maining phases also had distinct onsets but signal-to-noise ratios of < 2: 1). Since many of these phases had travel paths entirely within portions of the northwestern Pacific Basin already known to have an 8.3-km/sec waveguide (shaded area of Fig. 1), two major waveguides seem to be suggested for this part of the Pacific. Concerning the possible presence of the 7.8-km/sec phase for distances less than 30°, it seems reasonable that this phase would begin to make its appearance as the 30° distance is approached. Unfortunately, the only data available in the distance range of 19° to 30° were recorded on low-speed, rectified power level recordings of hydrophones. Later arriving phases on these recordings are extremely difficult to evaluate (6).

Although data relevant to the existence of a waveguide in the North Pacific comparable to the northwestern Pacific's 8.3-km/sec waveguide do not exist (because of a lack of stations at appropriate epicentral distances), worldwide observations suggest that such a waveguide should be present for that area as well as for large areas of the South Pacific. At this time, the extension of a dual waveguide hypothesis to the South Pacific, however, would not be reasonable since the 7.8-km/sec waveguide for this region would be based on only two poorly recorded phases at Easter Island.

Propagation of the 8.1- to 8.3-km/sec phase has generally been thought to occur along a discontinuity surface or within a waveguide which is close to the Mohorovicic discontinuity. Recently, explanations involving thin high-velocity layers have been proposed for this waveguide, as well as for additional upper mantle waveguides suggested by longrange seismic refraction observations in France (7). Such explanations may also be applicable to the suspected 7.8-km/ sec waveguide reported here.

Having now obtained evidence for high-frequency guided phases to distances well in excess of 3300 km (perhaps to 7600 km), it is my hope that the thought expressed in the following statement will receive additional, well-deserved attention: "It will be the challenge to both explosion and earthquake seismology for the coming decade to find an answer to the question of how P and S transmission can occur within the lower lithosphere over distances of more than 1000 km with nearly constant velocity of first arriving energy" (8).

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- See Bath (2)
- D. Walker, J. Geophys. Res., in press.
- The criterion used in selecting these 23 phases was merely the fact that they had distinct onsets. The phases are the only ones having such onsets in the currently available data set condrophone power level recordings. Of the 23 phases suspected as being guided arrivals, 16 were recorded by only one station; four were from two earthquakes, each recorded by two

stations at distances in excess of 30°; and three were observed by single stations at distances beyond 30°, but also recorded at distances less than 30°. Of these three observations at distances less than 30°, two have arrival times cor-responding to a higher velocity 8.33-km/sec waveguide; the remaining observation is found

- waveguide; the remaining observation is found close to the crossover point of the 8.33 and 7.76-km/sec travel time curves ( $\approx 18.6^\circ$ ). At distances less than 19° where the 7.76-km/sec phases would begin to arrive earlier than the 8.33-km/sec phases (because of the large nega-6. tive intercept), several arrivals corresponding to the 7.76-km/sec phase have been observed on a seismograph at Ponape (4). However, these observations may not be too meaningful because of the large standard deviation of the 7.76-km/sec line
- See Hirn et al. (2); R. Kind, J. Geophys. 40, 189 (1974); K. Fuchs and K. Schulz, *ibid.*, in press. 7 Hirn *et al.* (2) stated that thin, high-velocity strata could only be proposed if thin zones of low velocity were also included in the model implying "drastic modifications of current petro logical models of the lower lithosphere" an modifications to current estin strength of the lower lithosphere. modifications estimates of the
- See Hirn *et al.* (2), p. 381. I thank the staff of Honolulu Observatory for providing records from their Haleakala seismo graph, and R. Johnson for hydrophone record-ings. I thank G. Sutton and M. Odegard for reviewing this report, and E. McAfee for editorial assistance. Illustrations were provided by C. S. McCreery. Contribution No. 832, Hawaii Institute of Geophysics. This research was supported by the Office of Naval Research and NSF grant DES 75-14814.

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## **High-Resolution Soft X-ray Microscopy**

Abstract. X-ray micrographs of biological materials have been obtained with a resolution better than 100 angstroms by using x-ray resist as the recording medium. A high-resolution scanning electron microscope with a short-focal-length final lens, operating in the "low-loss" mode, is used to make the smallest features in the x-ray replica visible.

A resolution better than 1000 Å has recently been demonstrated by soft x-ray contact micrography by using polymethyl methacrylate (PMMA) resist for recording and a scanning electron microscope (SEM) for magnified viewing of the resist replica (1). We present here some new results which demonstrate a resolution better than 100 Å.

In PMMA breaking of bonds reduces the molecular weight and increases the dissolution rate in a proper solvent (2). Development in this solvent produces a relief replica of the object, where the higher elevations correspond to a higher absorption of the specimen. The limit of the resolution of an x-ray resist is the effective range  $\delta$  of secondary electrons which are produced in the resist by soft x-ray absorption (3). Measurements have shown that this range increases linearly with the energy E of the incident xrays and that a value  $\delta \approx 50$  Å is obtained for carbon  $K\alpha$  (E = 277 ev) x-rays (4). The highest resolution to be expected is for the wavelength range around 50 Å. For shorter wavelengths (higher energies) the resolution decreases because of the increasing range of secondary electrons; for longer wavelengths the resolution decreases because diffraction effects become dominant.

For our high-resolution experiments we have used carbon  $K\alpha$  radiation (wavelength  $\lambda = 44.8$  Å) and radiation from the DESY synchrotron in Hamburg, which was operating at an electron energy of 7 Gev and a current of 5 ma. The spectrum of the DESY synchrotron radiation was modified by reflecting it from a gold mirror at a glancing angle of 4° to eliminate the hard radiation with  $\lambda < 25$  Å. The effective exposure spectrum of the resist under this condition extends from about 30 to 44 Å (5).

Figure 1 shows a scanning electron micrograph of the resist replica obtained from a section of a salivary gland chromosome of Drosophila with carbon  $K\alpha$  radiation. The micrograph was obtained in a commercial SEM, and the finest details visible correspond to the resolution of this instrument ( $\sim 250$  Å).

Figure 2 shows the x-ray images of a section of the retina pigment epithelium



of the frog Rana catesbeiana. Ocular tissue from light-adapted frogs was immediately fixed after enucleation in 3 percent glutaraldehyde (10 hours) in 0.1M cacodylate buffer (pH 7.4). Tissues were washed in 0.1M cacodylate buffer, postfixed for 1 hour in 1 percent osmium tetroxide in 0.1M cacodylate buffer, washed in distilled water, dehydrated in acetone, embedded in plastic (Epon 812), and sectioned on a diamond knife. The sections, which were 700 to 900 Å thick, were placed in a droplet of water on a resist-coated silicon wafer and the specimen was heated to dryness. The sections were stained for 90 seconds with a continuous flow of 2 percent aqueous uranyl acetate. Synchrotron radiation from DESY was used for the expo-



Fig. 1 (left). Soft x-ray replica of part of a chromosome from the salivary gland of Drosophila in x-ray resist (PMMA). The exposure was about 10<sup>3</sup> joule/cm<sup>3</sup> (16 hours) with carbon K $\alpha$  radiation  $(\lambda = 44.8 \text{ Å})$ . The resist was developed in 1 : 1 mixture of methyl isobutyl ketone and isopropanol for 1 minute. This is a scanning electron micrograph made with a conventional SEM at a 60° viewing angle. Fig. 2 (above). Soft x-ray replica obtained from a thin plastic-embedded section of frog retinal pigment epithelium. The exposure was made with synchrotron radiation from DESY with an effective wavelength region  $\lambda = 30$  to 44 Å. The exposure dose was about 10<sup>4</sup> joule/cm<sup>3</sup>, the exposure time was 15 minutes, and the distance between the sample and the point of emission was 40 m. (a) This micrograph, taken in a commercial SEM, shows elliptical protuberances which are representative of melanin granules from frog retina pigment epithelium. (b) In this high-resolution low-loss micrograph of a melanin granule from a frog retina pigment epithelium cell, ultrastructural details can be clearly seen which measure less than 100 Å. The marker represents 1000 Å in (a) and (b).

sure. The SEM image in Fig. 2a was obtained with the conventional SEM. An SEM with a short-focal-length final lens and a LaB<sub>6</sub> cathode was used to obtain Fig. 2b (6). In this microscope the lowloss imaging method (7) is used, in which the image is formed by collecting highenergy electrons scattered from the sample surface, rather than the low-energy secondary electrons which are used in the conventional SEM. This results in a higher sensitivity to small changes in the surface topography and gives better contrast than is obtainable with conventional SEM's.

Structures with dimensions below 50 Å are visible in Fig. 2b. Some of the finest structures visible may be partly due to the metallization process (the resist is coated with a thin Au-Pd (60:40) film to make the surface conductive for the SEM inspection), but it is obvious from Fig. 2b that a resolution of at least 100 Å was obtained in the x-ray microscopy process.

We conclude that PMMA resist has a resolution which is better than 100 Å and has enough contrast to make features with these dimensions visible. A highresolution SEM of the type used to produce Fig. 2b is preferred for viewing the resist surface when high resolution is desired. The resolution obtained is very close to the resolution limit of organic resist films, which is determined by the range of secondary electrons and by the resolution limit due to diffraction in resist.

The photographs shown are two exam-

ples of the objects investigated so far. Others include heart of chick embryo cells, monkey retina sections (8), mouse and guinea pig brain sections, and tissue cultures of human central nervous system tumors (9). Many details of the structures in our micrographs have not been seen by any other method.

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