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Soft-x-ray microscopes

Major developments in sources, optics and detectors for soft x rays promise to help biologists realize the longstanding goal of high-resolution imaging of biological materials in their natural, even living, state.

Malcolm Howells, Janos Kirz, David Sayre and Günter Schmahl

Biologists have long dreamed of a microscope capable of imaging specimens in their natural state, at molecular or near-molecular resolution. Physicists have for some years known that the soft-x-ray photon has properties that suit it for use as a probe in such microscopy. With the advent of synchrotron radiation sources, and with other technical advances, the difficulties that impeded the development of soft-x-ray microscopy have begun to give way, and in 1983 the technique produced the first images ever obtained of a living cell at a near-molecular resolution of 75 Å.

In this article we summarize the methods and potential of high-resolution imaging with soft x rays, and we attempt to assess the technique's prospects for the future.[1] After a look at the general imaging properties of photons in the soft-x-ray range of 100–1000 eV, we will discuss the optical elements and techniques used in current soft-x-ray microscopes such as the one shown in figure 1. Finally we will consider possible future systems for three-dimensional imaging based on diffraction-pattern analysis and holography.

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The soft-x-ray photon

Three techniques dominate submicron biological imaging today:

▶ Optical microscopy, introduced by Antony van Leeuwenhoek in Holland around 1671, uses visible and near-ultraviolet photons. This microscopy is successful in imaging unmodified and living biological material, but the long wavelengths preclude any approach to molecular resolution.

▶ Diffraction of x rays, discovered by Max Laue in 1912 and first utilized for the imaging of matter by Lawrence Bragg in 1913, uses photons in the medium-to-hard x-ray region around 10 keV. This technique gives fully three-dimensional images of very high quality, at atomic or near-atomic resolution. Investigators today use x-ray diffraction on assemblies of atoms as complex as proteins, nucleic acids and viruses, but the technique requires these substances to be available in a nonbiological form as macroscopic crystals or oriented fibers.

▶ Electron microscopy, developed by Ernst Ruska and Max Knoll in Berlin in 1932, uses electrons in the energy range 5–2000 keV. While electron microscopy can handle noncrystalline biological specimens, it generally requires subjecting biological specimens to some form of cellular disassembly, as well as modification of composition by dehydration and decoration with heavy atoms.

Thus none of the existing techniques quite manages as yet the double feat of imaging an unmodified biological cell at molecular resolution. There are spectroscopies that give information on the atomic structure of unmodified cells, but these are not full imaging techniques.

The limitations of the various imaging techniques are due to the imaging particles themselves. Thus in optical microscopy, it is the wavelength of the visible-light photon that limits the resolution. In x-ray diffraction, it is the relatively low coherent-scattering cross section of the 10-keV photon (figure 2) that makes it necessary to use macroscopic specimens to obtain sufficient interaction for imaging. The requirement for internal order then arises through the need to maintain reasonable simplicity of structure. In electron microscopy, on the other hand, it is the large cross section of the scattering electron that makes it necessary to disassemble the cell, and it is the low contrast, arising from the weakness of the dependence of cross section on atomic number, that makes staining and dehydration necessary.

Interest in using the soft-x-ray photon as an imaging particle stems from the fact that it avoids these limitations. The photoelectric absorption cross sec-

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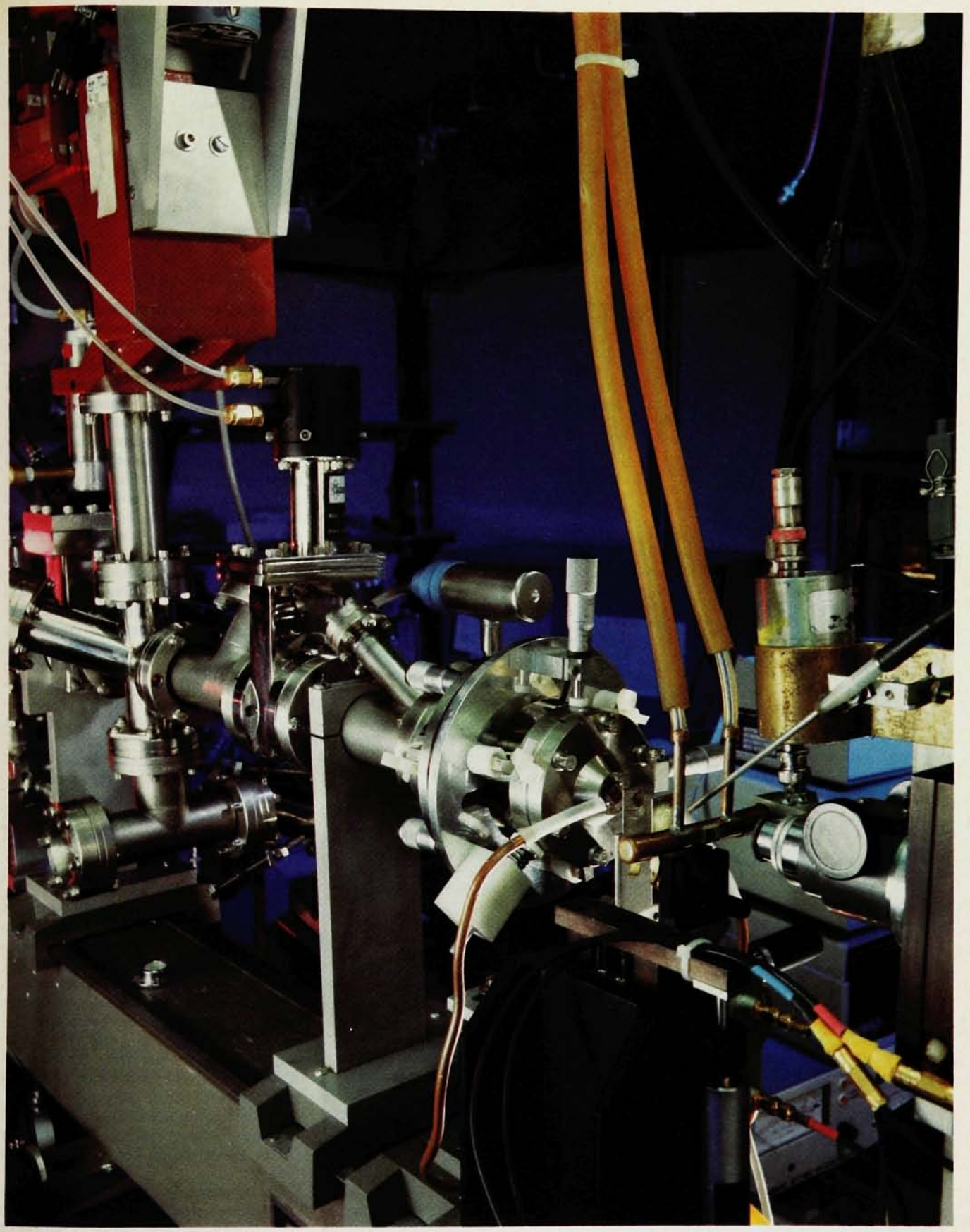


Scanning x-ray microscope at the National Synchrotron Light Source, Brookhaven National Laboratory. The x-ray beam in this Stony Brook experiment is incident from the left. The zoneplate that forms the scanning spot is mounted on the exit of the x-ray source. It is at the center of a diamond-shaped piece of silicon visible in the photograph. The specimen stage is in the foreground. (Photograph by Mort Rosen, Brookhaven National Laboratory.) Figure 1

Early history of x-ray microscopy

Few discoveries in the history of physics were exploited as rapidly as Roentgen's discovery of x rays in 1895. It was the high penetrating power of the new radiation that led to immediate success in applications, and the difficulties with designing optics for it that led to immediate frustration. The interested reader will find a rich source of leads and information on this early history in a book by Vernon E. Cosslett and William C. Nixon (see reference 20).

Contact microradiography, which we call contact microscopy in this article, was the first technique to be developed, and the basic arrangement has not changed since 1896 when G. Burch and F. Ranwez used it to examine botanical specimens for the first time. One places the specimen in contact with, or close to, a high-resolution detector, historically a photographic plate. After exposure and development, one examines the detector under a microscope. It is the microscope that is responsible for the magnification. In the early days, resolution was limited by the grain size of the detector. In the 1940s, Arne Engstrom and his collaborators at the Karolinska Institute in Sweden developed quantitative methods, using exposures at several wavelengths, for measuring the specimen's mass and the distribution of its elemental constituents (see reference 18).

Among the attractions of the contact technique are simplicity, a large field of view and minimal requirements on the x-ray source.

To improve the resolution, it was necessary to find a detector with higher resolution and a magnification scheme that is matched to its capabilities. The development of x-ray-sensitive polymers by Hans Konig and others in the 1950s, together with the use of electron microscopy for their examination, satisfied both needs (see reference 21).

The first technique to give magnification in the x-ray image itself was projection microscopy. As in a pinhole camera, a point source replaces the optics. In the projection microscope the specimen sits in the path of x rays diverging from a small source. The smaller the source the better the resolution, as long as one can ignore diffraction. In practice, however, a very small source means low flux and long exposure time, so one reaches a suitable compromise. Cosslett and his collaborators in Cambridge developed projection microscopy into a useful technique. Given a suitable microfocus x-ray source, in which x rays are generated by electrons focused to a very small spot on an anode, the scheme is simple to use, and it gained considerable popularity in the 1950s. In practice the resolution is not superior to that of the optical microscope, and to reach high resolution with soft x rays the specimen must be placed close to the source, limiting the field of view.

The first successful x-ray microscope to operate at a synchrotron radiation source was also essentially optics free. Paul Horowitz and John Howell built the instrument, a scanning microscope, at the Cambridge Electron Accelerator in 1971 (see reference 9). They used a pinhole 1–2 microns in diameter to define the fine x-ray beam that raster-scanned the sample. The size of this pinhole set the resolution. To increase the speed of data collection, Horowitz and Howell placed a condenser—an ellipsoidal mirror operating at grazing incidence—upstream of the pinhole. The microscope used electronic detectors that did not need to have any position sensitivity because the position of the beam was known. The microscope produced images based on fluorescence as well as images based on transmitted x rays. The closing down of the accelerator cut short this effort just as it started to bear fruit.

That grazing-incidence mirrors can be used as optical elements for soft x rays has been known since the 1920s. Thirty-five years ago, Paul Kirkpatrick and Albert Baez made the first serious effort to use such mirrors for microscopy (see reference 22). They found that aberrations in the grazing-incidence geometry are extremely difficult to control, and microscopy with grazing-incidence optics has remained a tool of limited and specialized utility. The advent of multilayer coatings permitting reflection at nongrazing angles is reviving interest in reflection optics. (See PHYSICS TODAY, April 1984, page 44.)

Subsequent to his work with mirrors, Baez first turned to the possibility of holography, and then to zoneplates, for image formation with x rays (see reference 23). These approaches are very much alive today and are undergoing rapid development, but a quarter century ago the technology was not ripe, and synchrotron radiation was not available to provide the needed coherent flux.

tion of soft x rays, plotted in figure 2, suits them well for viewing, in transmission, individual intact cells up to several microns thick; and the strong dependence of the cross section on atomic number and photon energy makes it possible to obtain contrast without removing water or staining. In particular, photons of wavelength slightly greater than 23.3 Å, the K edge of oxygen, have a sufficiently small reaction cross section with water to make dehydration unnecessary for most imaging purposes. This and the ability to avoid cell sectioning open the door to the imaging of unmodified and living specimens. Also, the fact that the cross section depends on atomic number and photon energy provides approaches to the important problem of identifying the composition of specimen features. Finally, soft-x-ray wavelengths are compatible with imaging at molecular resolution.

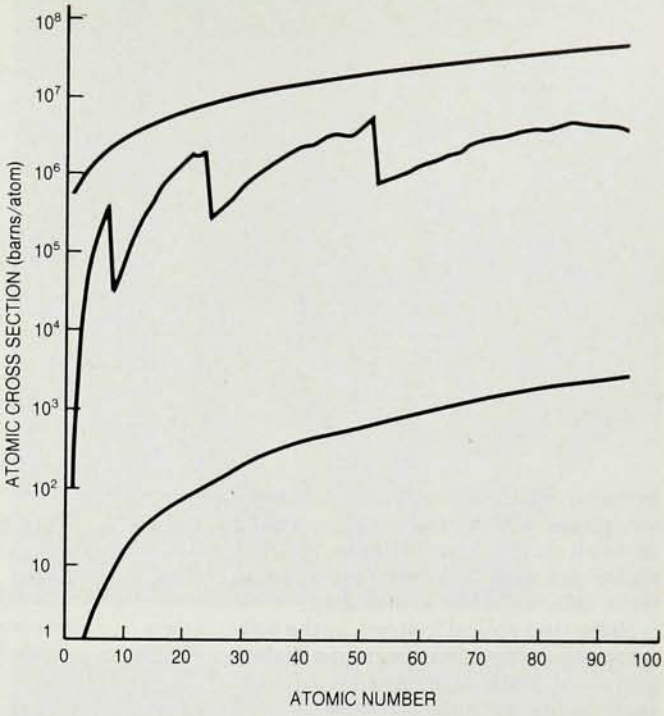
Physicists recognized most of the advantages of soft x rays by the 1950s. Exploitation of these advantages, however, has had to await the arrival of new soft-x-ray sources and improved optical devices.

Current microscopes

When soft-x-ray photons interact with atoms, photoelectric absorption is the principal reaction, followed in importance by coherent scattering. Microscopy with x rays is built mainly on these reactions and their consequences, such as photoelectron emission, fluorescence, diffraction, and rearrangement of valence electrons. Incoherent scattering in the soft-x-ray region is negligible for most purposes.

Table 1 summarizes the major soft-x-ray microscope systems—existing or under construction—of which we are aware.

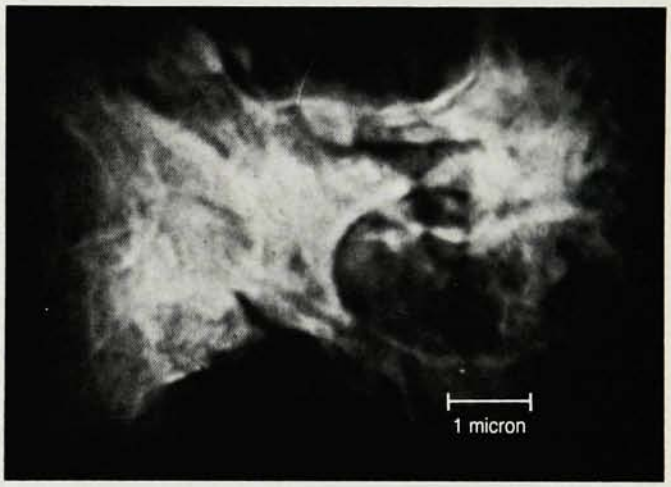
Optical elements. The first two groups of systems listed in the table are based on soft-x-ray optical elements, either of the transmission (zoneplate) or reflection (Wolter or Schwarzschild) types. (See the article by James Underwood and David Attwood on x-ray optics, PHYSICS TODAY, April 1984, page 44.) Zoneplates are inherently diffractive devices, as are the synthetic multilayer



Cross sections of major imaging reactions. Top curve: Total scattering cross section for 100-keV electrons. Middle curve: Photoelectric absorption cross section for 24-Å photons. Bottom curve: Coherent scattering cross section for 1.5-Å photons. Figure 2

reflective surfaces increasingly employed in reflection systems. Soft-x-ray optics is thus largely built on diffraction, replacing the refractive optics of optical and electron microscopy.

A zoneplate is a circular diffraction grating whose line density increases radially in such a way as to focus radiation. The focal length for the mth-order focus is approximately equal to $r_1^2/\lambda m$, where r_1 is the radius of the innermost zone. The resolution, as defined by the Rayleigh criterion, is $1.22\,\delta r_n/m$, where δr_n is the width of the nth, or outermost, zone. Physicists at the University of Göttingen have developed[2] a holographic method using ultraviolet lithography to fabricate zoneplates with outermost zone widths as small as 550 Å. Another method is electron-beam lithography,[3] in which a focused electron beam under computer control writes the zoneplate pattern onto a radiation-sensitive polymer. Other techniques under development include soft-x-ray interferometry,[4] in which one uses a parent zoneplate to produce a daughter zoneplate of higher spatial frequency, and the fabrication of zoneplates by sputtering alternating layers of two materials of differing absorption or refractive index onto a thin rotating wire 10–20 microns in diameter.[5] In the latter technique, one uses a special lathe to cut the coated wire into zoneplates, which can be etched with ions to the desired thickness. These or similar techniques will



Liver tissue. The Göttingen imaging microscope produced this image of a dehydrated liver specimen using 45-Å x rays from the BESSY storage ring. The exposure time was 10 seconds and the x-ray magnification 320X. (This Göttingen group image was made in collaboration with F. Pfannkuch.) Figure 3

Table 1. Soft-x-ray microscopes

Type	Details	Resolution (Å)	Source	Group responsible
Imaging	Zoneplate	500	Synchrotron radiation	Rudolph *et al.*, Göttingen
	Zoneplate	1 000*	Synchrotron radiation	Duke *et al.*, Daresbury
	Wolter	100 000	X-ray tube	Aoki *et al.*, Tsukuba
Scanning	Zoneplate	2 000	Synchrotron radiation	Rarback *et al.*, Stony Brook
	Zoneplate	500*	Synchrotron radiation	Niemann *et al.*, Göttingen
	Zoneplate	1 000*	Synchrotron radiation	Duke *et al.*, Daresbury
	Schwarzschild	<500*	Synchrotron radiation	Spiller, IBM
	Wolter	$<1\,000$*	X-ray tube	Franks *et al.*, Teddington
Contact	Image converter with emission electron microscope	500*	Synchrotron radiation	Polack and Lowenthal, Orsay
	Chemical resist	>50†	X-ray tube, synchrotron radiation, plasma	Feder *et al.*, IBM; and others

From reference 1.
*Anticipated
†See table 2.

probably make it feasible to fabricate zoneplates with outermost zone widths as small as 100 Å, but to date, the best plates are still a factor of five away from this, as table 1 indicates.

Reflection optical systems in the soft-x-ray region have mainly used a Wolter geometry, that is, ellipsoidal and hyperboloidal mirrors mounted to give total reflection at grazing angles of incidence.[6] The one-micron resolution reported in the table for the Tsukuba group's microscope is typical of the performance of systems of this type. Recently, with the development of artificial multilayers[7] capable of giving fair reflectivity at near-normal incidence, a group at DESY showed[8] that it is feasible to construct microscopes that use concentric spherical mirrors, an arrangement known as the Schwarzschild geometry. Wolter systems, too, benefit from the use of multilayer mirror coatings. The Teddington group mentioned in table 1 is designing its microscope to use multilayer Wolter optics as one option. Because such optics are narrow-band, however, the instrument will also be able to use grazing-incidence optics, whose wide bandwidth is necessary for absorption analysis techniques.

The recent progress in diffractive optics is largely due to progress in the construction of small diffractive structures. Soft-x-ray microscopy is thus in part a product of the modern microfabrication industry.

Imaging microscopy. In the Göttingen imaging microscope installed at the electron storage ring BESSY in Berlin, polychromatic synchrotron radiation reaches the condenser zoneplate 15 m from the source. The condenser brings the radiation to a reduced quasimonochromatic image of the source in the object plane of the microscope. The high-resolution micro-zoneplate generates a magnified image of the object in the image field. One may photograph the image directly or view it with the aid of a channel plate.

The Göttingen group has used its microscope to image both wet and dry specimens with a resolution of about 500 Å. They have used 45-Å x rays to make images in the first and second diffraction orders, and 24-Å x rays to make images in the first order. Figure 3 shows an x-ray image of a 4-micron-thick liver specimen imaged with 45-Å radiation.

Scanning microscopy. In scanning microscopy the specimen is mechanically scanned through a small beam spot whose size defines the resolution of the microscope. The scanning system positions the spot on the specimen and also keeps track of its location to construct the display, so the detector need not be position sensitive. This geometry makes efficient use of the x rays incident on the specimen, minimizing specimen damage. In addition, the optical system is simplified because one desires only a stationary spot on the optic axis; one need not consider problems such as off-axis aberrations. The photons not absorbed by the specimen are counted with a proportional counter.

The critical task is to form the small spot. Because one cannot increase the density of photons in phase space, a brilliant source is the route to rapid imaging. Synchrotron radiation is brilliant and has the added advantage of tunability. The procedure is to monochromatize the synchrotron radiation suitably and then collimate it with a small aperture. It is this secondary source that the optical system demagnifies to form the desired spot. A scanning microscope can use synchrotron radiation that has been collimated but not demagnified, but diffraction then limits the resolution to about one micron.[9]

There are two schemes for demagnifying the secondary source. One method uses reflection optics, where, as we noted, current interest centers on systems that use multilayer coated mirrors. The other method uses zoneplate optics. In this category are the Göttingen scanning microscope, which is about to start operation at the BESSY synchrotron radiation source in Berlin, and the Stony Brook microscope (figure 1), which is operating at the National Synchrotron Light Source at Brookhaven. The Göttingen instrument uses holographically made zoneplates as the monochromatizing and condensing element, and to form the final spot. The Stony Brook microscope uses a toroidal grating as the first element, and a zoneplate fabricated at IBM by electron-beam techniques as the final probe-forming device.

Scanning microscopes accumulate

Table 2. Contact microscopy

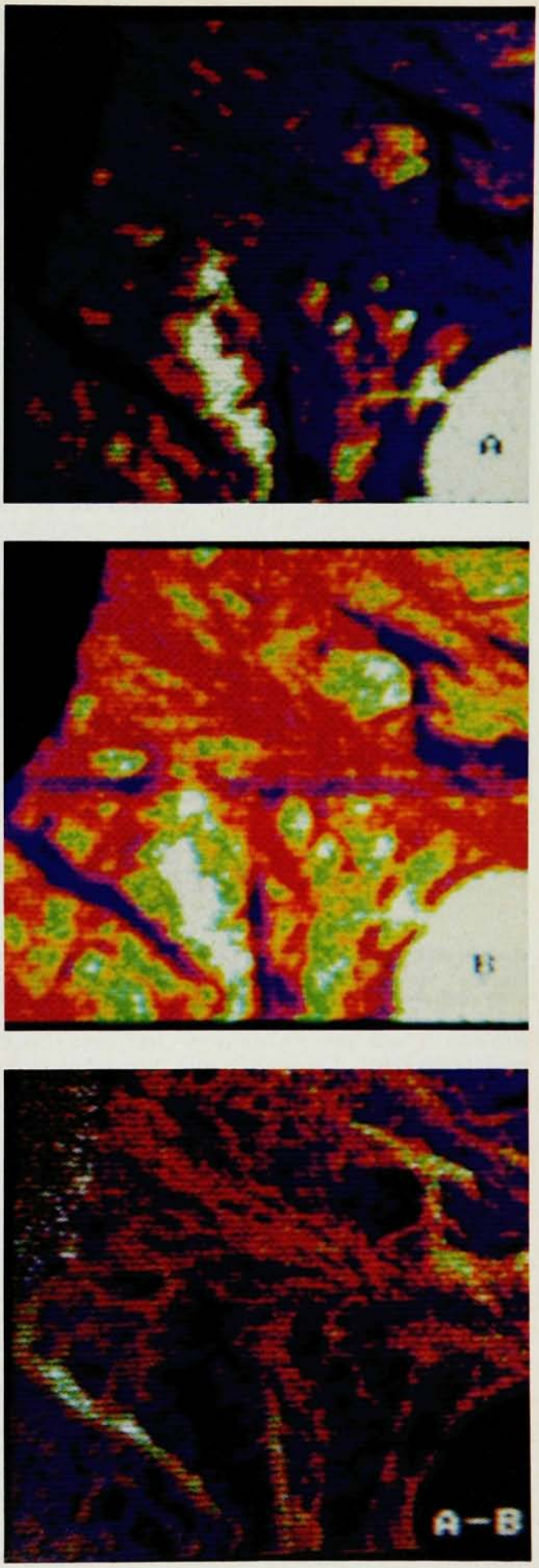
Specimen thickness (Å)	Separation n	Resolution (Å)
< 100	< 4	50
1 000	40	160
10 000	400	500

Approximate resolution at detector surface for 25-Å photons. Features near the exit surface of a specimen will be imaged at higher resolution.

Table 3. Possible future systems

Type	Details	Resolution (Å)	3D Imaging	Undulator needed?
Imaging	Improved optics	100	Multiple exposures	
Scanning	Improved optics	100	Multiple exposures	Yes
Contact		> 50	Multiple exposures	
Holography		100	Single exposure	Yes
Contact*	Image reconstruction	50	Single exposure	
Diffraction*		12.5†	Various	Probably

*Feasiblity not yet established.
†For $\lambda = 25$ Å

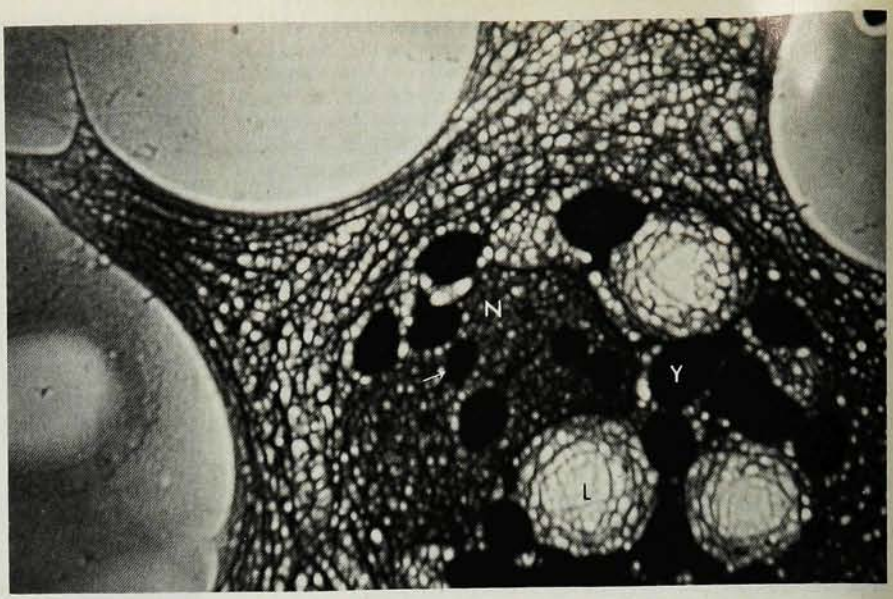


Human skull tissue, imaged with the Stony Brook scanning microscope. **a:** Image made with x-ray wavelength of maximum absorption by calcium. **b:** Image of same specimen, but with wavelength of low absorption by calcium. **c:** A calcium map, the result of subtracting the images in **a** and **b**. (From reference 19.) Figure 4

the image in digital form in a small computer, and are therefore particularly suited to image processing and quantitative measurement, as in the absorption microanalysis of a specimen, for example. The Stony Brook group makes two images in an interlaced fashion by rocking the monochromator across an element's absorption edge while taking data for each pixel. The difference of the two images shows the distribution of the element under study. Figure 4 shows two images of a thin section of human skull tissue, together with the subtracted image, which shows the calcium-rich regions.

Contact microscopy. Contact microscopy is based on the fact that any structural feature in a specimen throws a shadow due to absorption. One may record this shadow by placing a detector close to the specimen. The detector must have adequate spatial resolution to record the shadows of the smallest features to be imaged. Today there are soft-x-ray detectors with approximately 50-Å spatial resolution. However, in view of the fact that 50-Å-diameter shadows in the soft-x-ray region effectively disappear within a few wavelengths, it is more realistic to say that the approximate resolution of the technique is $\sqrt{n\lambda}$, where n is the number of wavelengths that the radiation must travel between feature and detector. For 25-Å x rays, this leads to the resolutions given in table 2.

The principal high-resolution detectors in use today are x-ray resists borrowed from microfabrication technology. These are organic polymers whose chemical bonding structure and solubility are altered by x rays. A brief immersion of the exposed resist in a solvent reveals a record of the x-ray shadow in the form of a height profile on the resist. One can examine this profile at high magnification in an electron microscope. Figures 5 and 6 show examples of the technique for a vertebrate muscle cell and a living human blood platelet, respectively. The latter image shows detail to approximately 75 Å, and is believed to be



Muscle cell. This contact image of a vertebrate muscle cell shows the nucleus (N), a lipid granule (L), a yoke granule (Y) and a nucleolus (white arrow). The lipid granule is about 10 microns in diameter. (Courtesy of Ping Ching Cheng, IBM.) Figure 5

the first instance in which a living cell has been viewed at that resolution.

As an alternative to photoresists, one may use a photocathode as a detector, following it with electron optics to magnify the image. The result is real-time contact microscopy. The Orsay group mentioned in table 1 estimates the resolution to be somewhat lower than that of chemical detectors, due mainly to the separation between specimen and photocathode imposed by the photocathode support membrane. An earlier instrument constructed[10] at Tübingen in 1955 had a resolution of about 1200 Å.

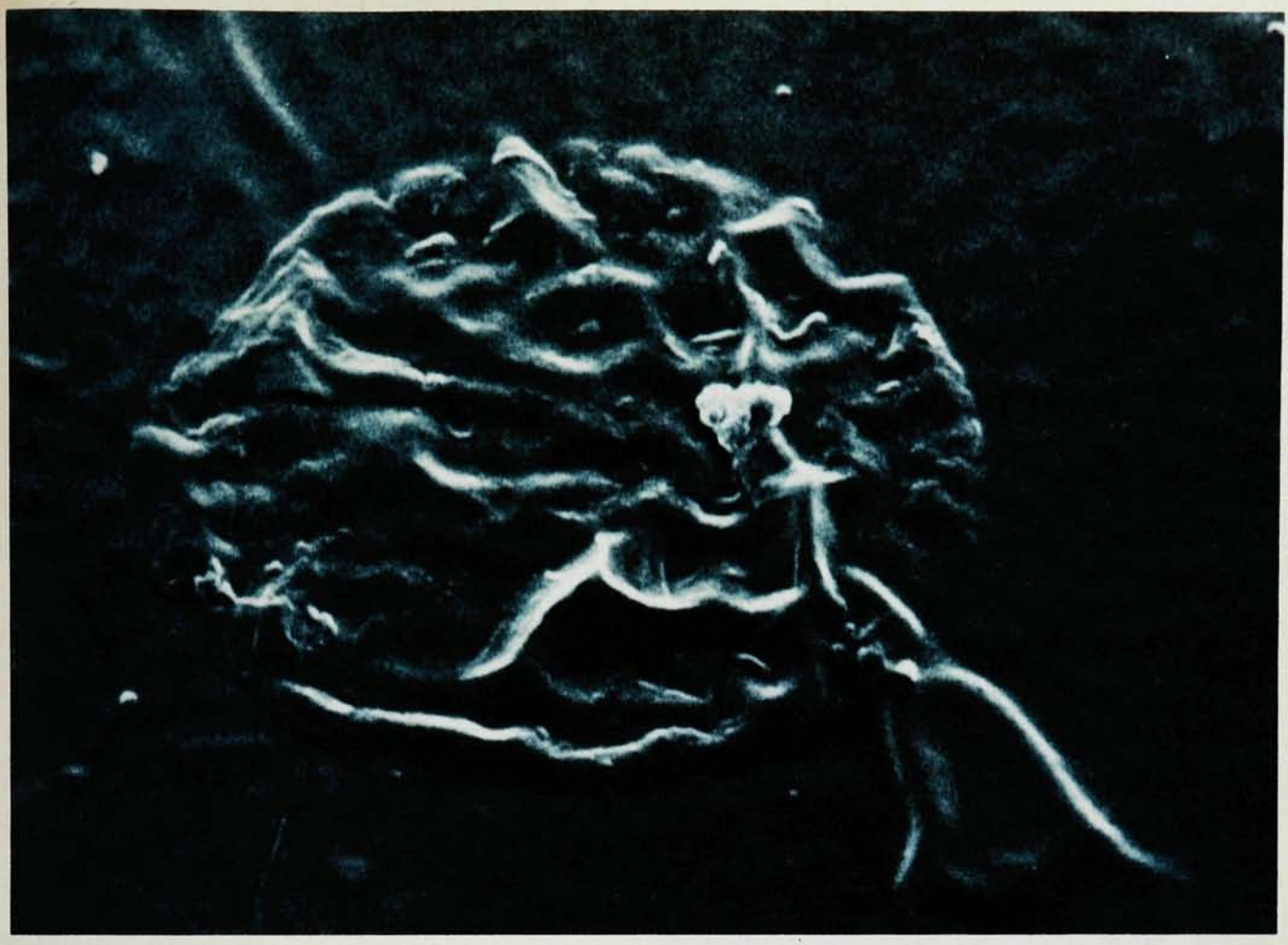
Contact imaging has now reached a fair degree of usefulness in biology. In particular, Ralph Feder at IBM and his colleagues have used contact imaging in several biological research studies, and the technique is a major tool in a current study[11] of the blood platelet. Usage today is at the level of a thousand images of biological specimens per year.

Sources. Ideally, soft-x-ray sources for microscopy are intense and tunable. High intensity is required for the fast imaging needed to obtain clear images of living moving cells. Tunability is required to exploit the energy dependency of the reaction between photon and atom. Bending-magnet synchrotron radiation from 0.5–1.0-GeV electrons in dedicated storage rings is currently the most widely used radiation (table 1). Such sources have their peak flux in the soft-x-ray domain, have no hard-x-ray component and are tunable when equipped with a monochromator. Examples are the ACO storage ring in Orsay, the vacuum ultraviolet storage ring at Brookhaven and the BESSY storage ring in Berlin.

A particularly important source characteristic for most forms of imaging is spectral brilliance, which is a measure of the degree to which the source is small and emits a collimated beam of monoenergetic radiation, that is, the degree to which the radiation is concentrated in phase and energy space. Spectral brilliance is important because the usable volume in phase and energy space is frequently limited in imaging techniques. We expect a major advance in the spectral brilliance of soft-x-ray sources in the near future, through the use of undulators in 1–5-GeV electron storage rings. These sources, which are being installed at a number of centers, can be several meters long; bending-magnet beam lines, by comparison, have only several centimeters of path length that contribute to the beam. (See Arthur Bienenstock and Herman Winick's overview of synchrotron radiation research, PHYSICS TODAY, June 1983, page 48.) Moreover, the brilliance of undulator radiation exceeds that of bending-magnet radiation, because its spectrum peaks while bending-magnet radiation has a white spectrum. Undulators should make possible new types of imaging that will come closer to the diffraction limit of resolution. We will discuss this below.

Also of great interest is the soft-x-ray laser, which combines high spectral brilliance with high pulsed power. Reports of such sources are now beginning to appear.[12]

Finally, it is important to have sources that are small enough to fit in the biology laboratory. Accordingly,



Living blood platelet. This flash contact image was made with x rays from a commercial z-pinch plasma source.[13] The platelet was imaged at the moment that it sent out a pseudopod, which is a structure that links to other platelets as part of the clotting process. (Courtesy of Ralph Feder, reference 11.) Figure 6

there is interest in pulsed plasma radiation sources, both of the gas-puff *z*-pinch and laser-produced-plasma types.[13] These are available commercially and have low brilliance but very high pulsed intensity. Used as sources for contact microscopy, which does not require especially high brilliance, they permit one to make exposures in nanoseconds, allowing stop-motion imaging of live biological material such as the blood platelet shown in figure 6. The exposure time here is much shorter than the seconds that are required with bending-magnet radiation and the milliseconds anticipated with undulator radiation.

Possible future systems

Table 3 gives some possible characteristics of future soft-x-ray microscope systems. In essence the table predicts that improved optics, new sources and the introduction of holography and related imaging methods will produce gains in resolution and three-dimensional imaging.

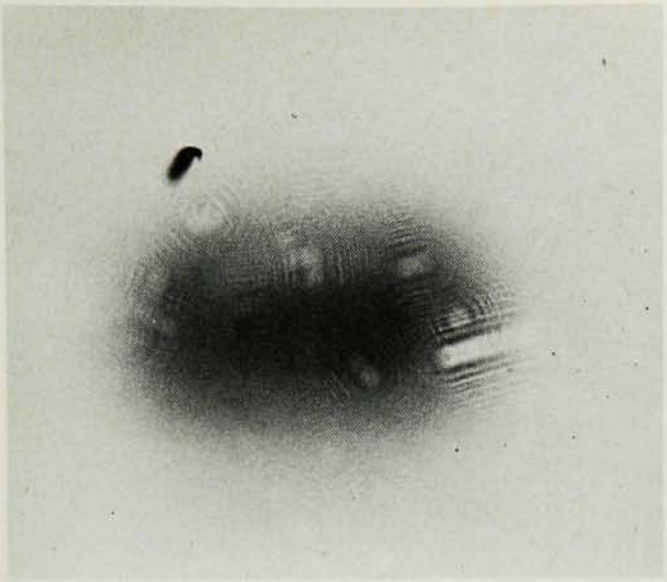
Holography. To do three-dimensional imaging using the principles of current x-ray microscopes, one must do repeated two-dimensional imaging using multiple orientations or focal planes. Such imaging is not possible for living specimens. Holography, with its ability to produce an image in three dimensions with a single exposure, offers the possibility of extending high-resolution three-dimensional imaging to living specimens as well.[14]

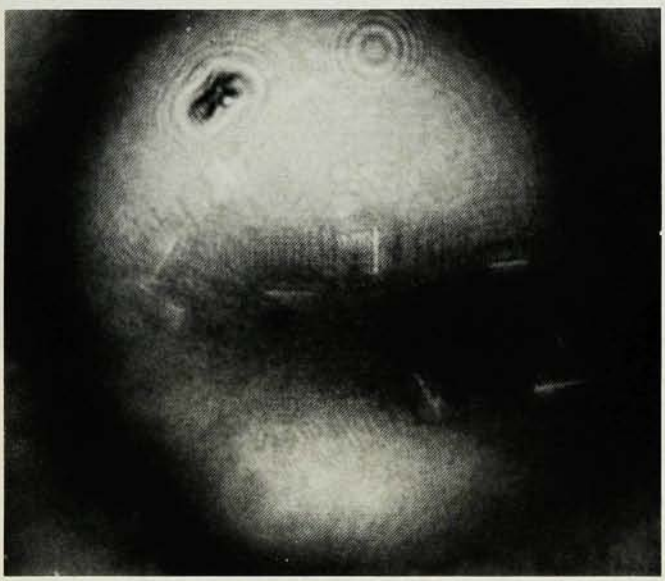
Figure 7 shows examples of soft-x-ray holography done[15] recently at Brookhaven's vacuum ultraviolet synchrotron radiation source. It shows better resolution than earlier x-ray holography experiments.[16] The improvement is due principally to the higher brilliance of the source, which makes possible a large increase in the coherence of the illumination deliverable at a given power level.

In current work using simple Gabor geometry and photographic film, with images reconstructed in the visible, the transverse resolution in the reconstructed images is on the order of 1–2 microns, corresponding to the angle to which fringes are recorded with adequate signal-to-noise ratio. The resolution limit for this type of holography is probably about 0.2–0.4 microns. To get better resolution, one might use a high-resolution resist instead of silver halide, or change to Fourier transform holography. Both of these steps appear to be within technical reach at present, and there are plans for further work on both.

The image produced by contact microscopy is an extreme near-field Gabor hologram. Reconstruction is difficult, but may be practical if it is possible to use a multifilm resist pack to make a set of holograms at various distances from the specimen. If successful, this technique could effect a major improvement in the contact method, avoiding the falloff of resolution with specimen thickness shown in table 2, while giving single-exposure three-dimensional images.

Diffraction imaging. In diffraction imaging the detector receives scattered photons only, rather than the coherent mixture of incident and scattered photons used in holography. The phase of the diffraction pattern must be established without the aid of a reference





Soft-x-ray hologram of 1–2-micron asbestos fibers, and reconstruction. Soft x rays produced this demonstration hologram (top); visible laser light produced the reconstruction. The reconstructed image had a magnification of 1.8. It was photographed through a light microscope to give an overall magnification of 300×. The time taken to record the hologram was about one hour. Figure 7

beam, meaning possible loss of the ability to image with a single exposure, depending on the phasing technique used. The diffraction imaging arrangement has the advantage, however, of allowing the detector to surround the specimen fully, with potential advantages in the resolution and three-dimensionality of the images. In recent work at the National Synchrotron Light Source at Brookhaven, Wen Bing Yun, Kirz and Sayre have recorded diffraction patterns—as yet limited to a maximum resolution of 300 Å—from single biological cells, namely diatoms.[17]

Elemental analysis

As we mentioned earlier, the photoelectric cross section shows a dramatic increase when the energy of the x-ray photon passes from below to above the threshold for inner-shell ionization (see figure 2). The detailed shape of this edge structure is a subject of active investigation, and can provide information about the chemical environment of the photoabsorbing atom. (See the article by Bernd Crasemann and François Wuilleumier on atomic physics with synchrotron radiation, PHYSICS TODAY, June 1984, page 34.) The tunability of synchrotron radiation sources makes it possible to exploit this effect conveniently in soft-x-ray microscopy. Two absorption images of the same specimen, taken with x rays of slightly different energies, will look very similar unless these energies bracket an absorption edge or involve a feature of the edge structure of an element present in the specimen. Then the two images will differ in those locations where the element is present, and the difference will contain information on the amount of the element and in some cases its chemical state. This technique, known as x-ray absorption microanalysis, was developed[18] in the 1940s, but today's new sources and imaging techniques make it possible to generate quantitative maps showing the distribution of major elemental constituents at high resolution. Figure 4 is an example.

Several light elements of major biological interest have absorption edges in the soft-x-ray range. These elements include carbon, nitrogen, oxygen, phosphorus, sulfur, chlorine, potassium, calcium and iron. Absorption microanalysis is not suitable for locating the heavier elements, especially if they are present in trace concentrations. For these elements fluorescence microanalysis provides the necessary sensitivity. Because fluorescence microanalysis uses harder x rays for which high-resolution optics do not yet exist, its spatial resolution is typically no better than 1 micron. The work[9] of Paul Horowitz and John Howell at the Cambridge Electron Accelerator is still the best example of image formation using the fluorescence signal. In their

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experiments, a small pinhole defined the beam with which they scanned the specimen. They detected x-ray fluorescence using either proportional counters or a Si(Li) detector. New facilities for x-ray-fluorescence imaging are under construction at the National Synchrotron Light Source at Brookhaven and at the Daresbury Laboratory in England.

From the physicist's point of view, table 3 gives an indication of what the future may hold for x-ray microscopy: more work in x-ray optics, in holography and related image science, and in storage rings and other x-ray sources, leading, one hopes, to a well-established set of techniques for producing three-dimensional images with resolutions of 10–100 Å.

For the biologist there is perhaps even more to be done if x-ray microscopy is to play the role that it appears capable of playing. Biologists have shown great resourcefulness in using the electron microscope, finding ways to turn its particular characteristics to their advantage. Experience already gained in x-ray microscopy indicates that a similar resourcefulness will be needed here as well. At the simplest level, our familiarity with the appearance of cellular structures in electron micrographs, gained over many years of study, must be reacquired for photon images, which look different because cross section depends on atomic number. At a deeper level, we will need to become familiar with the structures in unprocessed as well as processed cells. Finally and most important, if we are to realize fully the potential of a microscopy capable of imaging the living cell, we will need ways to bring not only structures but also *processes* to the microscope stage.

* * *

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References

1. The most useful current reference to the subject is G. Schmahl, D. Rudolph, eds., *X-Ray Microscopy*, Springer-Verlag, Berlin (1984). This book contains descriptions of the systems in table 1: See D. Rudolph, B. Niemann, G. Schmahl, O. Christ, p. 192; P. J. Duke, p. 232; H. Rarback, J. M. Kenney, J. Kirz, M. R. Howells, P. Chang, P. J. Coane, R. Feder, P. J. Houzego, D. P. Kern, D. Sayre, p. 203; B. Niemann, p. 217; P. J. Duke, p. 232; E. Spiller, p. 226; A. Franks, B. Gale, p. 129; F. Polack, S. Lowenthal, p. 251; R. Feder, V. Mayne-Banton, D. Sayre, J. Costa, B. K. Kim, M. G. Baldini, P. G. Cheng, p. 279. See also S. Aoki, Y. Sakanayagi in *Ultrasoft X-Ray Microscopy*, D. F. Parsuns, ed., Ann. N.Y. Acad. Sci. **342**, 158 (1980). The resolution for the Tsukuba instrument cited in table 1 is from a recent personal communication from S. Aoki.
2. G. Schmahl, D. Rudolph, P. Guttmann, O. Christ, in G. Schmahl, D. Rudolph, eds., *X-Ray Microscopy*, Springer-Verlag, Berlin (1984) p. 63; P. Guttmann, p. 75; J. Thieme, p. 91.
3. D. P. Kern, P. J. Houzego, P. J. Coane, T. H. P. Chang, J. Vac. Sci. Technol. **B1**, 1096 (1983); D. C. Shaver, D. C. Flanders, N. M. Ceglio, H. I. Smith, J. Vac. Sci. Technol. **16**, 1626 (1979); A. G. Michette, M. T. Browne, P. Charalambous, R. E. Burge, M. J. Simpson, P. J. Duke, J. Vac. Sci. Technol. **16**, 109 (1979); E. Kratschmer, D. Stephani, H. Beneking in *Microcircuit Engineering 83*, H. Ahmed, J. R. A. Cleaver, G. A. C. Jones, eds., Academic, New York (1983) p. 15.
4. G. Schmahl, D. Rudolph, B. Niemann in *Scanned Image Microscopy*, E. A. Ash, ed., Academic, New York (1980) p. 393; W. B. Yun, M. R. Howells, Brookhaven Laboratory Technical Report No. 35628, Brookhaven National Laboratory, Brookhaven, New York (1985).
5. D. Rudolph, B. Niemann, G. Schmahl in *High Resolution Soft X-Ray Optics*, E. Spiller, ed., SPIE Proc. **316**, 103 (1981).
6. H. Wolter, Ann. Phys. **10**, 94 and 286 (1952).
7. T. W. Barbee Jr, in G. Schmahl, D. Rudolph, eds., *X-Ray Microscopy*, Springer-Verlag, Berlin (1984) p. 144; E. Spiller, p. 226.
8. R.-P. Haelbich, W. Staehr, C. Kunz in *Ultrasoft X-Ray Microscopy*, D. F. Parsons, ed., Ann. N.Y. Acad. Sci. **342**, 148 (1980).
9. P. Horowitz, J. A. Howell, Science **178**, 608 (1972); P. Horowitz, Ann. N.Y. Acad. Sci. **306**, 203 (1978).
10. L. Y. Huang, Z. Phys. **149**, 225 (1957).
11. R. Feder, V. Banton, D. Sayre, J. L. Costa, M. G. Baldini, B. K. Kim, Science **227**, 63 (1985).
12. See PHYSICS TODAY, March, p. 17; Science **215**, 488 (1982); D. L. Matthews, P. L. Hagelstein, M. D. Rosen, M. J. Eckart, N. M. Ceglio, A. U. Hazi, H. Medecki, B. J. MacGowan, J. E. Trebes, B. L. Whitten, E. M. Campbell, C. W. Hatcher, A. M. Hawryluk, R. L. Kauffman, L. D. Pleasance, G. Rambach, J. H. Scofield, G. Stone, T. A. Weaver, Phys. Rev. Lett. **54**, 110 (1985).
13. D. J. Nagel, in *Ultraviolet and Vacuum Ultraviolet Systems*, W. R. Hunter, ed., SPIE Proc. **279**, 98 (1981). Pulsed radiation sources based on the plasma *z*-pinch are commercially available from Maxwell Laboratories in San Diego, Calif., and Physics International Co in San Leandro, Calif. Small sources based on laser-produced plasmas are available from XMR Inc in Santa Clara, Calif.
14. J. C. Solem, G. C. Baldwin, Science **218**, 229 (1982).
15. M. R. Howells, in G. Schmahl, D. Rudolph, eds., *X-Ray Microscopy*, Springer-Verlag, Berlin (1984) p. 318; M. R. Howells, M. Iarocci, J. Kenney, J. Kirz, H. Rarback, in *Science with Soft X-Rays*, F. J. Himpsel, R. W. Klaffky, eds., SPIE Proc. **447**, 193 (1983).
16. S. Aoki, S. Kikuta, Japan. J. App. Phys. **13**, 1385 (1974). Aoki informs us that Gabor x-ray holograms are now being recorded with an undulator at the Photon Factory at Tsukuba.
17. This possibility was discussed in D. Sayre, R.-P. Haelbich, J. Kirz, W. B. Yun, in G. Schmahl, D. Rudolph, eds., *X-Ray Microscopy*, Springer-Verlag, Berlin (1984) p. 314.
18. A. Engstrom, Acta Radiol. Suppl. **63** (1946).
19. J. M. Kenney, C. Jacobsen, J. Kirz, H. Rarback, F. Cinotti, W. Thomlinson, R. Rosser, G. Schidlowsky, J. Microsc. **138**, No. 3 (June 1985).
20. V. E. Cosslett, W. C. Nixon, *X-Ray Microscopy*, Cambridge U.P. (1960).
21. H. König, Z. Physik **129**, 483 (1951).
22. P. Kirkpatrick, A. V. Baez, J. Opt. Sci. Am. **38**, 766 (1948).
23. A. V. Baez, J. Opt. Sci. Am. **42**, 756 (1952); **51**, 405 (1961). □