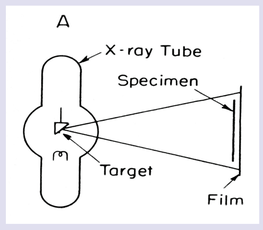
Early x-ray microscopy

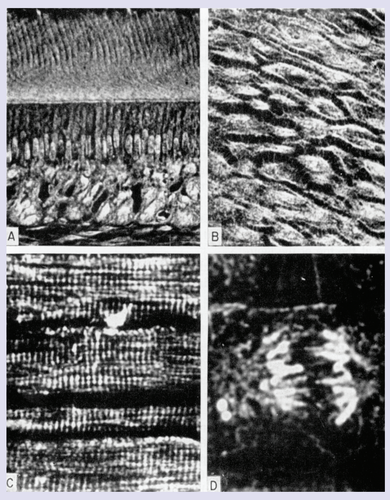
The earliest description of enlarged x-ray images was reported in 1897 (Heycock and Neville, 1898), only two years after the discovery of x-rays by Röntgen, and well before the physical properties of x-rays were known. In that case, radiographs were simply enlarged photographically. By the 1950s, the resolution had exceeded that of the light microscope and today reaches close to ten nanometers. However, it is the unique properties of x-ray imaging in regard to penetration, depth of focus and contrast mechanisms, rather than simply resolution, that make x-ray microscopy an important tool in structural research.

Contact microradiography

There are four types of x-ray microscopy. In early contact microradiography, the specimen was simply placed on a photographic emulsion and illuminated by x-rays (Goby, 1913). The image was developed, and then enlarged by standard photographic processes, which themselves were still under development. For some time, this technique was not considered to be microscopy, since no lenses were involved. For biological imaging, x-ray tubes were developed that generated the long-wavelength (1-100 nm) x-rays that are needed for high contrast, while providing sufficient brightness for a reasonable exposure time with fine-grain emulsions (Ehrenberg and Spear, 1951; Engström et al., 1957). The resolution was limited by the graininess of the photographic emulsion, which was 0.5-1 µm. Philips developed a compact, sealed-tube instrument in 1957 for commercial sale (van den Broek, 1957). Ladd et al. (1956) had some success in avoiding film grain altogether, by the use of a material that changes solubility after x-radiation (crystalline ammonium chloride). A replica of the "developed" image on the crystal was then viewed in the electron microscope. A similar method is still in use. A photoresist material such as PMMA is used for biological imaging with high-brightness synchrotron soft x-ray sources in the "water window", in some cases sometimes yielding a resolution on the order of 10 nm.



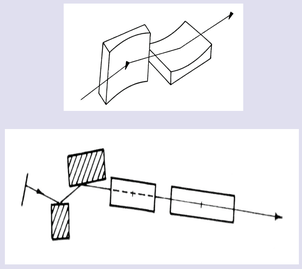
Schematic of contact radiography (from Cosslett, 1957)



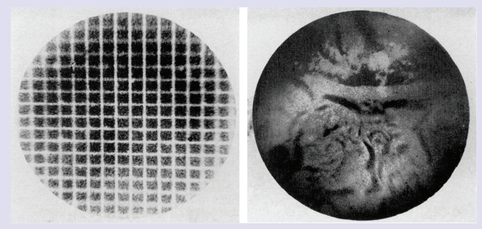
A-C: Contact radiographs of newborn rat material in 5µm thick paraffin sections. (A) Epithelium and enamel of incisor, 900X, (B) Skin, 1350X, (C) Skeletal muscle, 2560X. D: Mitotic anaphase division of onion root tip in 2µm thick paraffin section, 3500X. (Engström et al., 1957)

Reflection microscopes

Once it was generally understood (ca. 1912) that x-rays have wave properties, use of lenses to focus x-rays was considered. It was soon realized that a traditional microscope would have to be kilometers long, since x-rays are refracted only slightly. However, as first proposed by Jentzsch (1929) grazing-incidence mirrors could be used (Ehrenberg, 1947; Kirkpatrick and Baez, 1948). Before the introduction of zone plates in the late 1970s, this was the only type of true x-ray microscope in the sense of having x-ray optics. Early microscopes of this type, using two or four mirrors, which served to correct some of the aberrations inherent in grazing-incidence reflection, were built and achieved a resolution of about 1 µm (Pattee, 1957a). Resolution was limited by the smoothness of the mirror, which had to be on a molecular order for use with grazing incidence. After nearly two decades, development of soft x-ray optics using focusing mirrors would again find application.



Grazing incidence mirrors introduce strong coma aberration, which can be corrected by using two identical mirrors and an aperture stop, which however severely limits the field of view. A compound optical system uses two cylindrical mirrors, an objective at unit magnification and a projector, followed by two axially-symmetric tubes for additional magnification. (Pattee, 1957)

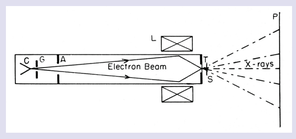


Images from a mirror microscope. Left: Silver mesh with 3µm wide grid bars. Estimated resolution 0.3 µm. Right: 7 µm thick section of earthworm gut, 400X. (McGee, 1957)

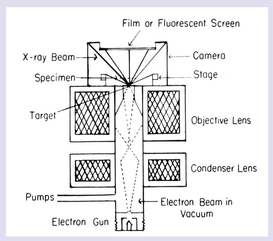
Point-projection microscopes

This method uses no lenses in the x-ray optics, but relies on a point source of x-rays projecting a "shadow image" (an early misnomer) of the specimen on a detector, originally a photographic emulsion, that is located some distance from the specimen. The challenge here is to make the source small, for high resolution, and bright, for a reasonable exposure time. This is the area of early x-ray microscopy that received the most attention, especially in the twenty year period centered on 1955.

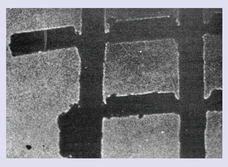
Hospital type x-ray tubes fitted with a small pinhole were used at first, but the low brightness made high-resolution work impractical. With the development of electron optics, it was soon realized by von Ardenne (1939) and Marton (1939) that a small x-ray source could be produced by focusing a fine electron beam on a thin metal target. This work was first taken up by Cosslett and Nixon (1951), and later by several others. The size of the x-ray source depended on the accelerating voltage of the electrons, the aberrations of the electron lens, and the thickness of the target. The electron optics were refined such that the x-ray source size was limited by the target thickness, and with a 0.1 µm thick target resolution on the order of 0.1µm was achieved at 10 kV with a 5 min exposure (Nixon, 1955). To increase the resolution beyond this, especially for biological work, even thinner targets, lower acceleration voltage (for better contrast due to longer-wavelength x-rays), and a smaller electron beam would be needed. These factors would decrease brightness and make the exposure time excessive. Early attempts were made to employ field-emission guns in the electron source for a point-projection x-ray microscope (Pattee, 1957b, Marton et al, 1957), with limited success. However, these were not followed up, partly because the state of the instrument was already adequate for most of its applications at the time.



Basic scheme for a point projection microscope (Cosslett, 1957)



System using magnetic electron lenses (Nixon, 1957)

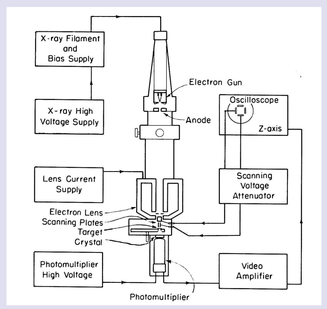


Resolution test of point-projection microscope. The width of the diffraction fringe indicates a resolution of about 0.1 µm. (Nixon, in Baez and El-Sum, 1957)

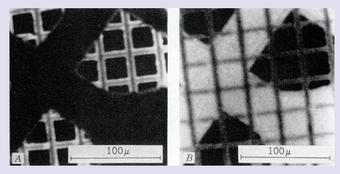
Scanning x-ray microscopes

In order to overcome the diffraction limit for resolution better than about 0.1 µm, the specimen in a point projection microscope has to be placed close to the target, on which a very small x-ray source is generated. This severely limits the field of view. Thus, in the 1950s, when development of the scanning electron microscope was underway, it was natural to consider scanning the x-ray source over the specimen. Pattee conceived (1953) and constructed (1957c) a microscope that scanned a fine electron beam over the x-ray target, on which the specimen was placed. X-ray photons were collected electronically with single-photon sensitivity and displayed on an CRT scanned in synchrony with the beam. In order to obtain an image with a sufficient signal to noise ratio when using the very thin target needed for high (better than 1 µm) resolution, a field-emission x-ray tube (see above) was under development.

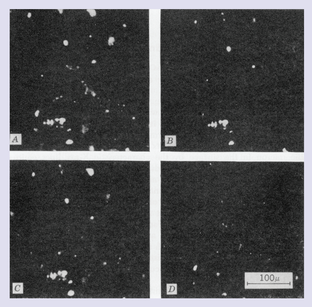
Similar technology was used by Duncumb and Cosslett (1957), but with the difference that the electron beam scanned the specimen directly, and the x-rays generated were collected either by a scintillator, followed by a proportional counter and pulse analyzer, or by a crystal spectrometer. In this way, images could be formed from x-rays characteristic of particular elements, and x-ray spectra could be collected at chosen points on the specimen. This paralleled the work at the same time on the electron probe microanalyzer, and is of course very familiar to us today. The major problems at the time were electron beam contamination and insufficient power supply stability.



Block diagram of a scanning x-ray microscope (Pattee, 1957a)



Scanning x-ray micrograph of 200 mesh Cu and 800 mesh Ag grids. (A) Image formed with Ag-L emission line. (B) Image formed with Cu-K emission line. (Duncumb and Cosslett, 1957)

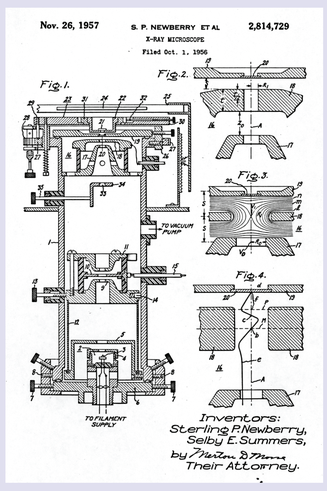


Impurities in Be foil revealed by x-ray emission scanning. (A) All energies, (B) Mn emission, (C) Ni emission, (D) Ca emission. (Duncumb, 1957)

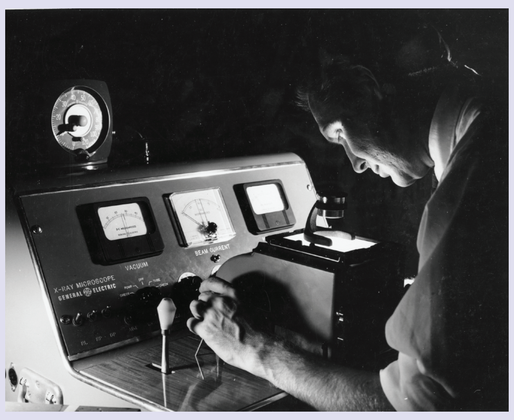
Early commercial x-ray microscopes

In the 1950s, the practical resolution of x-ray microscopy, using any method, stood at about 1 µm. Although not better than light microscope resolution, x-ray microscopy can be used to study the internal structure of objects opaque to visible light, and has a much greater depth of focus than the light microscope. It also has about ten times the penetrating power of typical electron microscopes. Three companies, Philips, Microray Laboratories of England, and General Electric produced point-projection x-ray microscopes during this period.

The GE model, designed by Newberry, was the first commercial x-ray microscope, announced in 1954 (Newberry and Summers, 1954). Newberry, a Charter Member of MSA (EMSA at the time), and had already built a magnetic transmission electron microscope in 1938. He also designed a small electrostatic electron microscope that GE planned to market in 1950s. The GE x-ray microscope uses electrostatic electron lenses and has an accelerating voltage of 5-50 kV. Six microscopes were sold by 1956 and about 100 units were produced, some of which are still operating. Some users fitted the microscope with a crystal spectrometer for recording x-ray emission lines from selected areas on the specimen. Also at this time, kits were made for converting a transmission electron microscope to an x-ray microscope (Siegel and Knowlton, 1957). In later years, x-ray microscope kits for SEM became available as well.



Patent drawing of the electrostatic GE x-ray microscope

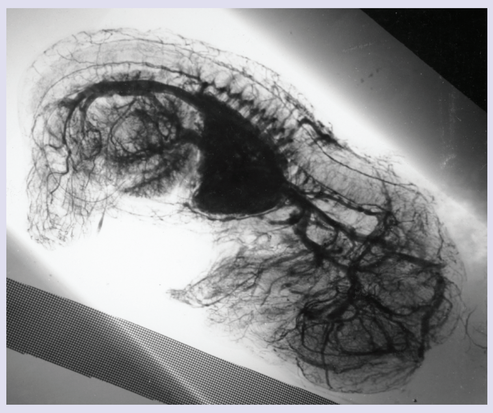


Sterling Newberry at the prototype GE x-ray microscope

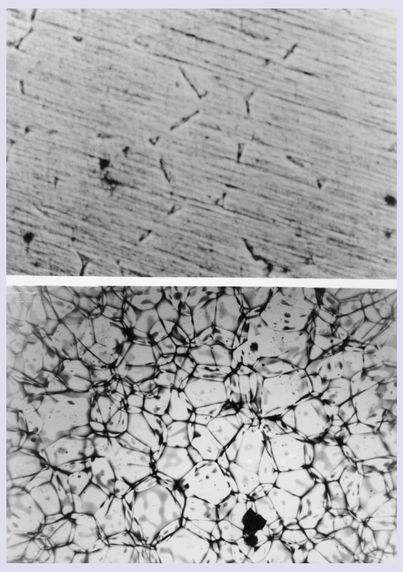
Early applications of x-ray microscopy

Taken on the GE microscope

Mosquito and mosquito head



Paraffin-embedded rat embryo, 5 mm thick, demonstrates great depth of field



Top: light metallograph of a polished aluminium tin bronze alloy. Bottom: x-ray micrograph of the same specimen, at higher magnification. X-ray and light microscopy complement each other to show both surface and internal featiures.

Acknowledgement

Inspiration for the creation of this poster is due to Sterling Newberry, who graciously contributed images and reference material from his collection, and reviewed the content of the poster.

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A prime source for material on early x-ray microscopy is:
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This is the proceedings of the Second International Symposium on X-ray Microscopy held at the Cavendish Laboratory, Cambridge, in 1956. The full papers in this book exemplify in detail the progress to that time, and further major advances were not made in the field until the 1970s with the application of focusing zone plates. In the references below, this volume is simply cited as "*Cambridge Proceedings*".

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