CHAPTER 1

INTRODUCTION

1.1. The use of X-rays for microscopy

The chief distinguishing characteristics of X-rays have special value for microscopy. Their short wavelength compared with visible radiation offers a correspondingly higher resolution than that of the optical microscope. Their relatively great penetration into matter gives the possibility of investigating internal structure, in both biological and inorganic specimens. The simple line-structure of X-ray emission, and the rapid variation of absorption coefficient with atomic number, provide two independent methods of analysis of the elements present. Unfortunately, however, there are serious difficulties in the way of focusing X-rays and it is only comparatively recently that some of these prospective advantages have been realised, with the development of methods of X-ray microscopy having a resolving-power approaching that of the best optical microscope.

The discovery of X-rays was made by Röntgen on 9 November 1895, and attempts were made at once to explore their use in microscopy. Röntgen (1895) himself recognized, in his original communication, the difficulties involved in attempting to focus X-rays with any sort of lens; he was unable to detect any refractive effect and concluded that it must, at best, be very small. The possibility of constructing an X-ray microscope was, therefore, dismissed and only some thirty years later was it realized that a mirror-system might be practicable, following upon detailed investigations of the reflexion of X-rays from polished surfaces. On the other hand, within a few months of Röntgen's discovery, X-rays were being used for the study of fine internal structure, by enlarging contact radiographs: in botanical specimens by Burch (1896) and by Ranwez (1896), and in alloys by Heycock & Neville (1898). From these beginnings the subject of microradiography grew, slowly until 1930, but with increasing refinement and width of application immediately before the Second World War and in

the decade since its end. The point projection method, in which the specimen is as close as possible to a fine focus, was not suggested until 1939 and has been realised in practice only since 1950. The development of both these non-focusing systems has suffered from technical limitations: the contact method requires a very fine-grained emulsion, the projection method a powerful fine-focus X-ray tube. In the meantime the possibilities of focusing X-rays, with curved crystals as well as with polished mirrors, have been thoroughly explored.

1.2. Contact microradiography

The simplest method of recording fine detail with X-rays is that of contact microradiography. The specimen is placed as close as possible to a photographic emulsion of very fine grain, at some distance from an X-ray source (Fig. 1.1), so that a one-to-one

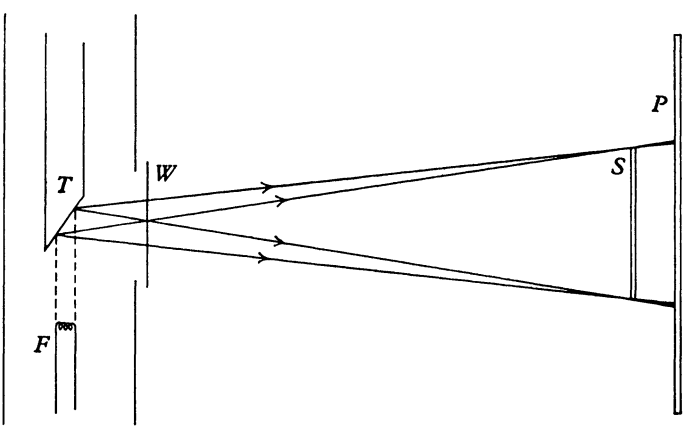

Fig. 1.1. Contact microradiography. *F*, filament; *T*, target; *W*, window; *S*, specimen; *P*, photographic emulsion.

image is obtained. The resulting negative is examined under a high-power optical microscope and selected regions are subsequently enlarged photographically. Being the easiest it was also the first method to be used for X-ray microscopy. After the early experiments, the first detailed investigation of its value as a research technique was made by Goby (1913*a*), who introduced the term 'microradiography'. For more than two decades its development

was almost entirely confined to France and mainly to biological applications. In the late 1930's the commercial availability of ultra-fine-grain emulsions led to a rapid extension of its use, particularly to metallography. Since 1947 it has been developed by Engström and his school as a powerful method of microanalysis in biology and especially in cytology.

For many purposes useful results can be obtained, at a resolution down to a few microns, with a normal type of X-ray tube and an emulsion of moderately fine grain. The resolution is determined by the penumbra width, as well as by grain size; to minimize the penumbra the specimen must be as close to the emulsion as possible and the angular size of the source must be small. For high-resolution investigations, therefore, a source of small size ($\sim$0·1 mm.) and high intensity is desirable, and suitable X-ray tubes have recently been developed. The specimen, usually a section a few microns thick, is laid directly on the photographic emulsion; a thin layer of nitrocellulose may be placed under it, or sometimes a strip of cellulose tape upon it, to facilitate its removal before the emulsion is processed. Great care is needed at all stages to avoid scratches or the deposit of dust on the negative; fine-grained emulsions are fortunately slow enough for all the procedures, including specimen mounting and removal, to be carried out under a safelight.

The advantages and limitations of the contact method are discussed in detail in chapter 2. Its apparent simplicity is complicated by the demands of specimen mounting and photographic processing in obtaining the original negative, and of high-resolution photomicrography in producing the final enlarged positive picture. The exposure-time is somewhat longer than in the projection method at the same resolution, but the field of view is much larger. The X-ray equipment is not so elaborate and costly as that needed for projection imaging, but the very best optical microscope must be available. The ultimate limitation to the contact method is in fact set by the resolving-power of the microscope used for the final enlargement, since the grain size is smaller than this in the best emulsions. With visible light the resolution limit is about 0·2μ, and it has already been closely approached in some of Engström's microradiographs (cf. Plate XVII). The use of the electron microscope for enlarging the image would require drastic treatment of the

emulsion, unless a radically different method of recording the image can be developed (§ 15.6). The greatest utility of the contact method promises to be for applications requiring only moderate resolution, of the order of a micron; that is to say, essentially as an extension of routine procedures in metallurgical and biological macroradiography.

1.3. Reflexion X-ray microscopy

The reflexion coefficient is very small for X-rays incident normally on a polished surface, since the refractive index for radiation of such short wavelength is very close to unity. However, the index is *less* than one, and so total reflexion takes place in the less dense medium, not in the denser as with light. This phenomenon of total external reflexion makes itself evident in a high reflexion coefficient for X-rays incident at low angle on a polished surface. Its use for image-formation was first suggested, and experimentally investigated, by Jentzsch in 1929, but the practical realization of a reflexion X-ray microscope was delayed until Kirkpatrick & Baez (1948) found an effective means of overcoming the strong astigmatism inherent in the process. By mounting two mirrors at right-angles to each other (Fig. 1.2) the astigmatism of the first is cancelled by that of the second. As focusing is very much stronger in the plane of incidence than in that normal to it, cylindrical instead of spherical surfaces can be used without loss of power and with gain in simplicity. The critical angle θ is between 89° and 90°, so that the glancing-angle for total reflexion ϕ is of order 30′; in Fig. 1.2 ϕ has been greatly exaggerated for clarity. The smallness of this angle makes the setting up of the mirrors highly critical.

The aberrations peculiar to such a non-centred system have been evaluated by Dyson (1952), Pattee (1953*a*) and Montel (1953, 1954). It is found that obliquity of the field can be corrected by suitably placed stops and spherical aberration by figuring the two mirrors of a pair, but coma only by resorting to a four-mirror system, again suitably figured. With a single pair of mirrors McGee (1957) has obtained a resolution of order 0·5 μ, at the low primary magnification of $\times 6$ (in the interests of short exposure) and with aluminium K radiation (8·3 Å), in the interests of larger glancing-angle and higher image contrast.

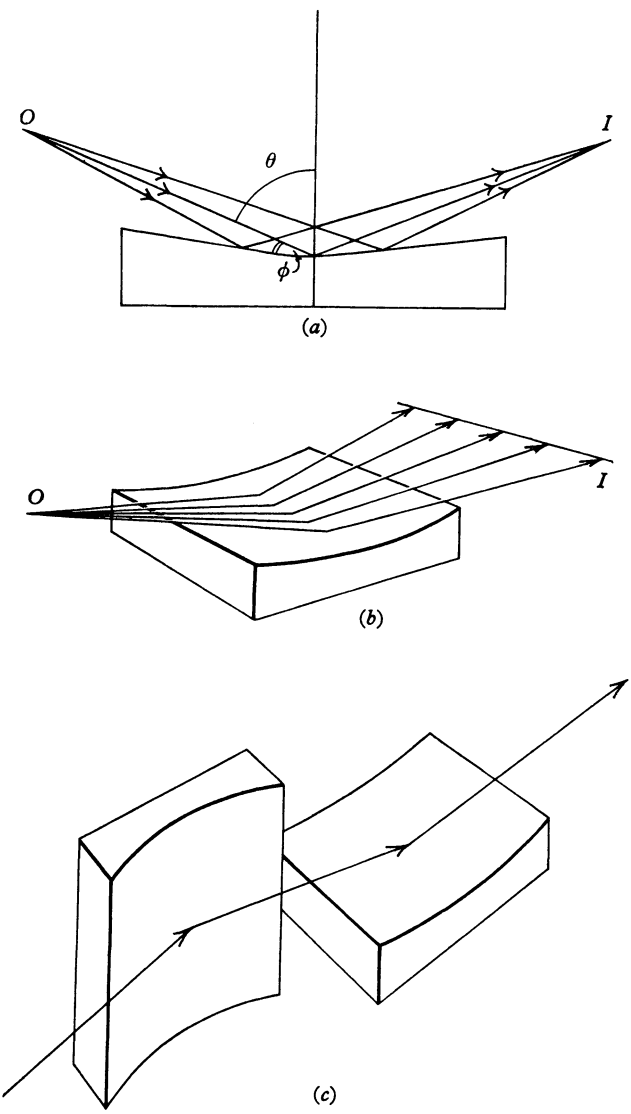

Fig. 1.2. Reflexion X-ray microscopy. (*a*) and (*b*) X-rays diverging from a source *O* are focused by a cylindrical surface to form an astigmatic image *I*; (*c*) arrangement of two cylindrical mirrors for eliminating astigmatism. (Kirkpatrick & Pattee, 1953.)

The development of the reflexion method is hampered more by practical difficulties than by any theoretical limitation. The ultimate resolution set by diffraction depends on the wavelength of the X-rays used and on the angular aperture, which can at maximum equal the glancing-angle for total reflexion. At a wavelength of 1 Å and critical glancing-angle of 0·4°, the diffraction limit is 85 Å. However, this would be attainable only over a very small field with the mirrors available owing to the effect of other aberrations. The first requirement is to find an acceptable compromise between width of field and resolution. Computation of the properties of a four-mirror system using elliptical and paraboloidal surfaces (Pattee, 1957*a*) predicts a resolution of 500 Å over a field of some 10μ at a wavelength of 4 Å. The technical difficulty then remains of figuring the surface to the required accuracy, which is of the order of 50 Å according to Pattee; in addition the surface must be free of local irregularities greater than 10 Å, to reduce X-ray scattering. These are much more stringent requirements than those encountered in optical technology. It can be expected that the necessary techniques of working and testing surfaces will gradually be developed, but it is likely to be some time before the reflexion X-ray microscope approaches its theoretical limit of performance. The problems involved are discussed in detail in chapter 4.

In principle an X-ray microscope can also be based on Bragg diffraction, the reflecting elements being curved lattices formed by bending thin crystals (Cauchois, 1946). Reflexion at normal or near-normal incidence is then possible. Although reasonably good images have been obtained at low magnification with a single mirror (Ramachandran & Thathachari, 1951), high-resolution microscopy is hindered by the lack of perfection even of single crystals over a sufficiently large volume and by the finite angular tolerance for Bragg diffraction. The various systems proposed, and their relation to the curved crystal systems used in focusing spectrometers, are described in chapter 5. An instrument devised by von Hámos (1934, 1953) combines some of the features of both spectrometer and microscope, and allows a partial qualitative analysis of the specimen to be carried out by fluorescent excitation (cf. § 5.2.2).

1.4. Point projection with X-rays

The simplest way of forming enlarged images with X-rays is to place the object close to a point source (Fig. 1.3); from the geometry of point projection, the magnification is the ratio of source–image to source–object distance. The resolution depends primarily on the diameter of the source, the exposure time on its intensity. One method of obtaining a point source is to place a pinhole in front of a macroscopic focal spot, as in the camera obscura. Early attempts were disappointing (Czermak, 1897; Uspenski, 1914) on account of the low intensity of the X-ray tubes then available, but the method was applied with greater success by

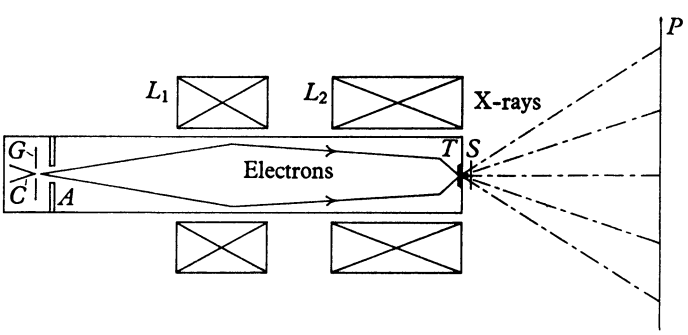

Fig. 1.3. Projection X-ray microscopy. The electron lenses $L_1 L_2$ form a reduced image at T of the cathode C; the X-rays emitted from T project an image of a specimen S on to the screen (or plate) P.

Sievert (1936) and more recently by Rovinsky & Lutsau (1957) using the modern type of tube developed for crystallographic analysis. A similar arrangement, without an object, has been used for many years for inspecting the shape of the focal spot and the distribution of X-ray intensity across it.

The use of a fine focal spot, instead of a limiting-pinhole was first proposed by Malsch in 1933. In 1939 Ardenne and Marton independently suggested that the newly developed electron optical techniques would make it possible to concentrate an electron beam into a minute focus, 1 μ or less in diameter, of great X-ray intensity. Experiments by Cosslett (1940) confirmed that a projection microscope of this type, with magnetic electron lenses, was a practicable

instrument but that the available intensity was severely limited by the lens aberrations. A first, low-power, model was constructed by Cosslett & Taylor (1948), and has since been developed for high magnification and a resolution approaching that of the ultra-violet microscope by Cosslett & Nixon (1951, 1952*a*, *b*, 1953).

The ultra-fine focus T is formed by two-stage demagnification of a cathode C by the lenses L_1, L_2 (Fig. 1.3). By setting the target in the wall of the tube and making it from thin metal foil the emitted X-rays can be utilized most efficiently. The specimen can be brought close to the focus, allowing a high magnification ($\times$ 50–500) and short exposure (0·5–5 min.) in a camera-length of a few centimetres. The size and intensity of the focus can be varied by changing the excitation of the electron lenses. In principle, it can be made as small as a few Ångström units in diameter, if the final lens aperture is stopped down to limit the effect of spherical aberration. At the same time the accelerating voltage must be lowered, to limit penetration of the electron beam into the target, which would enlarge the spot by elastic and inelastic scattering processes. Both measures diminish the X-ray intensity, so that the ultimate resolution attainable by point projection is closely related to the tolerable exposure time, at least until lenses can be corrected for spherical aberration.

The experimental resolution is also affected by Fresnel diffraction in the specimen, though this can in principle be reduced below any assigned limit by bringing it close enough to the source. In practice, this separation cannot be brought much below 1 μ, corresponding to a resolving-power of about 100 Å. A limit of the same order is set by the weakness of the absorption of X-rays in thin specimens: resolution is only of value so long as the resolved details produce visible contrast. These considerations set a limit to the useful resolving-power of the projection method, at least for the foreseeable future, of 100–250 Å. The best value so far obtained lies between 1000 and 2000 Å (Nixon, 1955*a*, *b*).

The projection method thus offers the prospect of better ultimate resolution than either the contact or reflexion methods. It also gives shorter exposure-time at given resolution (§ 3.8.3.) It shares with the reflexion method the advantage of readily allowing treatment of the specimen during microscopy and conversion to micro-

diffraction operation. Stereophotographs can be obtained, and microanalysis by absorption or emission carried out, more easily than in the contact method. The main disadvantage of the projection method is that it requires a special fine-focus tube, with highly stabilized electrical equipment to counteract the chromatic aberration of the electron lenses. On the other hand, photography of the image is simple and little or no subsequent enlargement is needed. The method is fully described in chapter 3.

1.5. Scanning methods

In all the methods described above the whole of the field of view is illuminated and its image recorded simultaneously. It is equally possible to explore it point by point, by scanning it with a fine electron beam ('probe'). The X-rays emitted (or transmitted, if the beam first falls on a target) are detected with a counter, the amplified signal from which is conveyed to a cathode-ray tube operated synchronously with the scan, so that an image of the area scanned is displayed on its screen (Fig. 7.8). Such a flying-spot system has the advantage of better heat dissipation in the target and of allowing electronic control of both magnification and contrast in the image. It was proposed by Pattee (1953*b*) for high resolution imaging by X-ray absorption, with the specimen in contact with a thin window-target, and by Cosslett (1952*b*) for microanalysis by emission. As discussed later (§ 15.3), the experimental realization of the former system is closely bound up with the availability of a high-intensity source of electrons (Pattee, 1957*b*).

In the alternative method the electron probe falls directly on the specimen and the X-rays thus excited are recorded. As originally proposed by Castaing (1951) the specimen was moved mechanically under a stationary probe. Greater speed and flexibility is gained if the specimen is fixed and is scanned by the probe (Cosslett & Duncumb, 1956, 1957). Since each element has a simple and characteristic X-ray line spectrum, direct microanalysis of the composition of a surface or thin foil can be carried out, point by point (§ 7.1).

1.6. Comparison with optical and electron microscopy

X-ray microscopy provides information supplementary to that obtained with the established optical and electron methods. The

physical respects in which it differs from them define its special sphere of usefulness: short wavelength, small but specific absorption, and a simple emission spectrum. In addition it shares with electron microscopy, in contrast to the light microscope, the possibility of microdiffraction on crystalline specimens and of stereophotography at high resolution on those of openwork structure.

1.6.1. *Penetration and absorption in matter.* The penetration of X-rays is high compared with visible light because of their much shorter wavelength (cf. Fig. 1.4) and it is high compared with that of an electron beam because of the absence of charge. Only those materials can be observed in the optical microscope which have

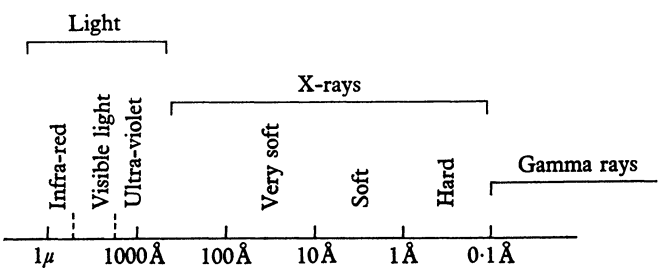

Fig. 1.4. Wavelength spectrum (1 Å = 10^{-8} cm.; 1μ = 10^{-4} cm.).

transmission bands between the wavelengths 0·1 and 0·7μ, that is, which are transparent in the usual sense of the word. In electron microscopy there are no specifically transparent substances, and at the highest voltage normally used (100 kV) the limiting thickness is about 0·2μ for compact material, but nearer 1μ for biological specimens. For X-rays of wavelength 1 Å, elements of medium atomic number are effectively transparent (35–40 % transmission) up to a thickness of 20–50μ. Images can be obtained through much thicker specimens, as in medical radiography, but with poor definition on account of X-ray scattering. In the thin specimens used in X-ray microscopy, scattering is negligible in comparison with absorption, except in certain metallurgical applications (cf. § 2.2).

As with visible light, the absorption of X-rays in matter is exponential and is expressed in terms of a linear absorption coefficient μ (cf. § 2.1), the value of which varies with the wave-