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by

R. Feder, E. Spiller, J. Topalian, A. N. Broers
IBM T. J. Watson Research Center, Yorktown Heights, New York

W. Gudat
Deutsches Elektronen-Synchrotron DESY, Hamburg

B. J. Panessa and Z. A. Zadunaisky
New York University Medical School, New York, N.Y.

J. Sedat
Yale University, New Haven, Connecticut

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HIGH RESOLUTION SOFT X-RAY MICROSCOPY

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New York University Medical School, New York, N.Y.

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

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

In PMMA breaking of bonds reduces the molecular weight and increases its 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 δ 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 x-rays and that a value $\delta \approx 50$Å is obtained for carbon Kα (E=277eV) x-rays (4). The highest resolution to be expected is for the wavelength range around 50Å. 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 Kα radiation (λ=44.8Å) 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 wavelength λ<25Å. The effective exposure spectrum of the resist under this condition extends from about 30 to 44Å (5).

Fig. 1 shows a scanning electron micrograph of the resist replica obtained from a section of a salivary gland chromosome of Drosophilia using carbon Kα-radiation. The micrograph was obtained in a commercial scanning electron microscope and the finest details visible correspond to the resolution of this instrument (~250Å).

Fig. 2 shows the x-ray images of a section of the retina pigment epithelium of the frog *Rana catesbiana*. Ocular tissue from light adapted frogs was immediately fixed after enucleation in 3 percent glutaraldehyde (10 hrs.) in 0.1 M cacodylate buffer (pH 7.4). Tissues were washed in 0.1 M cacodylate buffer, post-fixed for 1 hr. in 1 percent osmium tetroxide in 0.1 M cacodylate buffer, washed in distilled water, dehydrated in acetone, embedded in plastic (Epon 812) and sectioned on a diamond knife. The 700-900Å thick sections were placed in a droplet of water on a resist coated silicon wafer and the specimen heated to dryness. The sections were stained for 90 sec. with a continuous flow of 2 percent aqueous uranyl acetate. Synchrotron radiation from DESY was used for the exposure. The SEM image in Fig. 2a has been obtained with the conventional SEM. A short focal length final lens SEM with a LaB_6 cathode was used to obtain Figs. 2b (6). In this microscope the low-loss imaging method (7) is used in which the image is formed by collecting high energy 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 obtainable with conventional scanning electron microscopes.

Structures with dimensions below 50Å 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 the picture that a resolution of at least 100Å has been obtained in the x-ray microscopy process.

We conclude that PMMA resist has a resolution which is better than 100Å and has enough contrast to make features with these dimensions visible. A high resolution SEM of the type used to produce Fig. 2b is preferred for the viewing of 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 examples of the objects investigated so far. Others include heart of chick embryo cells, monkey retina sections (in collaboration with J. W. McGowan, University of Western Ontario), mouse and guinea pig brain sections and tissue cultures of human central nervous system tumors (in collaboration with L. Manuelidis, Yale University). Many details of the structures in our micrographs have not been seen by any other method.

FIGURE CAPTIONS

Figure 1 Soft x-ray replica of a part of a chromosome from the salivary glands of Drosophila in x-ray resist (PMMA) Exposure about 10^3 joules/cm^3 (16 hrs) with carbon Kα radiation (λ=44.8Å). Developed in 1:1 mixture of methyl isobutylketone and isopropanol for 1 min. SEM micrograph with a conventional scanning electron microscope at 60° viewing angle.

Figure 2 Soft x-ray replica obtained from a thin plastic embedded section of frog retinal pigment epithelium. Exposure with synchrotron radiation from DESY with an effective wavelength region λ = 30 - 44Å. Exposure dose about 10^4 joules/cm^3, exposure time 15 min., distance between the sample and the point of emission 40m. Fig. 2a, taken in a commercial SEM shows elliptical protuberances which are representative of melanin granules from frog retina pigment epithelium. Fig. 2b. In this high resolution low-loss STM micrograph of a melanin granule from a frog retina pigment epithelium cell, ultrastructural details can be clearly seen which measure less than 100Å in size. The marker represents 1000Å in a) and b).

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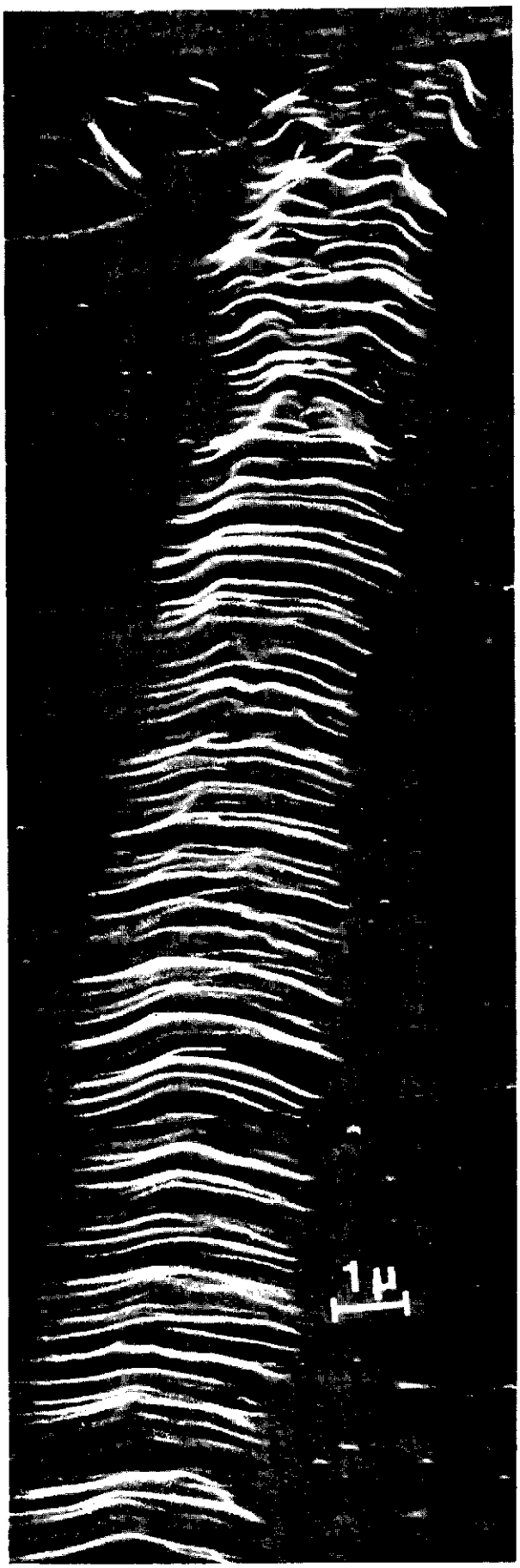
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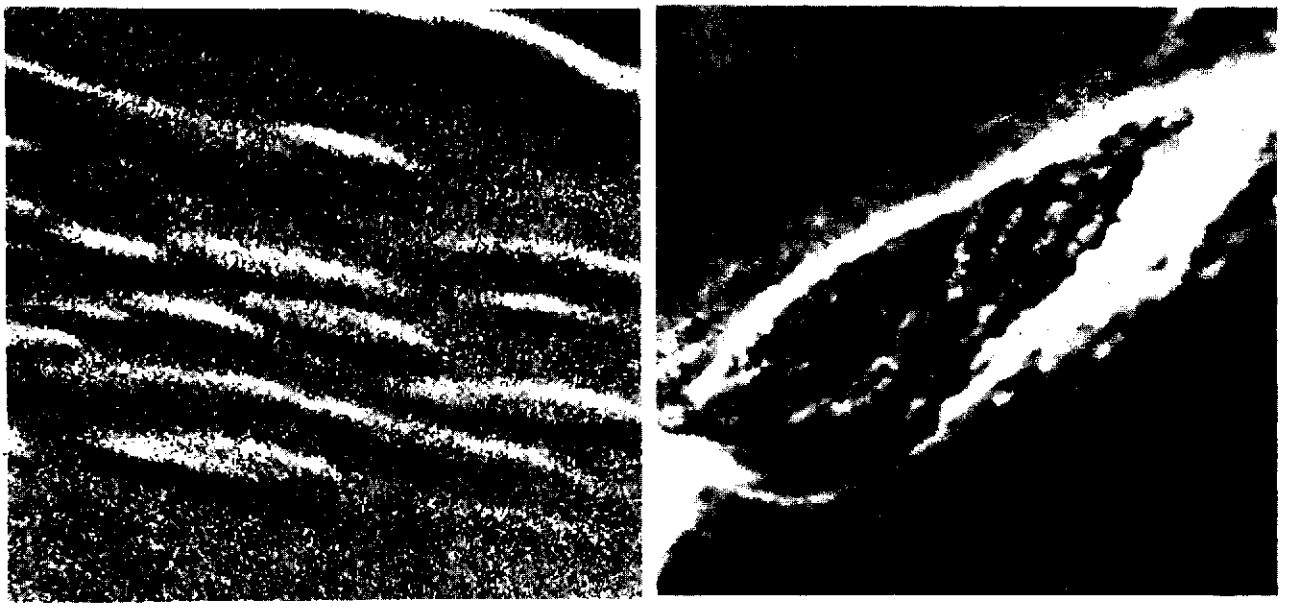

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