Use of KODAK TRI-X Pan Film and KODAK T-MAX 400 Professional Film in the Recording of Lattice Images of Labile Specimens with Electron Microscopy

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Introduction
While the resolving capability of the electron microscope has long been more than adequate to preserve detailed molecular structure for a great number of materials, the major limitation in achieving high resolution electron microscopy of beam sensitive materials has been the lack of a recording medium which will allow both high resolution and low dose. A typical beam-sensitive biological material is cellulose. Cellulose consists of 1,4-linked glucan polymer chains which are arranged in a crystalline state to form an insoluble submicroscopic rod-like entity known as the microfibril (Figure 1). In 1984, Sugiyama et al. first succeeded in recording the lattice image of cellulose microfibrils of Valonia macrophysa. Their success was based on the use of a 200 KV electron microscope and a high sensitivity electron microscopy film. It was subsequently shown by Reval (1985) that a conventional 120 KV accelerating voltage is adequate for recording lattice images of Valonia cellulose.

In the search for a recording medium with sufficient sensitivity and resolution to permit the imaging of the glucan chain lattice of cellulose, we decided to try electron microscopy with several standard panchromatic films. Because our Phillips 420 electron microscope was equipped with a 35 mm camera port, it was easy to test a number of standard films. To our surprise, we found that both KODAK TRI-X Pan Film and KODAK T-MAX 400 Professional Film provide superior recordings of the glucan chain lattice of cellulose from a variety of sources, in comparison with plate film ordinarily used for electron microscopy.

Applications
Cellulose microfibrils have been prepared from these representative sources: Ramie, typical of a vascular plant cellulose (Kuga and Brown, 1987a), Boergesenia, and Valonia (typical of giant marine algae), and cellulose microfibrils from the gram-negative bacterium Acetobacter xylinum (Kuga and Brown, 1987a; Kuga, 1987). Cellulose obtained from these sources was purified by successive treatments in hot alkali (0.5N NaOH) and acid (2N HCl) treatment. The microfibrils were then disintegrated into small fragments either by grinding them in liquid nitrogen, followed by a hot acid treatment, or by shaking in methanol containing acetyl chloride. The purified sample was washed thoroughly with water and mounted on a thin carbon film supported by a microneet. Bactracin (0.1%) was used as a wetting agent, and the grid was washed with distilled water after an initial drying in order to avoid any possible interference by Bactracin or other water-soluble crystalline materials.

For photographic recording, we found that the ordinary high speed panchromatic films KODAK TRI-X Pan Film or KODAK T-MAX 400 Professional Film worked very well. We used the 35 mm format of these films, and followed a standard development in KODAK MICRODOL-X Developer (undiluted, 8 minutes, 25°C). We also found that pushing with other developers did not improve the image quality because the gain in sensitivity was offset by an increase in grain size.

Figure 1. Disintegrated cellulose microfibrils of Valonia negatively stained to illustrate the rod-like structure of this crystalline polymer.

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Electron Microscopy Techniques

We used a conventional Philips 420 transmission electron microscope, with a standard hairpin filament. The microscope was stabilized at a 120 KV, and a very low level of electron illumination was selected. We used the smallest condenser aperture (15 μm) and a medium spot size. These conditions provided for a highly coherent electron beam required for lattice imaging, as well as a low level of illumination for the specimen search. We chose an objective aperture at least 50 μm in diameter and placed it concentrically with the optical axis, selecting an imaging condition which represents the 3-beam interference mode with axial illumination. The 50 μm objective aperture corresponds to a lattice spacing of 0.36 nm with the focal length of the objective lens being 2.7 nm. After introducing the objective and condenser apertures, the objective lens was precisely stigmatized by observing the granularity of a platinum-carbon matrix at high magnification (150,000X in 35 mm mode).

In the search mode, an area containing properly dispersed microfibrils could easily be observed using low magnification (5,600-9,200X in 35 mm mode) using a very low electron beam density (around 20-50 electrons/μm²/s). Using these conditions, unstained microfibrils have almost no contrast when they are in focus: however, they could be made more clearly visible by achieving phase contrast with an underfocus of 10-20 μm (Figure 2). Alternatively, we could achieve darkfield conditions using a tilted beam and observe the "glittering" of the crystal lattice by the Dragg reflections which indicates that these regions of the microfibril still contain a crystal lattice under the beam conditions used. Using a beam intensity of 20-50 electrons/μm²/s, we were able to observe microfibrils which remained undamaged for 10-30 seconds, making it possible to locate a suitable area in either brightfield or darkfield. Once the area was found, we used a low-dose unit, switching it to focusing mode before the specimen became severely damaged. Then the magnification was increased to an electron optical magnification of 15,000 to 41,000X. The "in focus" condition was obtained by observing the granularity of the micronef matrix or fresnel fringes at the edge of a micronef hole. The accuracy of such a focus in this condition was ±100 nm.

Recording of the Image

Using a magnification of 15,000-41,000X, it was possible to record the lattice images on 35 mm film. A typical electron beam exposure for recording was in the range of 80-200 electrons/μm²/s. These conditions gave sufficient contrast of the lattice fringes with KODAK TRI-X Pan Film or KODAK T-MAX 400 Professional Film, using an exposure of 1-5 seconds. In general, exposures were not made until at least one minute to allow for stabilization after the last operation of the translation mechanism, so that specimen drift was minimized.

Once the 35 mm film was processed, developed, and dried, a search for the lattice image of cellulose on the film was made using either an optical bench equipped with a laser beam, or a standard brightfield light microscope. If the lattice image is intercepted by the laser beam, a characteristic diffraction pattern for the periodic spacing is easily observed; however, it is difficult to locate on the negative precisely which microfibril is responsible for the diffraction pattern. Thus, the use of a light microscope proved to be a more practical method for locating the microfibrils with periodic structure.

Photomicrographs of the original negatives were on KODAK TRI-X Pan Film at a magnification of approximately 20X. The lattice images were printed at a final magnification of 1,000,000-3,000,000X. The calibrated magnification was determined by recording the lattice images of graphitized carbon (with a d-spacing = 0.34 nm).

Figure 2. This is an example of three microfibrils of Valonia suspended across the micronef of carbon. This electron micrograph made with Kodak T-Max 400 Professional Film (120 KV electron microscope, original magnification of 41,000X), is the primary negative which is examined with a light microscope to produce a secondary negative at a magnification of approximately 20X (as shown in Figure 1).
Lattice Images of Microfibrils

Figure 3 shows a typical lattice image of a microfibril from *Valonia*. The 0.53 nm lattice spacing is clearly observed. The *Valonia* microfibril has a maximum width of only 22 nm. This surface of the microfibril is parallel to the plane of the cell. Perpendicular to this surface is the 0.60 nm lattice plane which can occasionally be observed if the microfibril is sufficiently oriented on the grid.

Enhancement of the lattice image can be achieved by use of digital image processing. This involves digitization of the original photographic image, a medium high pass filtering operation, a Fourier transform, mask production, and reconstruction of the lattice image after masking. Such operations greatly enhance the periodic information within the beam labile specimen (Kuga and Brown, 1987b).

Significance of this Research

Using conventional films and methodology employed in transmission electron microscopy, it is now possible to record lattice images of beam-sensitive materials at a resolution of 0.34 nm. Beam-sensitive materials such as cellulose prove that this technique can be extended to other biologically labile macromolecules. Of particular interest would be a study of the surface topography of enzymes, glycoproteins, nucleic acids, microtubules, microfilaments, viruses, and structural proteins at 0.5 nm resolution or better. We are presently investigating the interaction of cellulases with cellulose microfibrils as a means of extending high resolution, low-dose microscopy to other systems. The usefulness of this technique extends beyond the field of biology. For instance, lattice imaging of beam-sensitive materials such as minerals, alloys, precipitates, solids, particulates, non-biological polymers, epitaxially grown crystals, electron beam lithographic masks, etc. may be possible with this technique. The use of unstained specimens and a practical high-sensitivity, high-resolution recording medium is of paramount importance for obtaining these high-resolution images. We do not yet understand why films designed for photon exposure are operating better than those designed for electron exposure; however, this technique should help extend the useful range of the electron microscope in the imaging of biological macromolecules.

The combined use of *Kodak* Tri-X Pan Film or *Kodak* T-Max 400 Professional Film in the 35 mm camera of our Philips 420 electron microscope was very efficient in recording lattice images of cellulose because of the ease in developing, low cost in comparison with plate films, rapid availability, greater sensitivity, and greater resolution.

Note: Most of the material for this article was taken from an original paper which recently appeared in the literature (Kuga, S. and R. M. Brown, Jr. 1987. Practical aspects of lattice imaging of cellulose. *Journal of Electron Microscopy Technique* 6: 349-356).

References


Kuga, S. and R. M. Brown, Jr. 1987a. Lattice imaging of Ramie cellulose. Accepted: *Polymer Communications.*
