Scanning Microscopy

Volume 1 | Number 3 Article 2

4-28-1987

X-Ray Microscopy and X-Ray Imaging

R. E. Burge
King's College London

A. G. Michette
King's College London

P. J. Duke
King's College London

Scanning Microscopy, Vol. 1, No. 3, 1987 (Pages 891-900)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/87$3.00+.00

X-RAY MICROSCOPY AND X-RAY IMAGING

R E Burge*, A G Michette and P J Duke†

Department of Physics, King's College London (KQC), Strand, London WC2R 2LS, UK

† Also at: SERC Daresbury Laboratory, Warrington WA4 4AD, UK

(Received for publication February 20, 1987, and in revised form April 28, 1987)

Abstract

Within a framework of an overview of the current status and potential of X-ray microscopy, a description is given of the development of the King's College scanning instrument which produced its first images in September, 1986. The instrument was mounted on the newly-built undulator beam line at the UK Science and Engineering Research Council's SRS synchrotron. There are consequently three sites worldwide where high-resolution X-ray microscopes with zone-plate optics are in operation. The other sites are BESSY-Berlin and NSLS-Brookhaven.

KEY WORDS: X-rays, microscopy, imaging, scanning, zone plates, synchrotron radiation, microradiography, holography, radiation damage, electron beam writing.

*Address for correspondence:
Department of Physics, King's College London (KQC),
Strand, London WC2R 2LS, UK

Phone No. (01) 836 5454
Ext 2154

Introduction

X-ray imaging was first demonstrated in the form of microradiography in the nineteenth century when a photographic emulsion was used to record the X-ray transmission of a specimen placed in contact with it. This method, now called contact microscopy, is in increasing use, photographic emulsions having been replaced by high-resolution photoresist layers as detectors and utilizing high-intensity synchrotron or plasma sources of soft X-rays. The information encoded by the detector in contact microscopy is limited by the structure of the detector surface and its observation requires either optical or electron microscopy. Cosslett and Nixon (7) provided a basis for X-ray microscopy by introducing a microfocus X-ray source and a projection system to produce image magnification; they achieved a point to point resolution similar to that achieved in optical microscopy. Further progress towards the realisation of X-ray microscopy at improved resolution had to await the development of synchrotron sources with appropriately high brightness and of suitable optical components for focussing X-rays.

Soft X-rays, meaning photons with energies less than about 1 keV (or wavelengths greater than about 1 nm), are of interest because of the need to match the high-resolution potential of thin specimens with sufficient beam-specimen interaction to produce adequate image contrast with limited beam damage. Currently there are three imaging methods which have been demonstrated, contact microscopy and X-ray microscopy as introduced above, and also the use of coherently scattered photons, for example X-ray holography of the Gabor type in which waves scattered by an object are superimposed coherently on the direct incident wave; in principle, coherent scattering methods allow reconstruction of the object in three dimensions as well as offering the possibility of resolutions in the range of a few nm due to their ability of using photons scattered at large angles in forming the image.

In essence, the contact imaging method has remained unchanged from its beginnings, apart from the increasing sophistication of methods of X-ray irradiation, specimen containment in contact with the photoresist layer, and photoresist

examination (4,8). The contact image is expressed as a relief structure following resist development, and the detailed characterisation of the relief structure, in relation to the X-ray absorption by the specimen, is still a research problem. An important extension of the contact method which uses coherently scattered photons has recently been suggested by D Sayre (private communication) where successive parallel photoresist layers are exposed downstream of the specimen. The images are related by a Fresnel propagator and may possibly be used to solve the phase problem for the complex object wave and to reconstruct the three-dimensional distribution of specimen absorption.

X-ray microscopy, meaning the dynamic observation of specimens under real-time viewing conditions, is required to supplement optical microscopy and electron microscopy. The ideal microscope would combine the flexibility of the optical microscope, which allows, for example, hydrated specimens to be examined in air at a spatial resolution limited to about 0.2 μm by optical wavelengths, with the atomic-dimension point to point resolution of the electron microscope, but avoids the preparation procedures in electron microscopy which are required to provide suitably thin specimens with appropriate electron contrast and also circumvents the mounting of specimens in a vacuum environment.

The X-ray microscope provides an imaging system which goes some way towards this ideal. The imaging of biological material by X-rays may be considered with the aid of figure 1, in which is plotted the X-ray absorption coefficients for carbohydrate, water, protein, air, and also gold at a range of X-ray wavelengths through the soft X-ray region. We note, first, the absorption coefficient for air at standard temperature and pressure is consistent with the design of specimen stages in air providing the X-ray path length is not more than a few mm, alternatively specimens can be mounted in a helium-enriched atmosphere. Second, the range of absorption coefficients for biological material containing water at this range of photon energies is consistent with a realistic specimen thickness of 5 μm to 10 μm. Third, the "water-window", between the K-absorption edges for O and C, provides a wavelength region across which, for hydrated biological material, little correction is necessary for the effects of water absorption on image contrast. The questions of suitable optical elements for microscope operation and the achievable spatial resolution are discussed below.

Holographic X-ray microscopy has been discussed recently by Howells (9). Holograms using synchrotron radiation were first made by Aoki et al (1) and an assessment of progress and future prospects is made by Howells et al (10). No work on X-ray holography using synchrotron radiation has yet been attempted in the UK. Published work shows that X-ray holography using the Gabor geometry and film detectors for recording the X-ray hologram followed by visible light reconstruction breaks down around the 0.5 to 1 μm resolution level. Further progress will require either holographic recording using a photoresist, with interpretation by electron microscopy, or the change to Fourier transform geometry.

X-ray sources

X-ray microscopy is under intensive development at the synchrotron sites BESSY-Berlin, NSLS-Brookhaven, LURE-Orsay, KEK-Tsukuba and Hefei. Both X-ray microscopy and holographic X-ray microscopy require intense photon sources of very high brightness and high spatial coherence. If chromatic optical elements are used the radiation incident on the specimen should have restricted bandwidth. The practical matter at issue is exposure time in relation to image spatial resolution, specimen deterioration and specimen vibration. For the holograms taken by Howells et al (10) using 3.1 nm radiation from the NSLS 750 MeV storage ring, exposure times using photographic film recording were in the range 3 to 100 minutes, and photoresist recording times were of the order of hours. For images (see below) using the 2 GeV energy Synchrotron Radiation Source at Daresbury on the 5U (undulator) beam line taken in September 1986, the coherent flux in an X-ray probe of 100 nm half width was about 2×10^4 photons s^{-1} per 1% bandwidth. Consequently a 128 x 128 point image had an exposure time of about 30 mins. Urgent efforts are being made to reduce this exposure time by improvements to the monochromator. A factor of ten increase in brightness is expected after the installation, currently in progress, of the High Brightness Lattice (HBL) at the SRS. The increased strength of the magnetic focusing in the HBL reduces the cross-section of the electron beam in the storage ring, thus reducing the effective source size as seen from any beamline.

A crucial factor in the current rapid development of X-ray microscopy worldwide has been the increasing availability of undulator beam lines of synchrotron sources and the high levels of the coherent photon flux incident on an X-ray focusing element following an undulator-monochromator system. Calculations for the spatially coherent flux at a wavelength in the centre of the water window with chromatic energy resolution $\lambda/\Delta\lambda \sim 500$, expected using the projected 6 GeV ESRF* synchrotron at Grenoble with a focused monochromatic spot of 10 nm diameter, predict flux levels between 10^9 and 10^{10} photons s^{-1}. For X-ray imaging directed towards spatial resolutions of 10 nm the pressure for source development is to increase the flux levels achievable in nanometer spot sizes in order to reduce exposure times to practical levels (say < 10s for 512 x 512 image points in a scanned image). A further factor of importance is the tuneability of the synchrotron wavelength, allowing images to be taken at, and to the side of, an elemental X-ray absorption edge thus allowing quantitative elemental analysis. Some selected absorption edges are listed in Table 1:

*ESRF: European Synchrotron Radiation Facility

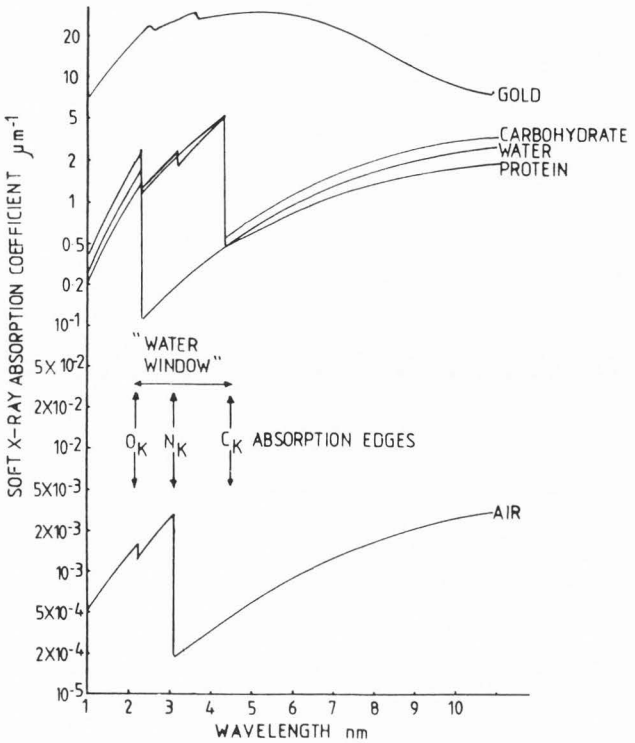


Figure 1. X-ray absorption coefficients for various soft X-ray wavelengths. The water window lies between the K edges for oxygen and carbon at wavelengths of 2.33 nm and 4.36 nm, respectively.

Table 1 K and L shell X-ray absorption edges (nm)

Element	K	L_I	L_{II}	L_{III}
C	4.36			
N	3.03			
O	2.28			
Mg	0.95			
P	0.58	6.56	9.12	9.19
S	0.50	5.37	7.58	7.63
Ca	0.31	2.83	3.55	3.58

Optical systems for X-ray microscopy

X-ray optical systems for X-ray microscopy may be based either on reflection, eg, as in the Wolter geometry (20,21) using grazing incidence illumination or the Schwarzschild normal incidence geometry using the enhanced normal incidence reflectivity of multilayers (3,17), or on transmission systems using diffraction, ie, Fresnel zone plates. The first X-ray microscopy using zone plates and synchrotron radiation was published by Schmahl and his group in 1984 (15).

High-resolution X-ray microscopic (as distinct from contact) imaging, defining this as a spatial resolution of 50 nm or better, has so far been produced entirely using zone plates. For this reason zone plate imaging will be considered in some detail; but the potential for further development is noted of the grazing incidence

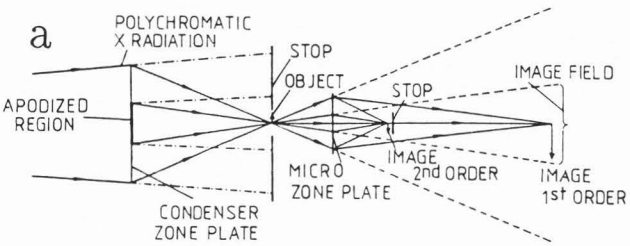


Figure 2(a). The optical arrangement (15) of the fixed-beam X-ray microscope mounted at the BESSY synchrotron, Berlin. A zone plate is used as a combined monochromator and condenser and the object is followed by an objective zone plate. Unwanted zero order and higher order foci from the zone plates are blocked by a combination of central obstruction of the condenser and a central aperture.

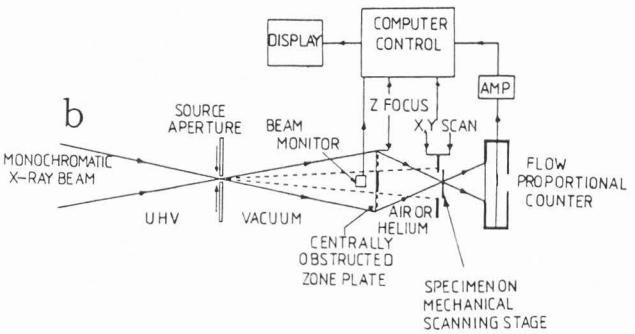


Figure 2(b). Optical system, following the monochromator, of the King's College scanning X-ray microscope (6). The probe-forming zone plate is centrally obstructed and unwanted diffraction orders are screened by an aperture (or collimator) from the specimen. Only the first order and zero order beams are shown.

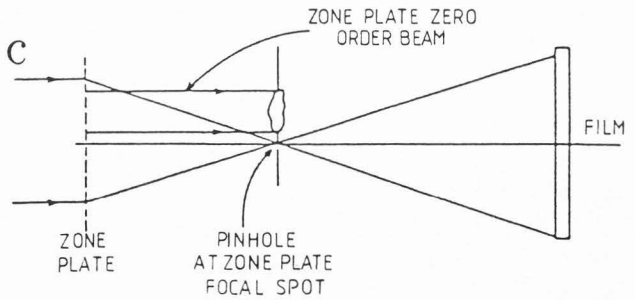
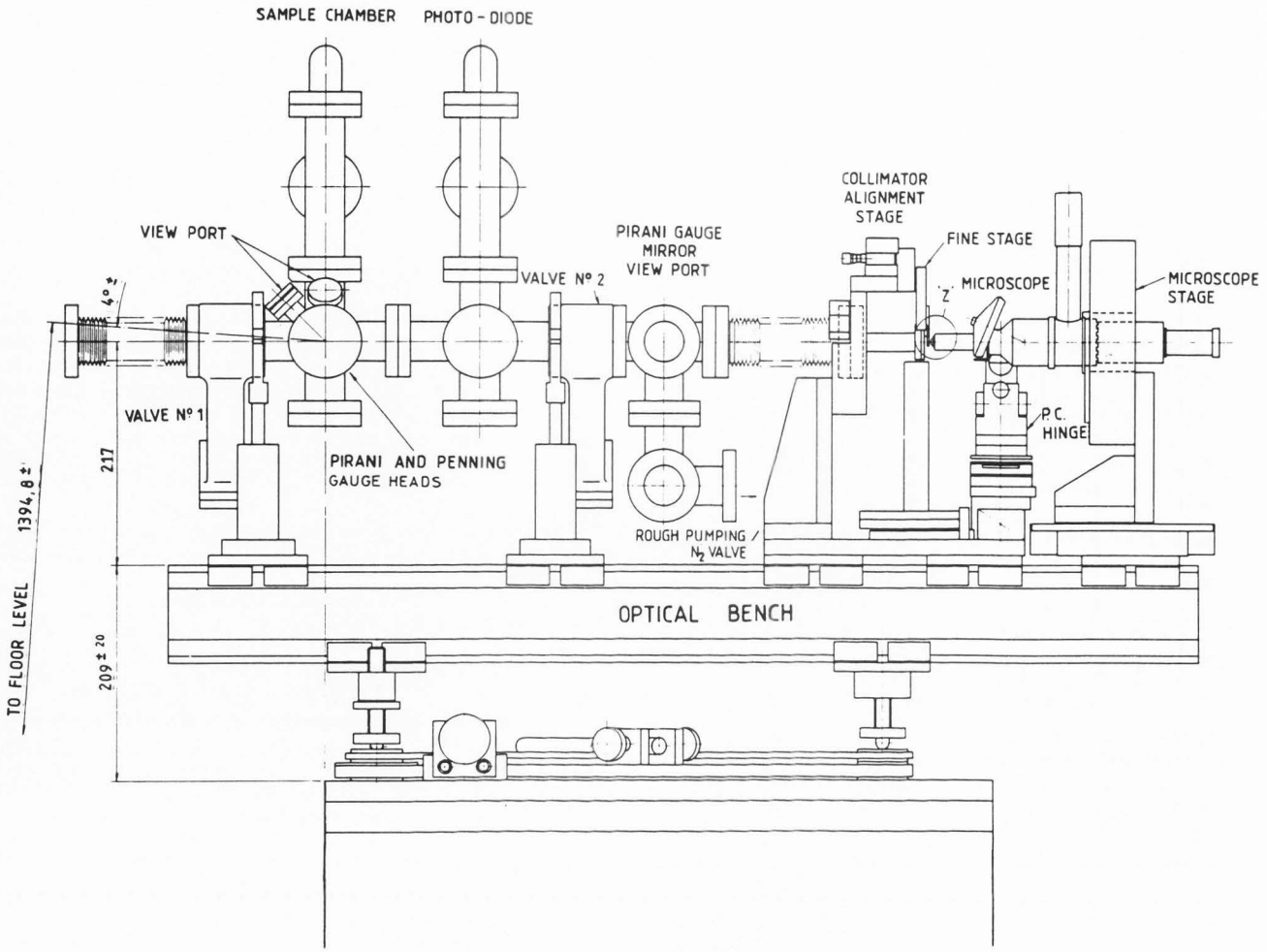


Figure 2(c). Proposed optical arrangement (9) using a zone plate to produce holographic X-ray imaging using Fourier transform geometry. Waves overlap from the zero-order object transmission and the pinhole at the first order focus of the zone plate.



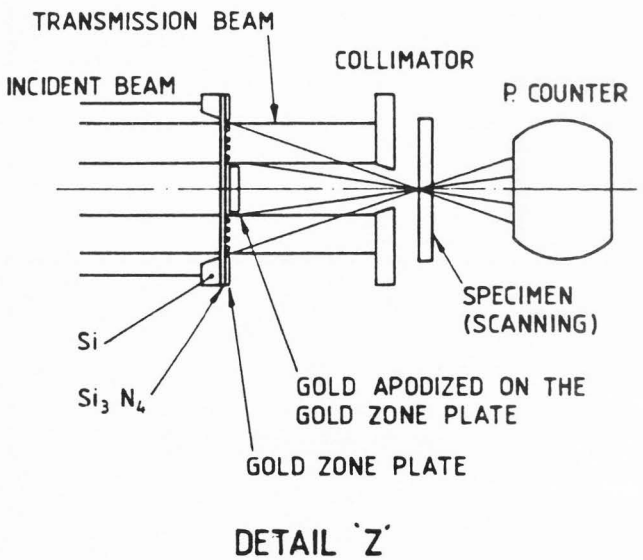


Figure 3. Diagram of King's College/SERC Daresbury X-ray microscope. The sample chamber at the left allows X-ray contact microscopy. The photodiode provides monitoring of the incident X-ray intensity. The detail Z shows a gold zone plate directly mounted on a vacuum window (200 nm thick silicon nitride). The entrance slit to the microscope is to the immediate left of the drawing and the system is evacuated up to the zone plate mounting. The specimen is usually mounted in air on a simple support attached to the specimen fine stage. The light microscope allows specimen observation and a Michelson interferometer using white light fringes assists the setting of focus. The detector is a proportional counter.

Fine stage movement (10 nm steps in x and y, range 40 μm) is by inverse piezo-electric stage drivers. The coarse stage has a range of about 4 mm in steps of 1 μm.

approach, especially in the hands of Franks and his colleagues at the National Physical Laboratory.

Three optical systems using zone plates are shown in figure 2. Figure 2(a) shows the fixed-beam microscope (15) mounted at BESSY, which has zone plates for both the combined condenser and monochromator and for image formation. Both zone plates are arranged to work in the first order focus; both the zero order and higher diffraction orders are screened from the object and image planes. Images are recorded by photographic film or channel plate. With a fixed beam optical system, the whole field of view is imaged simultaneously, which is important for dynamic observations. Further, the back focal plane of the objective is available for the installation of a phase plate; phase contrast X-ray microscopy is under development for a wavelength of 0.8 nm (G Schmahl, private communication).

Of course, monochromators need not be based on zone plates and the monochromator at SERC Daresbury is based on conventional grating dispersion. In figure 2(b), the optical system for a scanning X-ray microscope is shown, excluding the monochromator. Three scanning microscopes are in operation, at NSLS/Stony Brook, SRS/King's College London, and at BESSY/Gottingen. The zone plate produces a fine X-ray spot at the specimen, currently a minimum of 50 to 75 nm in diameter in all three microscopes, and the specimen is scanned in a raster across the beam. The minimum scanning increment is about 10 nm and stages operate at frequencies up to 1 KHz. As in figure 2(b) the zone plate in the scanning instrument has a central obstruction in front of the specimen to screen away all but the first order focus.

In the fixed-beam microscope the X-ray photons passing through the specimen are imaged by a zone-plate in first order focus. Since for current zone-plates the diffraction efficiency in first order focus is only about 5%, most of the photons traversing the specimen produce beam damage and not image information. In contrast, in the scanning instrument, all the photons in the illuminating cone which are transmitted by the specimen contribute to the detected signal and this is an important factor in favour of choosing a scanning system. However, the scanning microscope is less useful for the observation of specimens subject to rapid change.

The balancing of the advantages and disadvantages of fixed beam and scanning X-ray microscopes is reminiscent of similar discussions for optical and electron microscopes. Just as for these established microscopes, fixed beam and scanning X-ray microscopes are complementary and both are needed.

Figure 2(c) shows how a zone plate system may be used to produce a Fourier transform hologram (9). The reference source is provided by the focused point at the first order zone plate focus and the specimen is illuminated by the zero order beam.

In figure 3 is shown a block diagram of the King's College/Daresbury scanning X-ray microscope by which a diffraction image of an entrance aperture at a demagnification of about 1000 times is produced at the specimen by the zone plate.

The preparation and properties of zone plates

A zone plate is a circular diffraction grating with radially increasing line density. If the radius of the first zone is r_1, the first order focal length f_1 is given approximately by $f_1 = r_1^2/\lambda$, and the mth order focal length f_m by $f_m = f_1/m$ where m is an integer. Both real and virtual foci occur. A zone plate with n zones, $n > 100$, has object and image distances related in the same way as thin lens. Because of the dependence of focal length on wavelength, quasi-monochromatic illumination of a zone plate is necessary with $\lambda/\Delta\lambda \sim n$. The resolution δ of a perfect zone plate with outermost zone width dr_n is given by the Rayleigh criterion as $\delta = 1.22\, dr_n/m$. Zone plates are normally designed for use in the first order m = 1, hence improvement in point to point resolution directly concerns the reduction of dr_n.

Zone plates have been made by three methods: (i) a holographic lithographic technique useful for both condenser and objective zone plates superimposing two coherent ultraviolet sources at a substrate coated with photoresist has been developed by Rudolph and his colleagues (15): this method has a potential limit for dr_n of about 50 nm. This group has suggested a method to produce even finer zones by X-ray interference on superimposing first and second order radiation from a zone plate made by UV holography (15) but it seems unlikely that the mechanical and electrical stabilities at the BESSY synchrotron source will allow its practical implementation at present. (ii) Research groups in Japan (2), Aachen (12) and IBM Yorktown Heights (18,19) have developed E-beam lithographic procedures using photoresist to produce zone plates. The current limit on dr_n from this method, due mainly to instabilities in the electron scanning systems is about 50 nm. (iii) The use of electron beam writing by enhanced carbon contamination and a matrix of highly accurate local fiduciary marks (5) within a fabrication procedure using a scanning transmission electron microscope partly under microprocessor control and partly under operator control. At King's College zone plates have been drawn in carbon by this method with outermost zone widths of between 18 nm and 100 nm, outer diameters of up to 50 μm and with several hundred zones. From the point of view of the geometry of fabrication, and the accuracy of placement of the zones, the zone plates with dr_n = 18 nm are considerably in advance of the rest of the world, but the problem lies in the relatively low diffraction efficiency of a zone plate made in 200 nm thick carbon contamination on a carbon or silicon nitride supporting film 20 nm thick. Such a carbon zone plate has a first order diffraction efficiency of about 0.5%, as against about 5% for a gold zone plate. A carbon zone plate with dr_n = 18 nm and a diameter of 36 μm is shown in figure 4. Using the procedures of microelectronics, viz, replication into gold layers by exposing through the carbon zone plate using soft X-rays a photoresist layer on a gold film, followed by ion beam etch-

ing, gold zone plates with dr_n = 75 nm have been produced and used in actual X-ray imaging. The question of replicating still finer structures, eventually achieving dr_n = 10 nm is an active research problem. An alternative possibility is to fabricate ultra-fine zone plates directly by introducing other contamination than hydrocarbon into the path of the electron beam. Possible contaminants (13) are the gases WF_6 and WCl_6 for depositing tungsten or $Cr(C_6H_6)_2$ for the deposition of chromium. Some properties of carbon zone plates prepared at King's College are shown in Table 2.

Table 2 Properties of carbon zone plates

Radius (μm)	dr_n (nm)	Focal length mm at 3.1 nm	Depth of focus (μm)
25	100	1.600	± 6.4
23.5	75	1.125	± 3.6
21.5	75	1.030	± 3.6
22.5	50	0.710	± 1.6
28.5	30	0.550	± 0.6
18	30	0.345	± 0.6
18	18	0.210	± 0.2
25*	70	0.560	± 1.6

*A zone plate with every other absorbing zone missing has been constructed. This enhances the diffraction efficiency in the second order, m = 2, and provides a method to image at higher resolution; the diffraction efficiency for m = 2 may reach 2% at best.

Methods (ii) and (iii) of zone plate fabrication have not been applied to the fabrication of condenser zone plates which have a diameter of 2.5 mm. It would be difficult to maintain the necessary nm placement accuracy by any e-beam writing process for the zone plate rings across such an area.

The above consideration has been of amplitude zone plates, with either (in principle) totally transmitting or totally opaque zones. Considerably greater first order diffraction efficiency, up to 10% or 15% is expected for phase zone plates. Such zone plates are prepared to make a balance between phase effects and absorption effects in the metallised zones. A potential problem in microfabrication occurs since, for a germanium zone plate for example for a wavelength in the "water window", a thickness of about 400 nm is needed, corresponding, for dr_n = 10 nm, to an aspect ratio of 40:1.

Finally, mention should be made of the "jelly-roll" method of making zone plates (16) in which alternate layers of materials with different X-ray absorption coefficients are sputtered on a rotating wire 10 to 20 μm in diameter. Phase zone plates may be produced by cutting short pieces of the roll and using ion etching to produce the thickness required. This method is undergoing steady development but difficult problems have yet to be overcome of accurate zone placement and zone uniformity with increasing radial line density for large numbers of zones.

X-ray beam-specimen interactions: radiation damage

The principal interaction of soft X-ray photons with matter is atomic photoelectron emission, followed at a much lower level of importance, by coherent scattering (ie, diffraction of long wavelength X-rays). The latter has actually been observed (22) and provides a means to observe X-ray diffraction from isolated structures (as distinct from a crystal lattice).

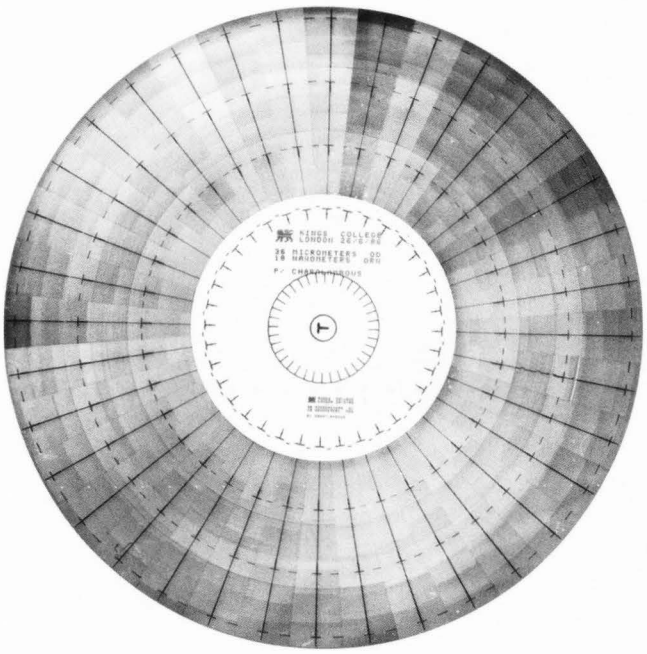
The emission of photoelectrons from a photoemissive surface placed behind the specimen in the manner used for contact microscopy followed by electron acceleration and electron imaging, has been developed (14), to produce real-time distributions of the X-ray intensity transmitted by the specimen. Considerable problems have been found in the practical development of the method due to mechanical instability and the resolution is limited currently to about 100 nm because of chromatic aberration in the electron images arising from the range of photoelectron energies.

The most important property of photoemission concerns the absorption edge structure and its relationship to elemental detection and quantitative analysis. For this purpose, the atomic number dependence of the cross-section for photoemission is very much more accommodating than the corresponding total mass scattering coefficient for electrons. X-ray fluorescence provides a further potentially useful signal as a consequence of electron rearrangement. X-ray fluorescence (XRF) spectroscopy is a well-established method for multi-element analysis of solids, powders and liquids. However, the efficient application of XRF requires much higher energy photons at the specimen than those used in X-ray microscopy.

Concerning radiation damage by X-rays with energy < 1 keV, there has been little detailed work on the molecular effects in this photon energy range which falls between the UV energy region and the hard X-ray/gamma ray region in both of which there has been extensive work. Up to the present, observed X-ray images have not been of a sufficiently high resolution to allow discussion of more than gross effects of radiation. Certainly, considering polymeric and biological materials, it would be unreasonable not to expect similar effects of radiation to those, for the same incident X-ray energies, which produce molecular changes in photoresists like PMMA when used as photon detectors and for X-ray lithography.

X-ray images

The first high X-ray resolution microscope images in the UK were taken with the King's College/Daresbury scanning microscope in September 1986, just prior to the SRS shut down for the installation of the high brightness lattice. Prior to September, the King's College zone plates had been tested in the scanning microscope at the Brookhaven synchrotron (by courtesy of Professor J Kirz), and subsequent to September some observations using King's zone plates were made in the fixed beam microscope at BESSY (by courtesy of Professor G Schmahl).



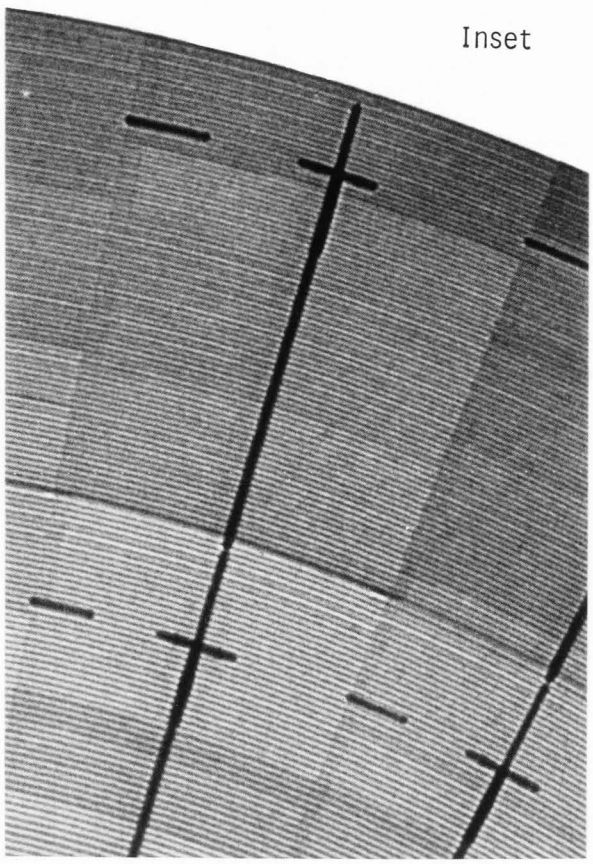


Figure 4. A high-resolution zone plate dr_n = 18 nm; zone plate diameter 36 μm; inset shows higher magnification image of outer zones - the short, thick lines are about 0.6 μm long) drawn in carbon contamination using (the prototype) Vacuum Generators HB5 STEM which, for 60 keV electron energy operation, has an electron probe of about 2 nm. Hydrocarbon contamination is introduced near the upstream surface of a Pt or Mo aperture disc covered with a thin support film. Initial work was carried out with 20 nm thick support films of evaporated carbon. Later work has used 40 nm thick films of boron nitride (courtesy of Dr R Feder), which had good transmission of soft X-rays and was smoother and flatter than the carbon films; these properties assist in the gold replication step.

The hydrocarbon molecules diffuse across the film surface and are "fixed" in position by the electron beam. The contamination structures, for a stationary beam, are normally about 150 to 200 nm high and 20 nm in diameter. To write structures of still smaller diameters requires the contamination level to be reduced. Control of the thickness of the contamination is affected by balancing the reducing bright field image signal against the increasing dark field as the contamination structures grow.

The placement accuracy currently achieved in writing the zone plates is to within a total error of about 10 nm across an aperture of 50 μm diameter. This accuracy is achieved by separately writing fields only a few μm in extent, each being related under microprocessor control to a local fiduciary mark (the 'T's' in the figure). The specimen is rotated mechanically by 10^{o} between patches; the total operation of writing a zone plate takes about 8 h.

The zone plates, following replication into gold, are apodised by evaporation of gold with a central spot about 20 μm in diameter following the alignment of the masks in a suitable jig.

The present purpose, which is essentially related to instrumental validation and future potential, will be served by showing an image from the first two of these phases of activity.

In figure 5 is shown an early X-ray image of a chick ciliary neurone (from the nervous system; specimen prepared by Dr J Pine) taken with the NSLS synchrotron, the Stony Brook scanning microscope and a King's College objective zone plate. Figure 5(a) is the original image and figure 5(b) is an image which has been subjected to computational procedures for noise reduction and for image sharpening. The procedure for image sharpening, for an (initially) unknown point spread function describing the X-ray probe, was carried out by a new method of blind deconvolution we have developed, followed by use of the maximum entropy algorithm (program courtesy of Dr J Skilling). X-ray images should be seen in the context of computer methods of image enhancement, noise correction and surface resolution.

Figure 6 is an image of wet, "living", bacterial spores taken with the King's College scanning X-ray microscope at the Daresbury SRS using a gold zone plate (46 μm diameter, 75 nm outer zone width). The specimen was provided by Dr S Ogawa. This image formed part of a study of the distribution of Ca in the spores and of an analysis of the effects of radiation damage.

Conclusions

The X-ray microscope has so far emerged as a working instrument at three synchrotron sites

(BESSY, Brookhaven and Daresbury), capable of producing images at a point resolution of about 50 nm. With further development, a resolution of 10 nm using zone plate optical elements appears to be possible. To achieve such a resolution, an outermost zone width of 10 nm is required together with a placement accuracy of the zones of about 3 nm right across the zone plate diameter of about 50 μm.

Contact microscopy is now considered to have a best resolving power of about 10 nm, limited by noise in present photoresists, (4,8). It seems likely that the much more flexible X-ray imaging microscopes with synchrotron radiation will supersede the contact method as the resolution improves apart from one possibility. The possibility may arise following the rapid (a few nanoseconds) X-ray exposure provided by X-ray emission from a plasma created by a pulsed laser source. The question at issue is whether the image of a beam sensitive specimen, for which the relaxation times for the processes of molecular damage are much longer than the pulse length, is rendered essentially without beam damage. An interesting alternative possibility is, of course, provided by an X-ray microscope mounted on a pulsed X-ray source where a similar advantage may accrue.

The methods of using coherently scattered photons, as a development of the contact method on the one hand and in the form of Gabor or Fourier transform holograms on the other, seem to be converging. It will be interesting to see whether three-dimensional reconstruction of X-ray holograms becomes a viable proposition as an alternative to the obviously practical methods of tilt series reconstruction or the imaging of successive focal levels through a thick specimen. The latter possibility may supplement the reconstruction of thick sections in electron microscopy at present realised by many serial sections.

The happy accident of the "water window" makes X-ray microscopy of high potential for the study of wet, possibly living, biological specimens at a resolution considerably improved over the light microscope. Indeed, the X-ray microscope falls in performance neatly between the light microscope and the electron microscope. In order to realise the potential of useful biological imaging, the problems of containment of wet, and of living specimens during examination remain to be solved. A glance at Table 2 shows that the higher resolution zone plates have very short focal lengths, at least as made currently, leading to problems for specimen mounting, cooling and tilting. Further development is in hand to increase the focal length by writing the zones to large diameters.

The use of absorption edges for elemental microanalysis is potentially important for a range of specimens, including biological specimens. A first study of the mapping of the distribution of calcium in bone has been published (11).

Application of soft X-ray microscopy in materials science generally may take two possible forms. First, transmission experiments using, eg, polymers, rubbers and resins, and second, scans of the X-ray probe across the surface of materials in order to generate photoelectron or fluorescence signals at high spatial resolution.

The current rapid progress in the development of X-ray microscopy has occurred due to the simultaneous occurrence of two factors. These are, the development of the intensely bright synchrotron source of photons of tunable energy, and second, the advances, due to the processes of microelectronics, in the fabrication of optical elements of nanometer dimensions. Further development calls for ever brighter photon beams and the replication of structures which are ever smaller in size and increasingly complex. The prospects for the development of phase zone plates are increasingly bright. Such optical elements will allow imaging with harder X-rays, leading to a wider range of applications in the science of materials. It is because of the increasingly likely broadening of the photon energy range that this paper is headed 'X-ray microscopy' rather than 'soft X-ray microscopy'.

Acknowledgements

The following have participated in the work of the King's College London group which forms the basis of the above account: Dr M T Browne, Mr C Buckley, Mr R Cave, Dr P Charalambous, Mr A J Freake, Mr A Hare, Mr C P B Hills, Dr J Kenney, Mr T Kuriyama, Mr D Lidiard, Mr W Luckhurst, Dr A McDowell, Dr G R Morrison, Dr K Ogawa, Mr A M Rogoyski. The assistance rendered by Professor J Kirz and his colleagues at the NSLS Brookhaven synchrotron source in connection with testing of the King's College zone plates, and by Dr R Feder and associates at IBM Yorktown Heights in relation to replication of our carbon zone plates into gold, were of critical importance to the successful development of a working UK microscope, and acknowledgement is given to their professional expertise and kindness. Financial support from the SERC and from the Rigaku Corporation, Tokyo, is gratefully acknowledged.

References

1. Aoki S, Ichichara Y, Kikuta S (1972). X-ray hologram obtained by using synchrotron radiation. Jpn. J. Appl. Phys. 11, 1857.

2. Aritome H, Aoki H, Namba S (1984). Focusing characteristics of X-ray zone plates fabricated by electron beam lithography and reactive ion etching. Jpn. J. Appl. Phys. Part 2, 23, L406-L408.

3. Barbee Jr. T W (1984). Multilayers for X-ray optical applications, In: X-ray Microscopy. Springer series in Optical Sciences, (Eds.) G Schmahl and D Rudolph, 43, 144-162.

4. Beese L, Feder R, Sayre D (1986). Contact X-ray Microscopy, Biophys. J. 49, 259-268.

5. Buckley C J, Browne M T, Charalambous P (1985). Contamination lithography for the fabrication of zone plate X-ray lenses. In: Electron-beam, X-ray and Ion-beam Techniques for Submicrometer Lithographies IV. Proc. SPIE, 537, 213-217.

6. Burge R E, Browne M T, Buckley C J, Cave R, Charalambous P, Duke P J, Freake A J, Hare A, Hills C P B, Kenney J M, Kuriyama T, Lidiard D, McDowell A, Michette A G, Morrison G R, Ogawa K, Rogoyski A M (1987). The King's College/Daresbury

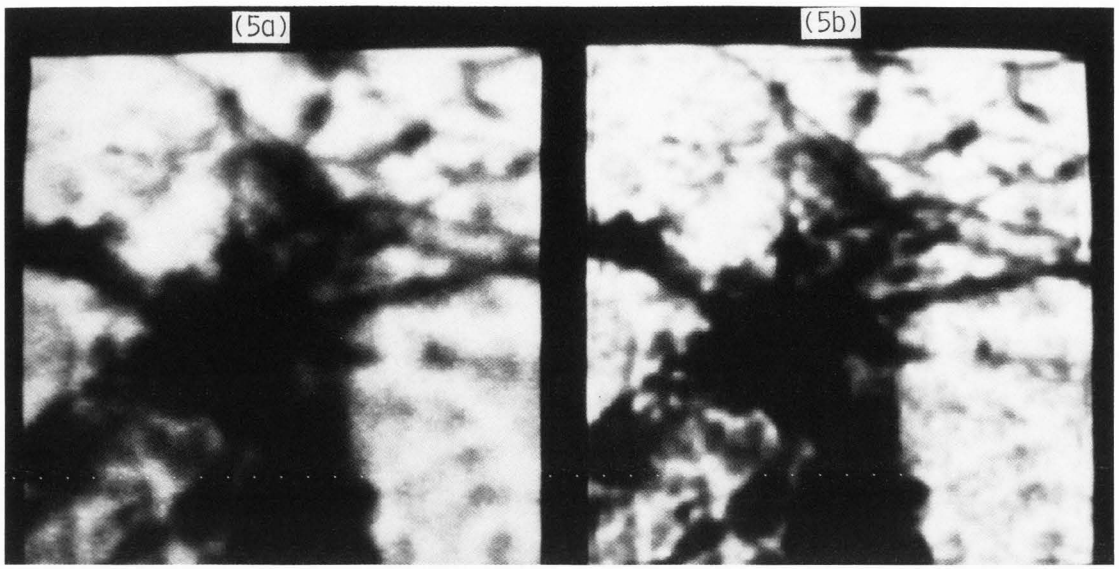


Figure 5. An early soft X-ray image of the chick ciliary neurone (specimen prepared by Dr J Pine), taken with the Brookhaven 750 MeV synchrotron, the Stony Brook scanning microscope and King's College zone plates. The agreement of Professor Kirz to publish this image is acknowledged. The image is 56 μm x 56 μm, pixel size 0.4 μm. Fig. 5(a) is the original image as recorded and Fig. 5(b) is an image after sharpening and noise reduction using the Skilling maximum entropy algorithm.

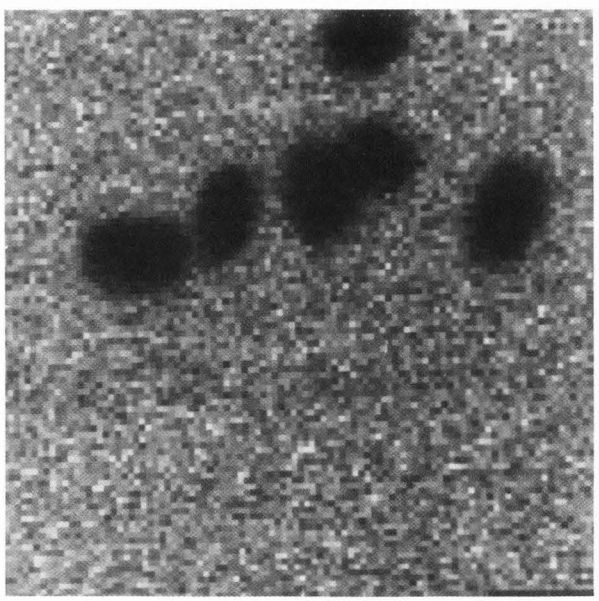


Figure 6. Image of wet bacterial spore taken at the SRS Daresbury with the King's College X-ray microscope at an X-ray wavelength of 3.1 nm. The image is 2.4 x 2.4 μm, pixel size 30 nm.

scanning X-ray microscope. In: Soft X-ray Optics and Technology. Proc. SPIE, 733 (in press).

7. Cosslett V E, Nixon W E (1960). X-ray Microscopy, Cambridge University Press.

8. Feder R, Mayne-Banton V, Sayre D, Costa J, Kim B, Baldini M, Cheng P C (1984). Recent developments in X-ray contact microscopy. In: Springer series in Optical Sciences, (Eds.) G Schmahl, D Rudolph, 43, 279-284.

9. Howells M R (1984). Possibilities for X-ray holography using synchrotron radiation. In: Springer series in Optical Sciences, (Eds.) G Schmahl, D Rudolph, 43, 318-385.

10. Howells M R, Iarocci M A, Kirz J (1986). Experiments in X-ray holographic microscopy using synchrotron radiation. J. Opt. Soc. Am. A3, 2171-2178.

11. Kenney J M, Jacobsen C, Kirz J, Rarback H, Cinotti F, Tomlinson W, Rosser R, Schidlovsky G (1985). Absorption microanalysis with a scanning soft X-ray microscope - mapping the distribution of calcium in bone. J. Microsc. (Oxford), 138, 321-328.

12. Kratschmer E, Stephani D, Beneking H (1983). High resolution 100 keV e-beam lithography. In: Microcircuit Engineering 83. (Eds) H Ahmed, J R Cleaver, G A C Jones. 15-22 Academic Press, New York.

13. Matsui S, Mori K (1986). New selection deposition technology by electron beam induced surface reaction. J. Vac. Sci. Technol. B4, 299-304.

14. Polack F, Lowenthal S (1981). Photoelectron microscope for X-ray microscopy and microanalysis. Rev. Sci. Instrum. 52, 207-212.

15. Rudolph D, Niemann B, Schmahl G, Christ O (1984). The Göttingen X-ray microscope and X-ray microscopy experiments at the BESSY storage ring. In: X-ray Microscopy. Springer series in Optical Sciences, (Eds.) G Schmahl, D Rudolph, 43, 192-202.

16. Rudolph D, Niemann B, Schmahl G (1981). Status of the sputtered sliced zone plates for X-ray microscopy. In: High Resolution Soft X-ray Optics, (Ed.) E Spiller. Proc. SPIE, 316, 103-105.

17. Spiller E (1984). A scanning soft X-ray microscope using normal incidence mirrors. In: X-ray Microscopy. Springer series in Optical Sciences, (Eds.) G Schmahl, D Rudolph, 43, 226-231.

18. Vladimirsky Y, Attwood D T, Kern D P, Chang T H P, Ade K, Jacobsen C, Kirz J, McNulty I, Rosser R J, Rarback H, Sku D (1987). High resolution X-ray zone plates. In: Soft X-ray Optics and Technology, Proc. SPIE, 733 (in press).

19. Vladimirsky Y, Källne E, Spiller E (1984). Fabrication of free standing X-ray transmission gratings and zone plates. In: X-ray Lithography and Applications of Soft X-rays to Technology, Proc. SPIE, 448, 25-37.

20. Wolter H (1982). Mirror systems with grazing incidence as image-forming optics for X-rays. Ann. Phys. 6th Ser. 10, 94-114.

21. Wolter H (1982). Generalised Schwarzchild systems of mirrors with glancing reflection as optical systems for X-rays. Ann. Phys. 6th Ser. 10, 286-295.

22. Yun W-B, Sayre D, Kirz J (1987). Observation of the soft X-ray diffraction pattern of a single diatom. Acta Cryst. A43, 131-133.

Discussion with Reviewers

F. Polack: The authors point out the importance of making zone plates with a high aspect ratio in order to achieve a high resolution with a good efficiency. Has the influence of the thickness of these structures on the point spread function of the zone plate been investigated?

Authors: This is a matter of current investigation. Indications are that, to a first approximation, the point spread function is not significantly altered for the aspect ratios presently being used.

F. Polack: To what extent can image processing restore images downgraded by zone plate imperfections?

Authors: This is not a simple question to answer, because it depends upon the type of imperfection. Loss of resolution caused by errors resulting in widening of the point spread function can, to a certain extent, be dealt with. We feel, at the moment, that our zone plates are sufficiently accurately made that image degradation is not an important factor.