Electron microscopy of intact tissues and cells
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There is an urgent need across biology and in medicine to obtain more detailed images of intact cells and tissues. An important goal is to reach ~2 nm resolution where protein molecule shapes can be recognised. Electron microscopy of thin sections cut from material embedded in plastic remains by far the most widely used method for such in situ studies. However, details smaller than ~ 50Å are generally not preserved by this method. This precludes easy integration of higher resolution structural information from component macromolecules and complexes into the cellular context, which is a major limitation to understanding cellular function. There have been few studies exploring how detail is lost during embedding and sectioning; however, it is often assumed that fixation and dehydration are the most damaging steps. Our collaborator, Michael Reedy, has monitored the preservation of insect flight muscle through fixation, dehydration and embedding by X-ray fibre diffraction. Remarkably, the best embedded blocks diffracted to 1.2 nm resolution (see Figure). However, Fourier transforms of sections cut from these blocks extended to only ~5 nm. This suggests that the cutting is the stage where much damage can occur. A major artefact of sectioning is compression in the direction of the cut, often by ~30%. Thus, if compression could be eliminated, preservation might be greatly improved. An oscillating knife ultramicrotome eliminates the compression, but the effect of this on resolution had not been measured. We cut sections, with and without, the knife oscillating, from the embedded insect flight muscle that give X-ray transforms extending to 1.2 nm. Sections cut with the knife oscillating did not show improved preservation over those cut without. Thus compression during cutting does not appear to be the major source of damage, which leaves unexplained the 50Å versus 13Å discrepancy between block and section preservation. The results nevertheless suggest that improvements in ultramicrotomy will be important for bringing thin-sectioning of plastic-embedded cells and tissues to the point where macromolecule shapes can be resolved.

Collaborator
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Publications

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