MICROSCOPY WITH X-RAYS

SYMPOSIUM IN CAMBRIDGE

IN the sixty years since the discovery of X-rays, very wide use has been made of them in industrial and medical radiography. These macroscopic studies of internal structure have proved of value both in research and for purposes of non-destructive testing. Only comparatively recently, however, has attention turned to the value of X-rays for microscopic investigation, and real progress towards a resolving power of optical order has had to wait until the post-war decade. Refinement of the normal radiographic technique, by using photographic emulsions of high resolution, has been accompanied by the development of a point projection method and by the use of reflecting mirrors to form an instrument more nearly akin to an optical microscope. The very short wave-length of X-rays, compared with visible light, holds out the ultimate prospect of a greatly improved resolution; but a more immediate advantage lies in the ability to examine specimens that are opaque at the usual optical wave-lengths. With X-rays it is also possible to get good micrographs from much thicker sections of transparent materials than can be tolerated in the optical microscope, on account of its very small depth of focus at high magnification.

These prospective advantages, as well as interest in the physical principles involved, have stimulated an increasing interest in the use of X-rays for micro-structural investigation. To bring together those actively working in the subject, a symposium on X-ray microscopy and microradiography was held in the Cavendish Laboratory, Cambridge, during August 16–21. It was sponsored by the International Union of Pure and Applied Physics, and received financial support from the United Nations Educational Scientific and Cultural Organization and from a number of industrial concerns in Britain. The response to its announcement was much greater than anticipated. In addition to those from the three main centres of activity, in Stanford University, California, the Caroline Institute, Stockholm, and the Cavendish Laboratory, participants came from nine other countries and papers, to be read in absence, from two others (Uruguay and the U.S.S.R.). In all, there were 125 members of the symposium and sixty-six papers were read.

The symposium was opened by Prof. N. F. Mott, as president of the International Union of Pure and Applied Physics and head of the Cavendish Laboratory. A general survey of the use of X-rays for microscopy by Dr. V. E. Cosslett (Cambridge) was followed by introductory accounts of the three chief methods: of the grazing-incidence reflexion technique by Prof. P. Kirkpatrick (Stanford), of contact microradiography by Prof. A. Engström (Stockholm) and of the projection method, using a point-focus tube, by Dr. W. C. Nixon (Cambridge). The following two days of the meeting were devoted to the problems of each technique in turn, including the special needs of micro-diffraction and of quantitative analysis by absorption, emission and fluorescence spectrometry.

Conditions of grazing incidence are new in optical theory. Considerable progress has been made at Stanford in the design of the form of aspheric surface best calculated to provide a practically useful compromise between width of field and resolving power. A four-mirror system computed by Dr. H. H. Pattee (Stanford) should give a resolution of 500 A. over a field of at least 20μ with X-rays of wave-length 4 A. However, to obtain this performance, the mirror contours must be true to within 50 A. and, to reduce scattering to a sufficiently small value, the surface must be molecularly smooth. The immediate problems therefore lie in the working and testing of mirrors and, once they have been made, in their precise alinement. New techniques were described for these purposes, as it is not enough simply to refine existing optical methods. An experimental microscope, reported by Dr. J. F. McGee (Stanford), incorporates two uncoated silica mirrors and two adjustable aperture stops. Using aluminium radiation (8 A.) a resolving power between 0·5 and 1μ has been obtained. In these systems the mirrors of an imaging pair are axially separated. M. Montel (Laboratoire de Chimie-Physique, Paris) has devised an elegant method of sectioning spherical mirrors and uniting two half-mirrors at the appropriate mutual angle, so as to eliminate astigmatism and anamorphism.

By contrast with reflexion microscopy, which is faced with severe technical problems, the contact method of microradiography is limited in performance only by the effective grain size of the photographic emulsion. The development of emulsions of resolving power 2,000 lines/in. now permits microradiographs to be obtained with a resolution approaching that of the best optical microscope. Prof. Engström described his work with softer X-rays (200–2,000 V.) than those usually employed. X-ray pictures of chromosomes and other cell components were shown which had a resolution of 0·3–0·5μ. An X-ray tube specially developed for these wave-lengths was described by Prof. P. J. Fitzgerald (College of Medicine, State University of New York), and an adapted commercial tube by Dr. A. Recourt (Philips, Eindhoven). Dr. B. L. Henke (Pomona College, California) has developed a tube having a gas-discharge type of electron source and a reflecting mirror for X-rays of 10–100 A. wave-length, in order to obtain better intensity in a region where efficiency of X-ray production decreases rapidly.

The point projection type of X-ray microscope, using magnetic lenses to obtain an electron spot as small as 0·1μ, was discussed by Dr. W. C. Nixon (Cambridge), Dr. J. B. Le Poole and S. P. Ong (Technical University, Delft) and Prof. B. M. Siegel (Cornell University). A resolving power approaching that of ultra-violet microscopy has been obtained by Nixon, with an exposure time of about 1 min. Smaller electron spots, certainly down to 100 A., can be obtained, but only with rapidly decreasing X-ray output, since the final electron lens has to be stopped down because of high spherical aberration. The need to increase the intensity of the electron beam, in compensation, was discussed by several authors. Dr. B. M. Rovinsky (Institute of Machine Sciences, Moscow) described a tube with ring cathode and needle-point anode, and the converse system using a point cathode and field emission was discussed by Dr. Pattee and by Dr. L. Marton (National Bureau of Standards, Washington, D.C.). The former has obtained high emission, but only at very high vacuum; the latter has worked at the vacuum more usually prevailing in a demountable apparatus, obtaining so far only small beam currents. In view of the difficulty of obtaining stable field emission in such circumstances, it appears at present that the best prospect for increased intensity in the point-focus tube lies in correction of the spherical aberration of the magnetic lens. The projection method has the advantage of direct enlargement on normal emulsions, but at cost of small field of view; the contact method gives a large field, but has greater difficulties of specimen preparation (since the specimen has to be very thin and placed almost directly on the emulsion) and of photographic processing. Even if a grainless film of reasonable speed can be developed, the optical viewing system will impose a limit on resolution—unless the emulsion can be stripped and viewed in the electron microscope. A possible technique of this nature was described by Dr. W. A. Ladd (Ladd Research Industries, New York), in which the 'emulsion' is a plastic film in which X-rays produce solubility changes depending on amount of exposure.

Even though X-ray methods may equal and perhaps exceed the optical resolving power, their main value in biology is likely to lie in the ability to distinguish different elements in the specimen, by means of differential absorption, emission and fluorescence. A large number of contributions was devoted to these methods of quantitative analysis. The Stockholm school has investigated in detail the accuracy attainable in absorption spectrophotometry, utilizing the characteristic absorption edges of the different elements. Dr. B. Lindström reported on analysis of sulphur and phosphorus in biological material, using a new high-vacuum apparatus and soft X-rays, and Dr. G. Wallgren described his studies in the mineralization of developing bone in human embryos. By direct microphotometry, analyses can be carried out on areas of tissue down to $3\mu^2$; a scanning and computing instrument for this purpose was described by Dr. O. Hallen (University of Gothenburg).

The projection method can also be adapted to microanalysis, and is especially useful for areas smaller than a few square microns, since photometry or photon-counting is carried out directly on an enlarged image. Mr. P. Duncumb (Cambridge) dealt with the design and operation of a flying-spot system for the analysis of thin layers by X-ray emission: the characteristic lines are detected and their strength measured by a proportional counter. An accuracy approaching 1 per cent can be obtained with a spot 1μ in diameter, and neighbouring elements in the periodic table can be separated if a crystal spectrometer is used. Mr. J. V. P. Long (Cambridge) described the adaptation of a projection X-ray microscope for the analysis of selected regions of a specimen, under a stationary spot, by fluorescence, absorption or emission methods at will. In emission, the specimen can simultaneously be viewed in transmitted light by an optical microscope. The limits of microchemical analysis by these methods were discussed by Prof. A. V. Baez (Redlands University, California). A detection limit of 1 per cent in a volume of $1\mu^3$ is equivalent to a mass sensitivity of 10^{-14} gm. of substance of unit density. At the moment, it is possible to determine elements of medium atomic number, down to aluminium or perhaps magnesium. Prof. Engström and Dr. Henke discussed the practicability of analysing separately the carbon, oxygen and nitrogen content of biological tissues with very soft X-rays, and it appears that this may be done with an accuracy of 5–10 per cent. Such a technique will be of revolutionary value in cytochemistry, just as the qualitative results obtained with microradiography are creating a new subject of microanatomy.

The sessions on applications of X-rays to microscopy, at the moment primarily using the contact method, covered a very wide range of subjects at the symposium. In biology, reports were presented of work on plant cancers and the growth of inorganic crystals in leaves, on the progress of osmium fixation in tissues, on the microstructure of wood and on insect flight musculature. In the medical field, investigations were reported of cancer of the breast and of the cervix, the human eye, the vascular patterns of human muscle and skin, growth of stone in the kidney and the embryonic stages of dentition. New techniques are now making it possible, particularly with the projection method, to observe changes in living tissue under experimental treatment. The metallurgical session was shorter, but contained accounts of the use of microradiography in studying segregation effects in ferrous metals and in light alloys, especially of aluminium, and the growth of oxide films. Preliminary studies of the distribution of heavy elements in rock sections were also reported.

Some discussion took place, formally and informally, on the need for further meetings on the subject of X-ray microscopy, for a specialized abstracting service and for clearing up matters of terminology. A small committee was set up to look into possible forms of organization, with particular regard to existing bodies and the need for connexion with the International Council of Scientific Unions. Its report will be prepared in time for discussion at the next symposium, which it is hoped to hold in Stockholm in 1959.

V. E. Cosslett

THE EIGHTEENTH INTERNATIONAL GEOGRAPHICAL CONGRESS

The eighteenth International Geographical Congress was held in Rio de Janeiro during August 9–18. The 1,006 delegates actually present included nearly four hundred from overseas, representing more than forty countries. The first congress was in 1871, yet this was the first to be held in the southern hemisphere, the first in the Tropics and the first in Latin America. It was opened in a blaze of pageantry by the President of Brazil in person at the Municipal Opera House. The delegates were welcomed by the President of the Organizing Committee of the Brazilian National Committee for Geography, and the British President of the International Geographical Union (Prof. L. Dudley Stamp, elected at Washington in 1952) gave a précis of his presidential address on the "Measurement of Land Resources". In order to secure some more precise measure of the capability of different types of land in differing climates and under differing types of management to support human life, he suggested the introduction of a 'Standard Nutrition Unit' of 1,000,000 calories annual production or 900,000 calories of net annual consumption. It was shown that a varied diet based largely on meat may require 3½ acres or more to