15. X–1 SOFT X-RAYS (23 TO 44 Å). By R. Rosser, Blacket Laboratory, Imperial College, London SW7 2BZ, U.K.

Soft X-rays (23 to 44 A) offer potential advantages over electrons for probing biological structure. The most exciting of these is the possibility of viewing hydrated biological specimens with a resolution equal to that of the scanning electron microscope. This arises because, in this wavelength range, protein is significantly more absorbing than water. Further, by taking pictures on either side of absorption edges and subtracting them, it should be possible to map individual elements.
Although less damaging than electrons, soft x-rays cause conderable disruption.Theoretical calculations indicate that damage will limit the resolution of a soft x-ray microscope to about 100 A.
The destruction appears to be caused by the creation of free radicals, which attack the chemical bonds, on a time scale of picoseconds to milliseconds. By using nano-second exposures, it may be possible to approach the Rayleigh resolution limit set by the wavelength of light being used (i.e. about 30 A).
For all forms of probing, intense soft x-ray sources are required. Synchrotrons provide the most readily available sources, though laser produced plasmas and gas-puff z-pinches offer possibilities. The first gives very short, intense pulses, suitable for ultimate resolution microscopy. The latter may provide a compact, inexpensive laboratory source.

15. X–2 OPTICAL METHODS OF SOFT X-RAY MICROSCOPY. By G. Schmahl, Forschungsgruppe Röntgenmikroskopie, University of Göttingen, Geismarlandstr. 11, 3400 Göttingen, Fed.Rep.of Germany

X-ray microscopy fills a gap between optical and electron microscopy. Using soft x-rays a higher resolution than with visible light can be obtained. In comparison to electron microscopy thick, wet, unstained specimens can be examined. This is especially advantageous for biological applications. The interest in x-ray microscopy is caused by the development of improved x-ray optics, high resolution x-ray resists for contact microscopy and the development of intense soft x-ray sources.

In this paper the development of different kinds of x-ray optics are described as zone plates, multilayered reflection optics and grazing incidence optics. The presentation concentrates on the development of zone plate condensers and high resolution zone plates suited for x-ray microscopy. In addition, the x-ray optical set-ups of an imaging x-ray microscope and of a scanning x-ray microscope are discussed. X-ray microscopy results are presented which have been performed with x-ray microscopes installed at the electron storage ring BESSY in Berlin.

15. X–3 APPLICATIONS AND EXAMPLES OF BIOLOGICAL SOFT X-RAY MICROSCOPY*. Ping-chin Cheng (鄭炳今), Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL, 60612

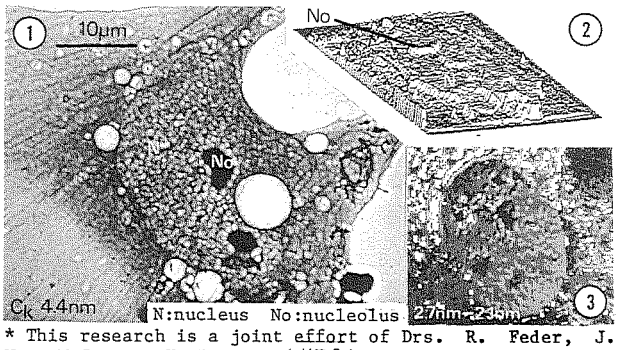
The recent advances in x-ray imaging technology indicate that x-ray microscopy could become a very important tool in biological research. At the present time, both optical and non-optical imaging techniques are available. It is the non-optical method (contact microscopy) on which this presentation intends to focus. Nearly all previous ultrastructural studies of cells (i.e., by TEM) have been performed on fixed and dehydrated specimens. Because water possesses an x-ray transparent window just below the oxygen absorption edge, x-ray microscopy can be a unique tool for the study of hydrated or even living cells. Wet cell images taken by contact microscopy, transmission, and scanning x-ray microscopy have been reported.

The investigations of various dehydrated biological specimens (i.e. cultured muscle cell, Xenopus laevis fibroblast(Fig.1, 4.4 nm image), bone marrow tissue, isolated nuclei, human platelet and plant tissue) by means of x-ray contact microscopy has been conducted by us and by others. X-ray-dense structures not visible by electron microscopy have been reported (Science, 212, p1398).

Studies of the absorption properties of biological specimens such as proteins and ADP have been conducted at Tantalus storage ring (USA). Knowing the x-ray absorption spectra of major biological compounds is essential for the precise determination of the absorption edges; this in turn is needed for microchemical analysis. Monochromatic radiation with energies above and below a certain elemental absorption edge have been used for microchemical analysis. The subtraction of images above and below an absorption edge were performed, and color-coding of the resulting digitized image was done by a computer (Fig.3, image taken at 2.7nm minus image taken at 2.1nm). In addition to the TEM-magnified x-ray contact image, a surface view of the photo resist by SEM was found to be useful for the image interpretation. The appearance of surface features depends on the viewing angle of the specimen, whereas multiple views are generally required for complete image interpretation. Furthermore, photographing under SEM at different viewing angles can be a time consuming task. However, isometric views of the resist's surface topography (Fig.2) at different viewing angles can be easily generated by a computer using a digitized TEM-magnified x-ray image as the database.

Stereoscopic pairs of electron micrographs have proven to be very useful in the study of cellular ultrastructure. Similar techniques can also be used in x-ray contact microscopy to provide 3-D information about a specimen.

Some of the valuable advantages to x-ray microscopy are the ability to handle hydrated specimens,thick specimens, and microchemical analysis. Further development is needed for complete realization of its potential.



* This research is a joint effort of Drs. R. Feder, J. Wm. McGowan, H. B. Peng (彭筱明), D. Sayre, D. Shinozaki and K. H. Tan (陳全華).