

Preface

Like most microscopists, I didn't fall in the mould as an undergraduate student. At the time I was certain I was destined to be a molecular biologist, never visualizing the actual samples I would be working on. I told myself that my research career would never involve any form of imaging, since I did not think it was interesting. A couple of years down the track I started my postgraduate studies and found myself, within 2 weeks of starting, in front of my first transmission electron microscope.

Almost immediately, I loved it, it must have been my inner artist shaping my future. However, I was frustrated by the fact that I was looking at a three-dimensional world in only two dimensions, so I set myself a challenge that I would one day progress to three-dimensional electron microscopy despite the fact that at that time it appeared to be nothing more than a good science fiction novel. Indeed, only a few specialized laboratories had the expertise to obtain three-dimensional datasets with an electron microscope. So instead, I spent the first decade of my research career honing my sectioning skills, as well as a few diamond knives, eventually progressing from single sections to a large number of serial sections. Since then a few years, along with a few laboratories and core facilities, have gone by, and the technologies permitting visualization of the "real" three-dimensional nanoworld have evolved and are now accessible to most researchers around the world.

Overall, it has been an interesting journey since the first book on electron tomography, edited by Joachim Frank, appeared in 1992. Even though no major new concepts have emerged in the last twenty years, the technology has evolved steadily. From stacks of thin serial sections to acquisition of tilt series in a transmission electron microscope, to even tilt series of serial sections, we have increased the resolution in the third dimension by an order of magnitude. While the microscopes have moved back from 400–1000 kV to 300 kV with the dissemination of field emission guns, the sample thickness of 300–500 nm has remained constant. Now it seems that the field is slowly moving towards scanning transmission electron tomography, which permits an increase in the thickness of the sample by at

least threefold to the micron range. Both techniques can be used on plastic sections or cryosections, but the latter avoids all the pitfalls and artefacts of chemical fixation. However, cryo-ultramicrotomy is not for the faint hearted and remains the “Holy Grail” for most of us. It also introduces a major artefact in the compression of sections and formation of crevasses. Yet new developments in the last 5 years will make this an artefact from the past with the preparation of focused ion beam cryo-lamellas instead of the cryosections. Though this is, for now, still only applied in a few laboratories that have the resources and more importantly the rare skill sets required. Dual beam microscopes have also seen an increased usage in biological sciences with the introduction of serial block face imaging where the microscope sequentially section and image the surface of the sample therefore creating a three-dimensional dataset at intermediate resolution between optical and transmission electron microscopy. The same techniques can be applied using a microtome inside the scanning electron microscope chamber creating virtual sections. Indeed the sections are actually accumulating on the surface of the knife and cannot be recovered.

So we have now moved full circle and are back to serial sections using the slightly less complicated tool that is the scanning electron microscope. Will this practice be the future of electron microscopy for the masses?

For now, all these techniques are complementary and one cannot replace fully the others. While plastic electron tomography is often seen as the poor parent of cryo-electron tomography, it is indisputable that the output in terms of number of samples per unit of time is far greater. In turn, cryo-ultramicrotomy is far faster than lamella preparation using focused ion beams, while block face imaging is for large samples at slightly lower resolution.

At the fringe of these three-dimensional techniques are applications that link them to the world of fluorescence microscopy and structural biology. On one side is array tomography, still using serial sections but with fluorescently labelled probes that let the scientist identify the otherwise grey blob. Or what used to be blobs, as subtomogram averaging is progressing fast, thanks to the direct electron detectors. This permits to do structural biology at a resolution not quite as high as single particle, but still below the 10 Å mark. The main advantage of this technique is to be able to place the structure in the context of the whole cell.

Furthermore, the acquisition of images is one thing, but the annotation and segmentation require some additional processing in order to be able to handle data with low signal-to-noise ratio (e.g. cryo-electron tomography).

Finally, the propagation of these techniques, their automation, and therefore the amount of data generated, have led to what are probably some of the biggest challenges the field has encountered and will have to face for the next few years: data storage and management. With these techniques going towards full automation some skills are likely to be lost, i.e. with the introduction of kits in molecular biology how many students still know what diatomaceous earth looks like and

what it is used for? Electron microscopy is one of the remaining technologies where skill is everything. Will we survive the new era of automation?

Putting together this book was an interesting and challenging adventure. I was lucky enough to find academic and technical experts willing to spend a large amount of time writing these chapters. For this and their dedication and patience, I would like to thank all of the authors.

Melbourne, Australia

Eric Hanssen

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