# PROSPECTS FOR LONG-WAVELENGTH X-RAY MICROSCOPY AND DIFFRACTION

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Our purpose in the first part of this talk is to call attention to certain advantages of the soft ($\lambda$ = 10-100A) x-ray photon in the imaging of biological material, which are not shared by other particles. The second part will briefly survey the status of some of the problems which arise in the use of these photons for this purpose.

## I. Properties of the Long-Wavelength X-Ray Photon as a Compositional Probe.

Examination of the reaction cross-sections[1] for photons in the soft x-ray region (Fig. 1) shows that these particles are well suited for the mapping of compositional features in intact, wet, unstained, and possibly living single biological cells or organelles. This arises as follows:

(1) Total reaction cross-sections correspond to mean free paths in biological materials of the order of 1 $\mu$m;

(2) Total cross-sections vary abruptly with specimen composition, due to existence of absorption edges;

(3) Photon absorption is the dominant reaction (absence of multiple scattering).

Sayre et al.[2] examined these effects in detail and concluded that an adequate signal/noise ratio for the imaging of 100A diameter features in 1 $\mu$m-thick wet unstained biological materials can be obtained at exposure levels of $\sim 10^4$ J/g. For electrons of the energies employed in electron microscopy, they concluded that exposure must rise to levels of $\sim 10^7$ J/g to achieve the same signal/noise ratio in the same materials. (Studies of radiation damage[3] indicate that exposures of $10^4$ J/g, although destroying function in biological material, do not seriously affect structure visible at 100A resolution. Such structure is destroyed by exposures of $10^7$ J/g.) Similar conclusions[4] apply to the mapping of the concentration of a particular atomic species Z in intact wet biological materials, except that for $Z \lesssim 11$ the method of electron energy-loss analysis can achieve exposures similar to those with soft x-rays.

High-resolution structural input to cell biology today comes mainly from studies on non-intact cellular material. Although this information is of extreme value, there seems to be agreement among cell biologists on the desirability of having techniques for the imaging of untreated cellular material.

## II. Problems Attendant on the Use of These Photons.

### A. Sources.

Until fairly recently, there existed no high-intensity laboratory sources of photons in the wavelength range under discussion. This problem has now been solved in most respects through the development of synchrotron radiation and plasma[5] sources. These allow $\sim 10^4$ J/g of fairly monochromatic radiation to be put on a sample, in seconds or minutes in the case of synchrotron radiation, and in $10^{-8}$ to $10^{-7}$ seconds with a

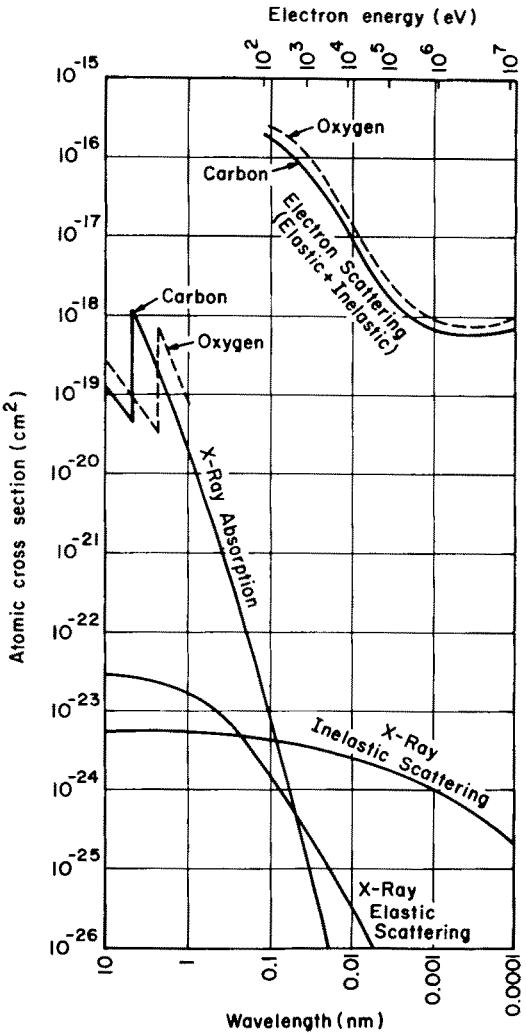
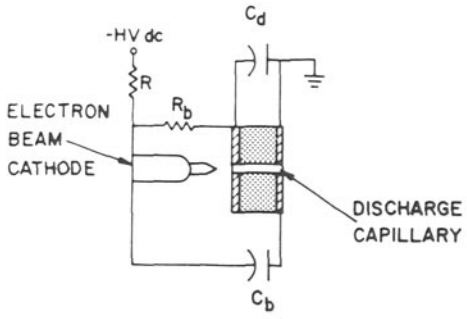
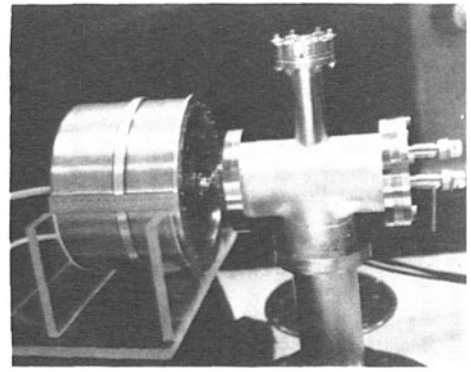



Fig. 1. Cross-sections for reactions of photons and electrons with carbon, as functions of particle wavelength λ. For comparison, portions of the corresponding curves for oxygen are also shown (dashed curves).



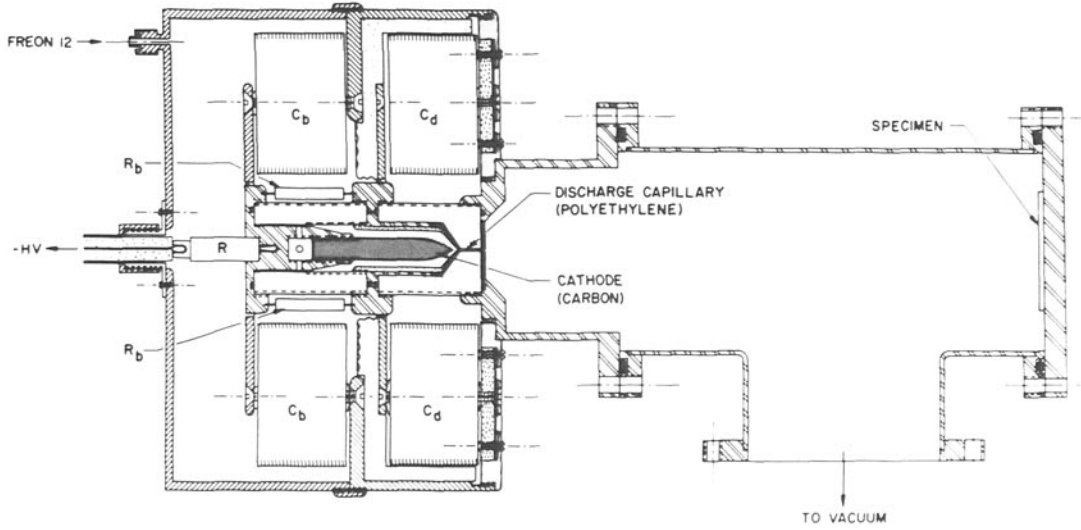



Fig. 2. The McCorkle-Vollmer pulsed plasma source. Upper left, schematic diagram of the source. Below, diagram showing some of the construction details. Upper right, photograph of the source. (Courtesy R.A. McCorkle.)

pulsed plasma source. The plasma source (Fig. 2) is also sufficiently compact and inexpensive to become widely available should the need develop. The synchrotron radiation source has the advantages of continuous tunability, narrow beam divergence, etc.

B. Imaging.

In the above study[2] it was assumed that every event of interest (absorption or non-absorption of the photon; elastic scattering, inelastic scattering, or non-scattering of the electron) could be detected and correctly assigned to the appropriate resolution element of the specimen; i.e., that the laws of optics and the quality of available components will allow an ideal image-forming system to be constructed. Somewhat similar idealizing assumptions were made in the study[4] on the mapping of atomic species.

*Contact Microradiography.* For thin specimens, or for resolutions which do not approach $\lambda$ too closely, the above assumptions can be quite well met at present by the use of high-resolution detectors in contact microradiography[6]. In this method (Fig. 3) irradiation produces an image of the specimen in a "grainless" film of photon-sensitive material with which the specimen has been placed in contact. (To read out the full information contained in the image, the image is examined in an electron microscope.) To date the most widely used photosensitive material is polymethylmethacrylate (PMMA), which undergoes a change in solubility upon exposure to radiation. For photons with $\lambda$ = 40A, the inherent resolution of PMMA is ~50A, with quantum efficiency approaching 1 (see the review by Spiller and Feder[7]). Accordingly, whenever diffraction effects can be ignored (thin specimens or applications in which resolutions do not approach $\lambda$), the conditions assumed above[2] are approximately met in this form of microscopy. Images of thin specimens with resolutions $\lesssim$100A have been obtained experimentally in this way[8]. Some biology is now being done with the technique[9,10] (see Fig. 4).

For high-resolution work with thick specimens the high-angle portion of the diffraction pattern must be processed more correctly than is done by the simple contact method. Proposals for doing this include deblurring by holographic or other processing and the replacement of the contact technique by scanning or conventional microscopy using wide-angle x-ray optics. (See the recent review of soft x-ray microscopy by Kirz and Sayre[11].) Although these proposals appear to have good potential, it may be some time before any have been sufficiently developed to produce an improvement over the simple PMMA-based contact method.

*Soft X-Ray Diffraction.* The above discussion has concerned microscopy, in which an image is formed directly by the experimental apparatus. An alternative is to adopt the practice of x-ray diffraction, in which the experiment is asked only to capture as much of the diffraction pattern as possible, and the image formation is carried out subsequently by computation. At $\lambda$ = 1.5A, this technique, although slow, is extremely successful in the imaging of 3-dimensional structures at the full theoretical resolution of $\lambda/2$. There appears to be no fundamental reason why the same technique cannot be applied at $\lambda$ = 30A. There would be a general scaling upward of distances in the image, i.e. the minimum resolvable distance would scale upward from ~0.75A to ~15A and the diameter of the structures imaged (imagible field of view) would similarly move upward from its present practical maximum of ~200A to perhaps 4000A. The method would thus cover a size-range of great interest in cell biology. One quantity which would scale downward is specimen size, because of the larger total reaction cross-sections at 30A; the appropriate diffracting structure would be the single biological cell or organelle. The diffraction would thus be micro-diffraction. It is not yet clear to the author what difficulties of instrumentation would result.

Physically the mechanism of diffraction changes from photon scattering at 1.5A to photon absorption at 30A. Fortunately the mathematics remains largely unchanged, with the diffraction pattern still being approximately a Fourier transform. (The function transformed is no longer $\rho(x)$ but $|m(x)-1|$, where m is the complex index of refraction, with $\mathrm{Im}(m) = \lambda/4\pi s$; here s is the mean free path for absorption of the photons. For the approximation to be good, $|m-1|$ must be small (Rayleigh-Gans theory). For biological materials, m for $\lambda$ = 30A is of the order of 0.999-0.001i.) Thus the general scheme of structure analysis at

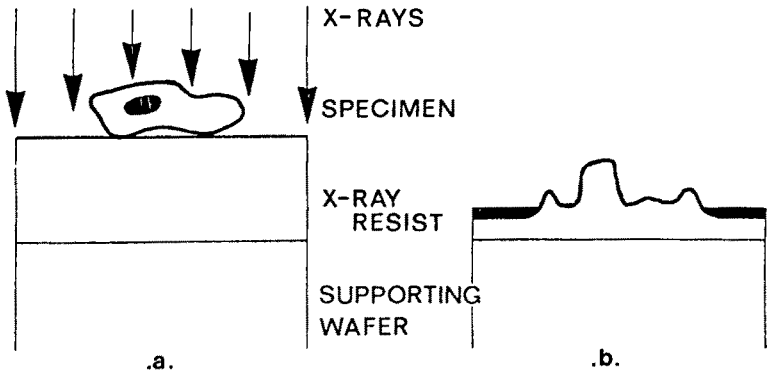



Fig. 3. Contact microradiography. (a) Irradiation of the x-ray resist through the specimen. (b) The resist after development. The projected image of the specimen is recorded in the resist profile and can be read out, after light metallization, in an SEM.

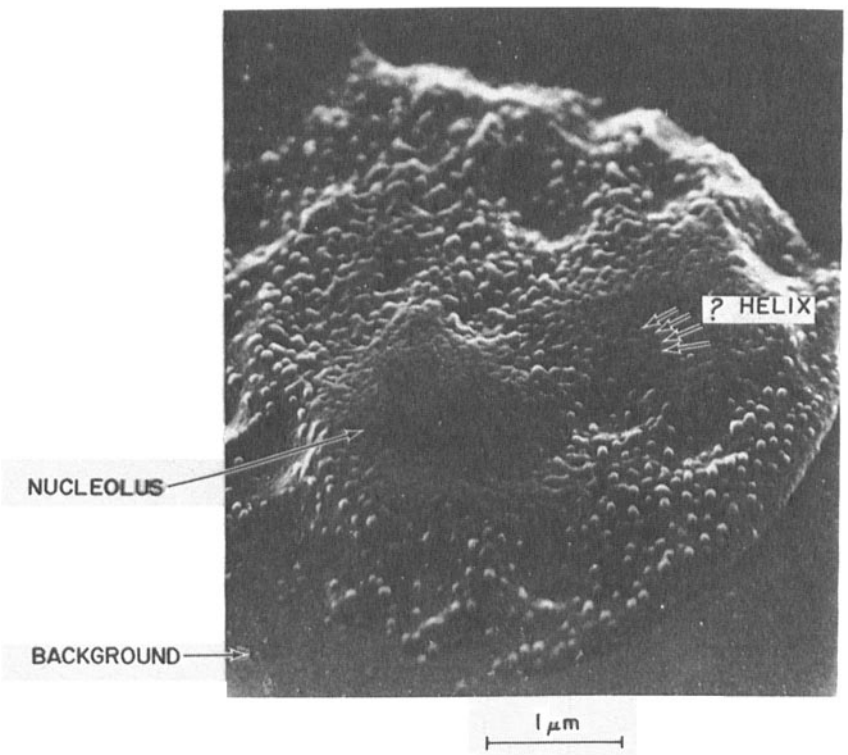



Fig. 4. Contact microradiograph of human interphase nucleus from glioblastoma tissue culture in PMMA, made with carbon Kα radiation. The x-ray source was operated at 5kV and 40mA, with a target-to-specimen distance of 18 cm and exposure time of 40 hours. (Courtesy L. Manuelidis (Yale University), J. Sedat (University of California at San Francisco), and R. Feder (IBM Research Center).)

1.5A carries over to the 30A case. In particular, the central problem of the analysis is that of supplying the phase of the diffraction pattern. It is suggested that at the scale of sizes involved, it should be possible to use artificially fabricated micro-objects of known structure to act as phasing references in a modified form of heavy-atom phasing. It should be noted also that the diffraction pattern will be continuous (not discrete), the specimen being non-crystalline.

Experimentally, diffraction patterns using synchrotron radiation at $\lambda = 46.8$A have recently been obtained[12] from 1 $\mu$m latex spheres. Exposures for collecting the small-angle diffraction pattern from assemblages of spheres were 2 minutes. The demonstration that the large-angle pattern can be collected from single objects remains to be done.

*Imaging in 3 Dimensions.* The thickness of the contemplated structures implies that 3-dimensional imaging will normally be necessary for comprehensibility. Contact microradiography lends itself naturally to the taking of stereo projections through tilting of the specimen-film pair (or more generally to the collection of n tomographic views), but is subject to the limitation on resolution noted above. Three-dimensional imaging without this limitation would also be provided by diffraction, as well as by microscopy with wide-angle optics (focussing on successive layers), or by holography.

In principle the exposure of $\sim 10^4$ J/g noted above is sufficient to establish the structure in 3 dimensions. However, the above techniques (holography excepted) require an increase in total exposure, and thereby in damage to the specimen. It is not clear as yet whether a 3-dimensional non-holographic method operating at $10^4$ J/g can be realized physically or not.

C. Specimen Chambers.

To realize the full potential of soft x-ray imaging, the specimen should be maintainable in a normal environment at 1 atmosphere. Fortunately, mean free paths in air at STP are several mm. for $\lambda > 31$A (absorption edge of nitrogen), so that the design of suitable specimen chambers employing thin windows or differentially pumped small apertures should not be difficult.

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