Into the X-Ray Microcosm

New techniques may reveal the most minute view yet of living tissues and create new micro-circuits etched in sharpest relief

BY JOHN H. DOUGLAS

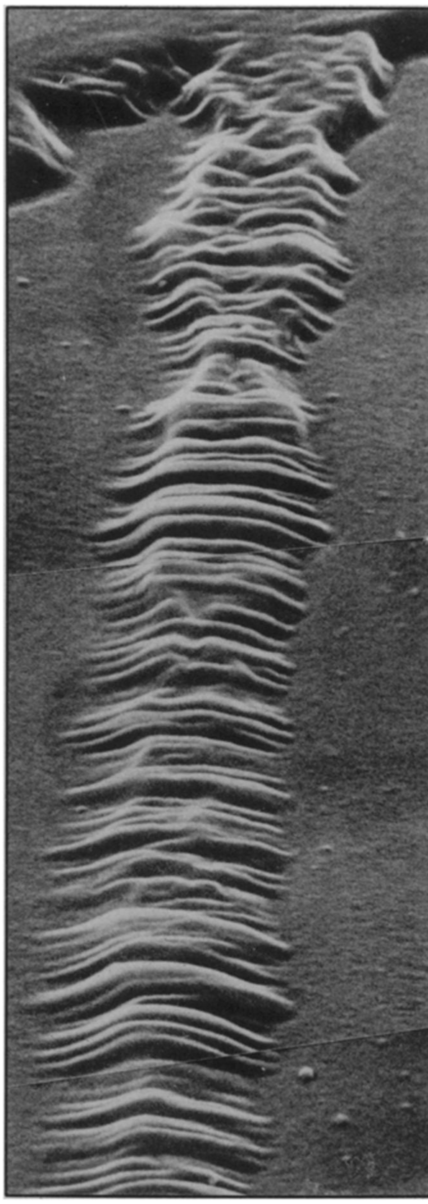
Like a range of thin mountains rising abruptly from a flat plain, the fine structure of a chromosome stands sharply revealed by a new technique for exploring ever smaller worlds—X-ray microscopy. Another technique based on the same principles—X-ray lithography—can sculpt a microscopic circuit pattern in high relief, it's elements crowded together like a miniature subdivision along a freeway. Both processes were pioneered by scientists at the Research Division of IBM, Yorktown Heights, N.Y.

The X-rays offer several advantages over electron beams now commonly used in advanced microscopic techniques. In particular, electrons destroy delicate biological specimens, which must therefore be considerably altered from their natural state before examination in an electron microscope. Elaborate sample preparation is required and observation is conducted in a high vacuum. With X-rays, however, living specimens can be examined in nearly natural conditions.

In the new technique, a specimen is placed on top of a thin plastic film, called a "resist," and exposed to an intense beam of X-rays. Some rays are absorbed, while others pass into the resist and alter its internal structure. Later, when the resist is placed in a solvent, those parts that received the most radiation are etched away the fastest. A physical profile thus emerges on the surface of the resist, whose features depend on the absorption characteristics of the original specimen. The resist is then photographed by a scanning electron microscope.

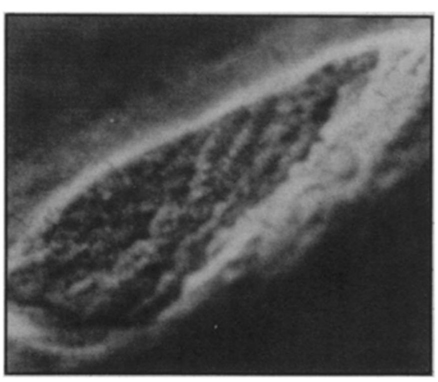
The result is an image roughly 25 times more detailed than that produced by the best light microscopes from biological samples and involves much more natural conditions than examination with an electron microscope of comparable resolution. To demonstrate this ability, Barbara Panessa and Jose A. Zadunaisky of New York University prepared a thin section of pigment cell from a frog retina for examination by the IBM team. The photograph obtained was compared to one from a conventional electron microscope. The researchers found that structures as small as 100 angstroms were clearly resolved and some unidentified objects only 50 angstroms across were visible.

One of the first uses of the new technique may be to help map sites of genes along chromosomes. John Sedat of Yale collaborated with the IBM team in examining a chromosome from the salivary gland of a fruit fly using X-rays. They detected structures as small as 250 angstroms wide. Such data will be compared with that gleaned from other experiments to learn more about how chromosomes are made up and how they function.

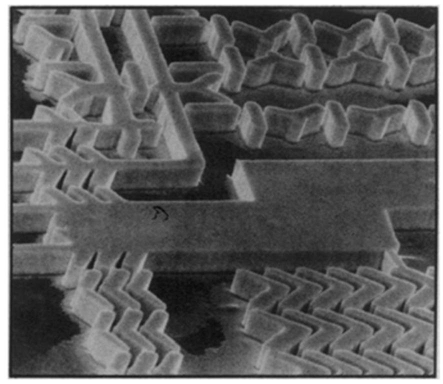


Photographs: IBM

Section of chromosome from fruit fly.



Slice of pigment cell from frog retina.



Magnetic bubble circuit etched by X-rays.

Practical uses are also being discovered for X-ray lithography. Here the advantage, compared to electrons, is that individual microscopic features can be much more sharply formed, and thus packed closer together. While a photograph of a tiny magnetic bubble circuit made by X-rays shows elements 3 microns high, 1 micron wide, with sharp edges, a circuit made by an electron beam would not have as high relief. Also, if an intense beam of X-ray is used, the circuit can be produced in one quick step, while lithography with electron beams requires a lengthy scanning process.

Obtaining a sufficiently intense X-ray beam, however, is no easy matter. Taking a microscopic picture with an ordinary X-ray source, for example, might require 10 hours. To obtain the micrograph of a frog retina, the IBM team had to use synchrotron radiation coming from the DESY machine in Hamburg, Germany. Wolfgang Gudat of the DESY facility cooperated in the experiment, which required an exposure of only one minute. Still shorter exposures—a few thousandths of a second—should be possible with specially constructed electron storage rings, and several proposals to build these are now pending before the National Science Foundation.

The new X-ray techniques were developed by IBM scientists Eberhard Spiller, Ralph Feder and John C. Topalian. The final high-resolution photographs were taken by Alec N. Broers in his specially built scanning electron microscope at the IBM lab. □