one never completes a full cycle. The physical spacing of the fringes can be made arbitrarily large by expanding the radius of the sphere. Thus we could use an ordinary film of arbitrarily large grain size, as long as the tradeoff between sensitivity and resolution was favorable.

Recording Methods for Fraunhofer Holography

High-resolution Fraunhofer holography requires a grainless recording medium. In fact, no recording medium is truly grainless, it is at least grainy on the atomic scale. However, there are ways of obtaining resolution far better than that available from the finest-grain film. One is to convert the x-ray image into an electron image by means of a photo-emitting cathode, and then enlarge the image with an electron microscope. This has the additional advantage that the electron microscope could be time-gated, thereby extracting information only during the part of the laser pulse when the best hologram was being generated. The other approach is to use a high-resolution recording medium. This could be done using a photoresist material, many of which have been developed for high-resolution lithography.[6]

An example of such a material is polymethyl methacrylate (PMMA). Photons striking this plastic break polymer chains leaving the material locally less resistant to etching agents. Bjorkland[7,8] used PMMA in holography experiments at vacuum-ultraviolet wavelengths. Feder et al.[9] have demonstrated that resolution of 100 Å can be obtained with PMMA in X-ray shadowgrams. We are most interested in wavelengths in the 30-50 Å range. This is optimum for the use of a photoresist such as PMMA. At higher energies the resolution degrades because of the increasing range of secondary electrons, and at lower energies the resolution degrades because of diffraction effects.[10] Holograms registered on a photoresist can be read out using an electron microscope and the image can be reconstructed either by optical laser illumination of the electron micrograph or computer analysis.[11]

Direct use of an electron microscope to register and enlarge the hologram has its difficulties. An electron microscope imposes a trade-off between quantum efficiency and resolution. The modulation transfer function degrades with increase in acceptance angle of the microscope and energy dispersion of the photoelectrons.[12] This general statement is true over a large range of designs. However, an intuitive feel can be acquired by noting that the spherical aberration of an electron microscope increases as the cube of its acceptance angle.[13] The quantum efficiency decreases as the square of the acceptance angle. Similarly, the chromatic aberration increases as the product of acceptance angle and energy dispersion.[14] We might reduce the chromatic aberration by using electronic gating to limit the energy dispersion. However, the problem of spherical aberration is not easily circumvented.

It is possible to build lensless electron microscopes. One such conceptual device is shown in Fig. 4. The photoemitter consists of a spherical shell, radius r_1, within which the hologram is recorded. The figure shows a typical Fresnel transform holography setup. The screen or recording medium consists of a spherical shell at a much larger radius r_2. The magnification should be given by the ratio of r_2/r_1. An accelerating high voltage is applied to the inner shell while the outer shell is grounded. The electrons are accelerated nearly radially from the inner shell to the outer shell and blurring is owing exclusively to initial tangential velocities. However, if the energy corresponding to the initial tangential velocity is E_0 and the potential on the inner sphere is V_0 then the dispersion in angle is given by

$$\Delta\theta \doteq 2 \left(\frac{r_2 - r_1}{r_2}\right) \sqrt{\frac{E_0}{V_0}} \quad . \quad (3)$$

This approximation is good when $E_0 < V_0 \times 10^{-3}$. The detailed expression is very complicated.

Examining this expression we immediately notice that the case of least blurring occurs as r_1 approaches r_2. This is the case of least magnification, i.e., parallel planes. However, in accelerating the electrons one has also decreased the divergence angle by a factor of $\sqrt{E_0/V_0}$. So we might instead consider using parallel plates to accelerate the electrons into the optics of a conventional electron microscope.

However, it is difficult to conceive of any practical configuration that would result in an acceptable blurring. For example, if we want the blurring, $r_2\Delta\theta$, to be only 100 Å, and we have a distance between plates, $r_2 - r_1$, of only 10 μm, and a tangential energy of only 1 volt, then we need 4 000 000 volts between the plates. This is clearly impractical.

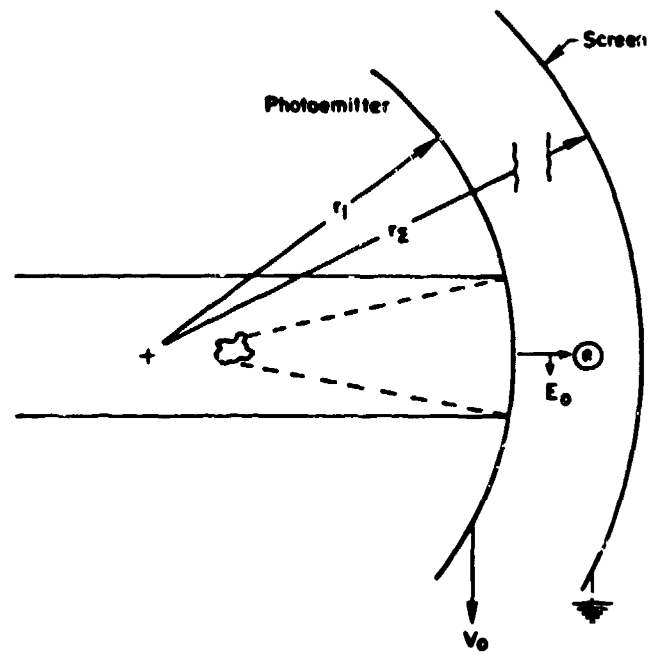

Figure 4: Conceptual lensless electron microscope with Fraunhofer holography.

A potential problem with Fraunhofer holography is low contrast ratio. This is particularly true if the specimen is not a very efficient coherent scatterer. The problem can be circumvented by mounting the specimen over a hole in an absorbing foil, which will attenuate the reference beam. The uniformity of the "shadow foil" must be such that spatial and temporal coherence of the reference beam are not drastically altered.

Fourier Holography

The trouble with lensless Fourier holography is that (in the figurative sense) it is not lensless. Figure 5 shows a typical setup for lensless Fourier holography. In order to obtain the spherical reference wave we must have a lens that focusses to a pinhole in the shadow plane. In the x-ray regime we do not use a lens, but rather use a Fresnel zone plate. With this type of experimental configuration, it is easy to prove that the hologram resolution is limited to the finest spacing on the Fresnel zone plate. This derives from the fact that the finest spacing on the zone plate determines the maximum angle of the spherical segment generated. The finest zone plate spacing allowed by current technology is about 800 Å.[15] This means that using this technique we could obtain resolution no better than 800 Å.

An alternative could be to use a coherent scattering reflector to generate the reference waves.[16,17] Figure 6 shows such a reflector, in this case a paraboloid that would generate spherical reference waves, enclosed in a spherical shell recording surface. For best contrast ratio, the paraboloid would have to be approximately the same size as the object (for example, 100 μm for a 100 μm biological specimen). The reference scatterer need not be a paraboloid. In principle, the hologram could be unfolded for any convex reference scatterer as long as we knew its shape and dimensions to within a fraction of a wavelength.[18] Presently, spherical microballoons can be made 100 μm in diameter with a surface smoothness better than 100 Å. These balloons can be measured to within 50 Å. So using a reflector that would work in the 20-50 Å region is just on the edge of technological feasibility.

Intensity Requirements

We define the stagnation time for a heated region by

$$T_{stag} \sim \frac{\delta}{\sqrt{C_v \theta}} \tag{4}$$

where δ is the linear dimension of the region, C_v the specific heat at constant volume, and θ the temperature. Under the assumption that the hologram can be recorded without blurring for a period on the order of T_{stag} time, the required intensity is given by

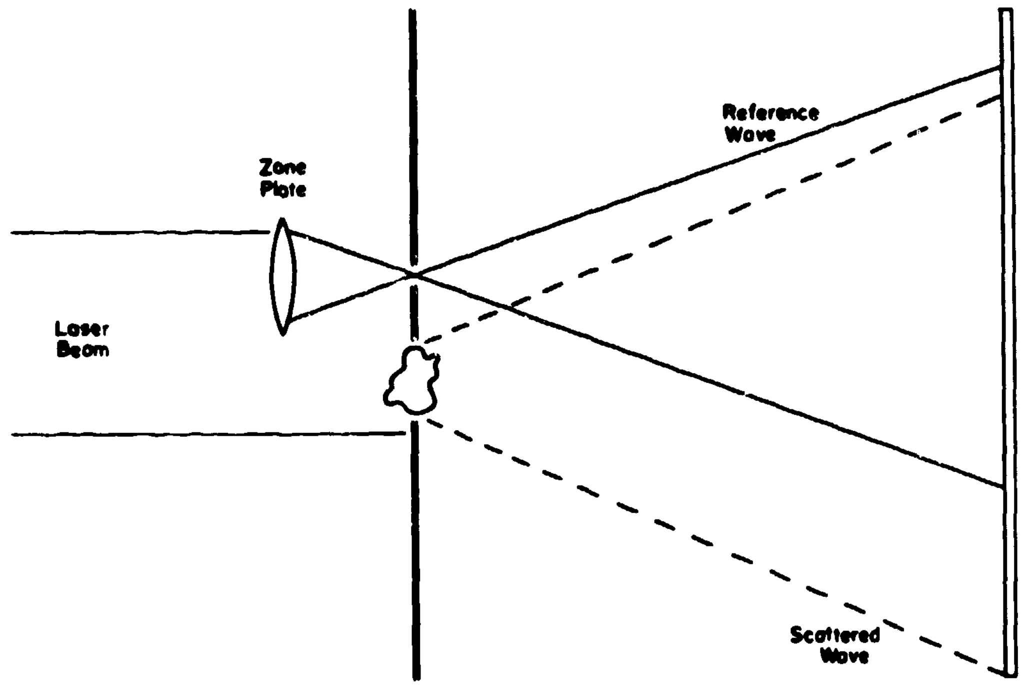

Figure 5. Typical setup for lensless Fourier holography.

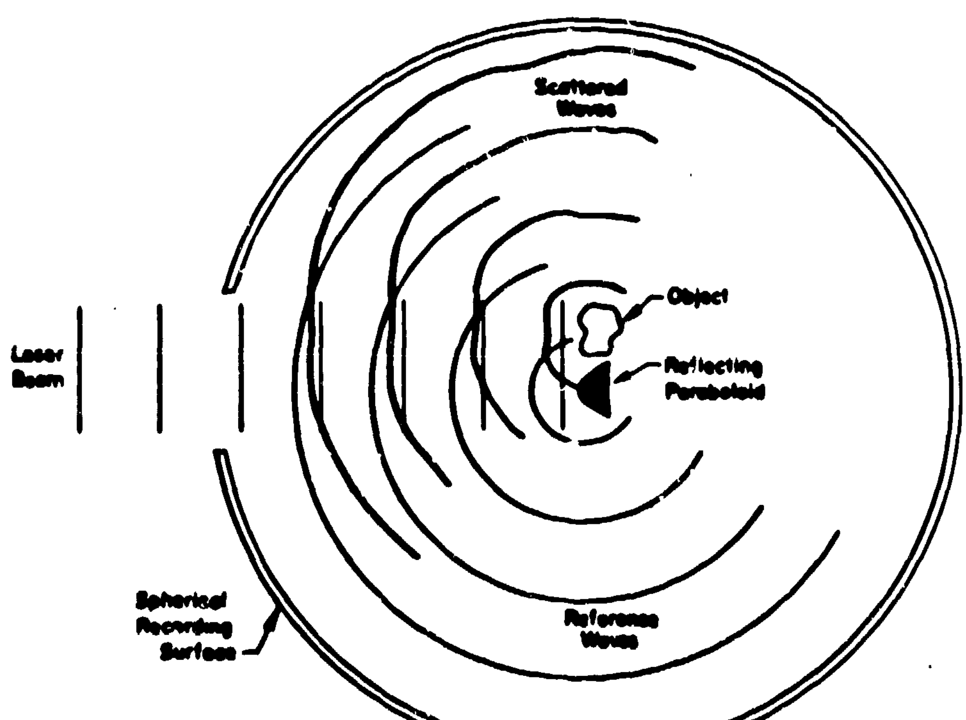

Figure 6: Fourier transform holography with a parabolic reflector.

$$I \sim \frac{1}{K}\sqrt{\left(\frac{Nh\nu}{\varepsilon\sigma_{elas}\,\rho}\right)^3 \frac{\sigma_{abs}}{\delta^{11}}} \tag{5}$$

where N is the number of photons to be recorded per resolution element, $h\nu$ is the photon quantum energy (ergs), ε is the detector quantum efficiency, i.e., the fraction of photons recorded, σ_{elas} is the elastic cross section (cm^2/atom), σ_{abs} is the absorption cross section (cm^2/atom), ρ is the density (g/cm^3), K is the number of atoms per gram, i.e., $6.02 \times 10^{23}/A$ (atoms/g), and δ is the linear resolution element (cm).

The exposure time (the time during which the hologram is recorded) must be less than the stagnation time. This can be accomplished by arranging for the laser pulse length to be shorter than the stagnation time, either by designing a laser with a natural short pulse or using any of a variety of mechanisms for chopping the pulse. A gated recorder, such as any of the electronphotoemissive schemes, could also provide a shutter and allow the laser pulse length to exceed the stagnation time. With a reflective-reference scheme such as that shown in Fig. 6, we could select a coherent-scattering resonance and laser intensity such that the resonance saturated in a time less than the stagnation time. This resonance would be used both in the reference reflector and the object, and would provide a natural shutter. When the resonance saturated, both the object and reference scatterer would become transparent to the laser beam, and generation of the hologram would cease. Unfortunately, this shutter scheme cannot be used very effectively with Fresnel holography and a photoresist recording surface, for after the resonance saturates (bleaches), the laser will continue to expose (darken) the photoresist. The laser pulse length could be a factor of two or three greater than the saturation time, but any further exposure would unacceptably degrade the contrast ratio.

The required intensity goes as $\delta^{-11/2}$. This derives from the increase in intensity required to get the same number of photons scattered off a smaller resolution element and the shorter stagnation time as the resolution element becomes smaller. This equation is only applicable in the region where the specimen is behaving like a fluid and there is local thermodynamic equilibrium. But it does give a feeling for the rapid increase in intensity required as higher resolution is sought.

The intensity is strongly dependent on the elastic cross section and somewhat more weakly dependent on the absorption cross section. To make holography practical on the 30-50 Å scale, we must use resonant coherent scattering. The resonant cross section is given by the Breit-Wigner formula[19] and to within multiplicity factors is approximately

$$\sigma \sim \lambda^2 \left(\frac{\Gamma_{res}}{\Gamma_{nat}}\right)^2 \tag{6}$$

where Γ_{res} is the resonant linewidth and Γ_{nat} is the natural linewidth. Figure 7 compares λ^2, which is a reasonable approximation to the resonant cross section, with the cross section for photoelectric effect, coherent scattering, and incoherent scattering in nitrogen.

To see how important it is to use resonant coherent scattering, let us consider a concrete example. Suppose that we were trying to make a hologram of a microscopic structure of solid nitrogen to a linear resolution of 50 Å. Further, suppose that we wanted to scatter coherently 10^3 photons from each resolution element. Using nonresonant coherent scattering, the elastic cross section is about 4×10^{-22} cm^2. The absorption cross section is about 4×10^{-20} cm^2. This gives an intensity of approximately 10^{18} watts/cm^2 and a stagnation time of 10^{-14} s. This is clearly unreasonable.

On the other hand, if we were able to prepare the nitrogen in a He-like state, we could use the 2P-to-1S resonance in He-like nitrogen that occurs at about 431 eV. In the He-like state, the atoms would have no Auger transitions, although there may be some contribution to the linewidth from inelastic collisions with free electrons,[20] and from Stark broadening.[21] However we expect that the resonant linewidth would closely approach the natural linewidth. Even though the temperature would be nearly 50 eV, Doppler broadening would be negligible. Therefore, the elastic cross section would be about 10^{-16} cm^2. This would give an intensity of 10^{10} watts/cm^2 and an observation time of about 10^{-11} s. It reduces the intensity requirement by eight orders of magnitude and makes the stagnation time 10 picoseconds which seems at least tractable. Also, the illumination on the recording surface is about 0.1 J/cm^2 which is within the dynamic range of practical photoresists.[22] Although Doppler broadening is negligible, Doppler shifting of the coherently scattered photons may alter the phase relationship with the reference beam at the recording surface. This could impose an upper limit on the distance from the specimen to the recording surface.

Formula 5 is not strictly applicable to this case, however. The formula would assume that the nitrogen starts cold in the He-like state. In fact, we have to apply enough energy to bring it to that state, and during that time it is dilating hydrodynamically. If we could ride on an absorption resonance of about 3×10^{-17} cm^2 until we reached the He-like state, then an intensity of about 3×10^{12} watts/cm^2 would be required, a stagnation time of about 1/3 picosecond would be encountered, and the fluence on the recording surface during registration of the hologram would be about 1 J/cm². Doing this however requires a fortuitous overlap of resonances. An alternative might be to direct a second laser at the specimen, perpendicular to the laser that would resonate with the He-like state. The second laser would be tuned to the K-edge and heat the specimen to the desired population of the He-like state. This would improve the contrast ratio and does not depend on any fortuitous overlap of resonances. The second laser would have to supply about 10^{13} watts/cm², however.

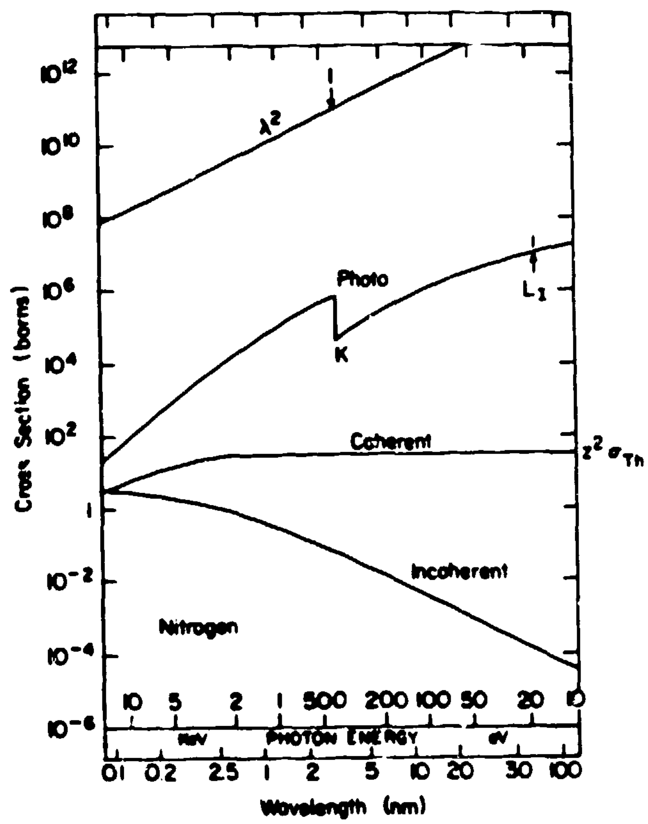

Figure 7: Cross sections for photoelectric effect, coherent scattering, and incoherent scattering in nitrogen compared with λ^2 which is the resonant scattering cross section to within multiplicity factors.

For a more realistic example we should look to cold resonances. Figure 8 shows the x-ray absorption cross section of molecular nitrogen in the range from 400-435 V as reported by Bianconi et al.[23] A resonance occurs at about 401.3 eV owing to the promotion of a 1S electron to the first unfilled (π_g2P) orbital.[24] While the total cross section of this resonance exceeds 22 Mb, its fluorescent yield, that is the fraction of its cross section that is coherent scattering, is only about a part in 10^3. However a real cell is not constructed of pure nitrogen and the accompanying atomic species provide a heat sink for energy deposited by noncoherent scattering processes. This somewhat suppresses the rate of dilation, and increases the stagnation time. This resonance is, of course, for N_2, but one would expect that a similar resonance would be encountered for nitrogen bound in the proteins of a cell, and that it would occur at a similar energy. This is because if a first vacant orbital exists, it will differ from the one in N_2 only by a fraction of the molecular binding energy. For example, the corresponding resonance in N_2O occurs at 401.2 eV,[25] only 0.1 eV from the one in N_2. For a realistic cell containing appropriate concentrations of H, C, O, and N, the theoretical resolution would be on the order of 200 Å because the strong absorption of the N_2 resonance makes protein concentrations appear almost black. To record the hologram on a standard photoresist, we would need a laser intensity on the order of 10^{10} w·cm^{-2}. A detailed numerical simulation will have to be undertaken to determine the hydrodynamic behavior of local concentrations of proteins and thereby indicate the actual resolution that can be achieved. However, because of the rapid dependence of intensity on the size of resolution element, we can somewhat confidently say that a resolution better than 2000 Å could be obtained.

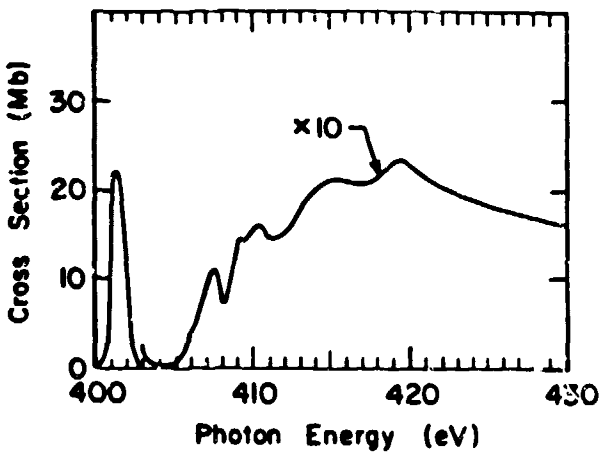

Figure 8: K-shell absorption spectrum of N_2 as determined photoelectrically by Bianconi et al. Notice enormous resonance at 401.3 eV for the $1S \rightarrow \pi 2P$ transition. (Adapted from figure in Ref. 23).

Possibilities

We have chosen nitrogen as an example because it seems to be the most likely atomic species we would like to detect within a cell. Looking at nitrogen would distinguish some of the more interesting aspects of cellular structure while ignoring the water, the sugars, and the lipids. This means we could see the membranes, the cytoskeleton, the nucleus, the mitochondria, and various other organelles. This is a distinct advantage over the electron microscope which in vivo does not distinguish the water from the structural elements without special preparation. In general, electron microscope samples must be freeze-dried and sputtered with a high-Z element such as osmium or uranium. It is generally thought that the cellular organization is radically disturbed by this process.

One of the most exciting possibilities is to take snapshots of mechanical processes within the cell on picosecond time scales. For example, one might be able to see the penetration of vesicles of neurotransmitter through the presynaptic membrane.[26] Also, with resolution of 100 Å or so, one might be able to detect switches in molecular conformation such as those thought to be responsible for signal promulgation in retinal cells.[27]

Conclusions

By process of elimination it appears to us most practical to use Fraunhofer holography with a photoresist recording surface. The recording properties of photoresist are optimum in the wavelength region where we could use resonant coherent scattering from nitrogen. To have reasonable contrast ratio, we need a "shadow foil" that is sufficiently homogeneous that it does not significantly alter the temporal and spatial coherence of the reference beam. The problems attending preparation of such a foil seem less stressing than those of making and measuring a suitable reflector for spherical Fourier holography. We also find that we must use resonant coherent scattering to obtain holograms at reasonable intensities.

Acknowledgments

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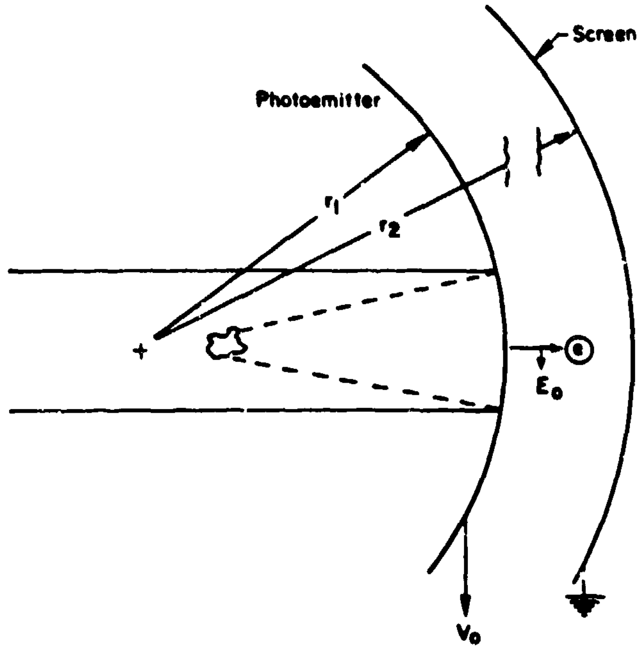

Figure 4: Conceptual lensless electron microscope with Fraunhofer holography.

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Fourier Holography

The trouble with lensless Fourier holography is that (in the figurative sense) it is not lensless. Figure 5 shows a typical setup for lensless Fourier holography. In order to obtain the spherical reference wave we must have a lens that focusses to a pinhole in the shadow plane. In the x-ray regime we do not use a lens, but rather use a Fresnel zone plate. With this type of experimental configuration, it is easy to prove that the hologram resolution is limited to the finest spacing on the Fresnel zone plate. This derives from the fact that the finest spacing on the zone plate determines the maximum angle of the spherical segment generated. The finest zone plate spacing allowed by current technology is about 800 Å.[15] This means that using this technique we could obtain resolution no better than 800 Å.

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We define the stagnation time for a heated region by

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where δ is the linear dimension of the region, C_v the specific heat at constant volume, and θ the temperature. Under the assumption that the hologram can be recorded without blurring for a period on the order of T_{stag} time, the required intensity is given by

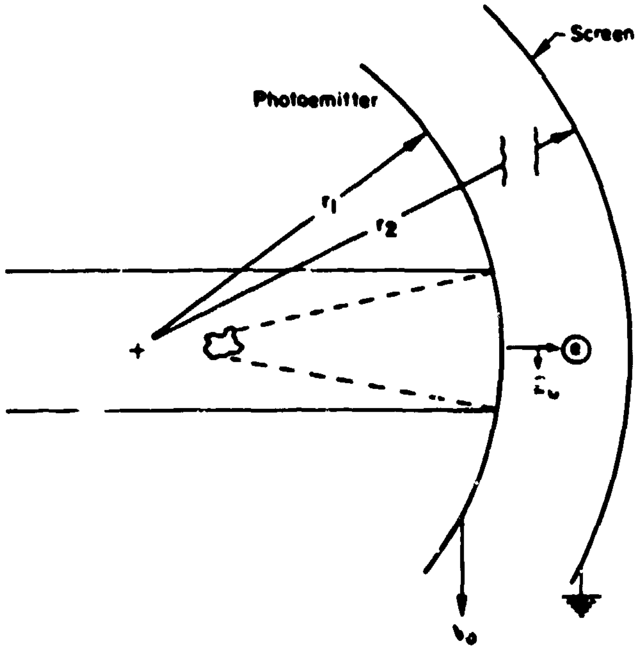

Figure 4: Conceptual lensless electron microscope with Fraunhofer holography.

A potential problem with Fraunhofer holography is low contrast ratio. This is particularly true if the specimen is not a very efficient coherent scatterer. The problem can be circumvented by mounting the specimen over a hole in an absorbing foil, which will attenuate the reference beam. The uniformity of the "shadow foil" must be such that spatial and temporal coherence of the reference beam are not drastically altered.

Fourier Holography

The trouble with lensless Fourier holography is that (in the figurative sense) it is not lensless. Figure 5 shows a typical setup for lensless Fourier holography. In order to obtain the spherical reference wave we must have a lens that focusses to a pinhole in the shadow plane. In the x-ray regime we do not use a lens, but rather use a Fresnel zone plate. With this type of experimental configuration, it is easy to prove that the hologram resolution is limited to the finest spacing on the Fresnel zone plate. This derives from the fact that the finest spacing on the zone plate determines the maximum angle of the spherical segment generated. The finest zone plate spacing allowed by current technology is about 800 Å.[15] This means that using this technique we could obtain resolution no better than 800 Å.

An alternative could be to use a coherent scattering reflector to generate the reference waves.[16,17] Figure 6 shows such a reflector, in this case a paraboloid that would generate spherical reference waves, enclosed in a spherical shell recording surface. For best contrast ratio, the paraboloid would have to be approximately the same size as the object (for example, 100 μm for a 100 μm biological specimen). The reference scatterer need not be a paraboloid. In principle, the hologram could be unfolded for any convex reference scatterer as long as we knew its shape and dimensions to within a fraction of a wavelength.[18] Presently, spherical microballoons can be made 100 μm in diameter with a surface smoothness better than 100 Å. These balloons can be measured to within 50 Å. So using a reflector that would work in the 30-50 Å region is just on the edge of technological feasibility.

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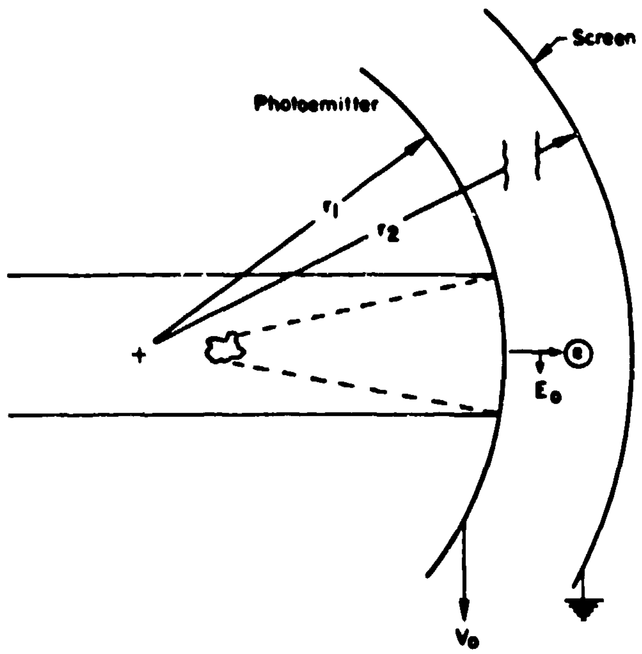

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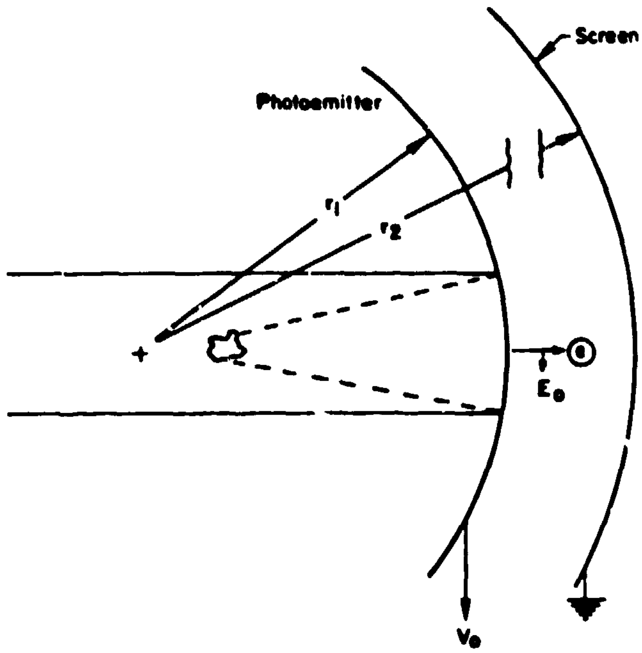

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