Letter

Bringing electron microscopy back into focus for cell biology

The use of electron microscopy (EM) in cell biology is declining precipitously despite the incredible ability of a wide array of EM techniques to provide information at the molecular, organellar and cellular levels*. The information made available by EM is usually not available by other approaches. Furthermore, the combination of cryo-based specimen preparation and stereology now makes it possible to carry out many precise quantitative and kinetic experiments using EM. I feel it is important to revisit some reasons why these techniques are still under appreciated.

Hans Geuze, a pioneer in the application of EM immunocytochemistry in membrane trafficking, recently wrote a short but influential essay entitled: 'Is there a future for electron microscopy in cell biology?'[1]. He summarized the enormous contributions the field of EM made to cell biology in the 1950s to 1970s – but pointed out that it has since suffered a slow but steady decline, in particular over the past decade. This is most dramatically illustrated by the continued closures of once-thriving EM facilities all over the globe, often because of an alarming lack of interest.

I predict that we will see a slow reversal of the decline of EM and that it will rise to its former levels of importance, if not popularity. Indeed, the cryo-EM field is already expanding rapidly and powerfully. This is because many high-resolution structural specialists have come to appreciate how beautifully the data obtained using cryo-EM can complement those from X-ray crystallography, with the two sets of information often overlapping perfectly to broaden the useful range of resolution. As a result, cryoelectron microscopists are now very much in demand.

*This letter is a modified version of a plenary lecture at the 12th European Congress of Electron Microscopy, Brno, Czech Republic, 9–14 July 2000 and is also published in the congress proceedings.

It seems no coincidence that the loss of interest in the use of EM for cell biology, starting in the early 1980s, began as new technology emerged for light microscopy (LM), especially video and high-resolution confocal microscopy. Now, staggering amounts of new LM-derived data appear almost daily. One cannot deny the aesthetic beauty of many of these images as they appear in striking multicolour. Furthermore, the methods are being constantly improved – witness the awe-inspiring potential of new technology such as fluorescence resonance energy transfer (FRET) to identify biochemical reactions in living cells (see Ref. 2, and admire the forthcoming 'GFP in Motion 2' CD-ROM compiled by Beat Ludin and Andrew Matus, 2001).

Although some of this LM technology is now being pushed to achieve resolution even below 100 nm (Ref. 3) and offers the possibility to identify molecules in living cells, it has limitations for interpretation that need to be taken more seriously by the cell-biology community. To localize a labelled molecule, one obviously needs not only to visualize the label directly but also to identify the reference space (to use a term widely in use in stereology – see below). By even routine EM approaches, membrane profiles, actin filaments and COP vesicles, for example, can be visualized directly (although a second marker antibody might be necessary for unequivocal identification). By LM, this is not possible in the context of a cell. A 5-nm gold particle that identifies the bound antibody is easily seen by EM and can be used to ask questions in which the reference space can be well defined. Pushing LM labelling beyond any sensible limits should be no substitute for the use of EM approaches, which, after all, were used to identify most of the cellular organelles in the first place. For more discussion of the potential misuse of LM in cell biology, see Ref. 4.

It is a huge misconception that EM can only provide a static picture of events. Most biochemical experiments that seek to provide kinetic data often compare, for example, a protein at a few different time points, as in a pulse–chase experiment, rather than follow the protein in real time. There is absolutely no reason not to use the same approach with EM. Indeed the classical autoradiographic experiments of Palade, Leblonde and many others used precisely this approach very successfully, and it has also been extensively used in combination with immunogold labelling of cells fixed at different times after initiating a condition[4]. It is such a pity that most cell biologists interested in topics such as membrane trafficking have no idea how powerful and precise these kinetic EM approaches can be, provided they follow the rules of systematic sampling (see, for example, Ref. 5).

It seems to me that, in cell biology of recent times, researchers have made a friendly agreement not to scrutinize each other's LM data too closely at the refereeing stage. If the author calls the 'perinuclear blob' a Golgi complex, let it be a Golgi complex, and so on. This problem is exacerbated when journals print immunofluorescence images to the size just at, or often below, that point at which the relevant information can be reasonably seen, while the postage-stamp-sized micrograph sits in a sea of empty space! The question of whether a signalling molecule, for example, localizes to the cytoplasmic surface of the membrane, in the actin cortex or on the membrane of an early endosome compartment just beneath the plasma membrane is obviously crucial to evaluating its function. But even the best confocals cannot attain this resolution, which is trivial to achieve at the EM level.

So this is a wake-up call to those cell biologists who need EM, even though they pretend otherwise. We are facing an information revolution from many angles; new LM technologies are flooding the market, with confocal microscopy justifiably 'on a roll'; but when will EM get a slice of this pie? The answer is simple – as soon as the majority of cell-biological laboratory chiefs realize how simple most EM techniques are in their application. They must also appreciate, however, that trained electron microscopists need to be an essential part of the team in order to obtain the best data and make the correct interpretations. Although, at

http://tcb.trends.com 0962-8924/01/$ – see front matter PII: S0962-8924(01)01949-3

first glance, a thin section through a cell looks simple in the EM, within minutes of examining it carefully, beginners will face bewilderment. They might occasionally see a structure recognizable from a textbook, but most of what they see is garbled because the interpretation of two-dimensional projections of cells is complex. Thus, although the world infrastructure for EM in cell biology has been declining, I feel that the services of well-qualified electron microscopists will remain very much in demand.

It is frustrating to be aware of the power of modern stereology to provide efficient and unbiased approaches for interpreting two-dimensional data but to see these methods only rarely applied in cell biology. Stereology is a mathematically based approach for quantifying structural parameters such as surface areas, volume, length and the number of objects from two-dimensional sections[6–9]. Even many EM specialists are not familiar with the full power of these methods, and, in the cell-biology community, it is especially under appreciated. And yet stereology, using state-of-the-art systematic sampling approaches, has undergone a major revolution, especially since the early 1980s, that has made available an array of approaches for quantifying structural information. These can be applied throughout the biological sciences. In a only few disciplines, such as pathology and brain research, has the use of stereology become a routine procedure.

The development of EM has led to the introduction of colloidal gold and a reliable method to make a precise size of gold particle[10]. Two important approaches were developed to prepare sections that could be successfully immunolabelled using gold. The first is the cryosection method pioneered by Tokuyasu, and the second (by Kellenberger, Carlemalm and colleagues, see Ref. 9) relied on the development of new acrylic resins that could be infused into the sample and embedded at low temperatures. Specimen preparations for EM revolutionized the development of cryo-EM and the ideas that are associated with this new approach[11]. The realization that it was possible to arrest living processes in a cold vitreous state by means of cryo-EM also led to pioneering technology for high-resolution scanning EM as well as to high-pressure freezing approaches (especially by Martin Müller, Heinrich Hohenberg, Andreas Staehelin and colleagues) that can be used to prepare vitrified, large bulk specimens for evaluation by transmission EM or scanning EM. The potential of these approaches is often significant, but again they are poorly appreciated. First-rate contributions by specialists such as Heuser, Orci, Mueller, Plattner, K. Fujimoto and many others have shown how freeze–fracture and shadowing approaches can also provide spectacular details of organelles. (This is why so many endocytosis specialists start their talks with a famous 'Heuser-o-gram'.) Finally, watch out for new developments in localizing lipids accurately by EM. Recent work by Slot, Geuze and collaborators, and others, is opening up the possibility to faithfully follow previously inaccessible lipids such as cholesterol.

At present, only a few specialist laboratories have the infrastructure and personnel required to perform many of the above-mentioned techniques. Moreover, the future of some of them is in doubt, mostly through lack of interest, or appreciation, in the enormous potential of the technologies. And, as Geuze pointed out[1], many specialists (like himself) are nearing retirement age, and there is a serious danger that a lot of experience and technical expertise is not being passed on, as it should.

So it must be appreciated that the EM world indeed offers a large number of interesting options for cell biologists. The specimen can be (almost) perfectly prepared, and sections, or whole mounts, can be labelled with antibodies and visualized at very high resolution (at best, overlapping with the lower limits for useful cryo-EM). Using these sections, the whole arsenal of modern stereology is available to provide a vast array of quantitative information about the cell or tissue of interest as well as the distribution of defined antigens. With both sections and isolated particles, the recent development of electron tomography must also be considered as a significant breakthrough that will open up many hitherto poorly understood structures[12,13]. Organizations such as EMBO, FEBS and the Royal Society offer practical courses covering many of these methods, and visits to the specialist labs are to be encouraged.

To answer the question posed by Hans Geuze[1], I personally believe that there is a big future for EM in cell biology. The information obtained from EM methods provides a foundation that opens the door for many other approaches to be subsequently applied that, in turn, open new questions for EM. The most pressing issues facing electron microscopists are how not to lose existing technology and expertise, to build up infrastructure and to spread the gospel – as soon as possible.

Gareth Griffiths
EMBL, Postfach 10.2209, 69012 Heidelberg, Germany.
e-mail: Gareth.Griffiths@EMBL-Heidelberg.de

References

1 Geuze, H.J. (1999) A future for electron microscopy in cell biology? *Trends Cell Biol.* 9, 92–93
2 Bastiaens, P.H.I. and Squire, A. (1999) Fluorescence lifetime imaging microscopy: spatial resolution of biochemical processes in the cell. *Trends Cell Biol.* 9, 48–52
3 Klar, T.A. *et al.* (2000) Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission. *Proc. Natl. Acad. Sci. U. S. A.* 97, 8206–8210
4 Griffiths, G. *et al.* (1993) The immunofluorescent era of membrane traffic. *Trends Cell Biol.* 3, 214–219
5 Jahraus, A. *et al.* (1998) *In vitro* fusion of phagosomes with different endocytic organelles from J774 macrophages. *J. Biol. Chem.* 46, 30379–30390
6 Gundersen, H.J.G. *et al.* (1988) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS* 96, 379–394
7 Gundersen, H.J.G. *et al.* (1988) The new stereological tools, disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* 96, 857–881
8 Mayhew, T.M. (1991) The new stereological methods for interpreting functional morphology from slices of cells and organs. *Exp. Physiol.* 76, 639–665
9 Griffiths, G. (1993) *Fine Structure Immunocytochemistry*, Springer-Verlag
10 Slot, J.W. and Geuze, H.J. (1985) A new method for preparing gold probes for multiple labelling immunocytochemistry. *Eur. J. Cell Biol.* 38, 87–93
11 Dubochet, J. *et al.* (1988) Cryo-electron microscopy of vitrified specimens. *Q. Rev. Biophys.* 21, 129–228
12 Baumeister, W. *et al.* (1997) Electron tomography of molecules and cells. *Trends Cell Biol.* 6, 81–85
13 Koster, A.J. *et al.* (1997) Perspectives of molecular and cellular electron tomography. *J. Struct. Biol.* 120, 276–308