Published in final edited form as:

Curr Opin Microbiol. 2005 June ; 8(3): 316–322.

Bridging the imaging gap: visualizing subcellular architecture with electron tomography

Sriram Subramaniam

Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health (NIH) Bethesda, MD 20892, USA

Abstract

Transmission electron microscopy is a powerful tool that is used to explore the internal structure of tissues, cells, organelles and macromolecular complexes. By integrating data from a series of images in which the orientation of the specimen is progressively varied relative to the incident electron beam it is also possible to extend electron microscopic imaging into the third dimension. This approach, commonly referred to as electron tomography, has been greatly aided in recent years by advances in technology for imaging specimens at cryogenic temperatures, as well as by substantial progress in procedures for automated data collection and image processing. The intense pace of developments in this field is inspired, in a large part, by the hope that the quality of the data will ultimately be good enough to allow interpretation of tomograms of cells, organelles, bacteria and viruses in terms of the three-dimensional spatial arrangements of the constituent molecules.

Introduction

To appreciate the growing interest in the use of three-dimensional (3D) electron microscopy for subcellular imaging, it is useful to begin by reviewing the scope of other imaging methods that are commonly used in biology. As schematically illustrated in Figure 1, X-ray and nuclear magnetic resonance (NMR)-based imaging technologies have had outstanding success in imaging at both ends of the biological size spectrum, which spans a ‘molecular weight’ range of over 27 orders of magnitude. Thus, technologies such X-ray crystallography and NMR spectroscopy have the capability of delivering structures at atomic resolution whereas, similar principles, when applied to computerized tomography and magnetic resonance imaging provide valuable information at resolutions in the millimeter range. By contrast, the vast majority of our knowledge of biological structures that are of intermediate size has come from light microscopic and conventional electron microscopic methods, which are useful in biological imaging over a size range spanning nearly 20 orders of magnitude. Although the entire range of interest in biological imaging is almost completely spanned by these four methods, there is still a small, but extremely important, gap in imaging. This relatively narrow gap, which covers the overall molecular mass size range of $\sim 10^6$ – 10^{10} Daltons (Figure 1), is of great interest to the field of cell biology because this is the size range of cellular entities (e.g. large and dynamic multi-protein complexes) and small subcellular organelles (e.g. mitochondria) as well as of a large spectrum of whole organisms (e.g. enveloped viruses and bacteria). In general, objects of interest within this size range are either too large or too heterogeneous to be accessible for analysis by X-ray crystallographic methods. However, structural information about these macromolecular assemblies at the molecular and the near-atomic level is essential for understanding and interpreting their function at the cellular level.

Emerging methods in cryo-electron microscopy have the potential to be particularly valuable in covering this imaging gap. For radiation-resistant specimens, such as metals, resolutions greater than 1 Å have already been achieved by direct imaging with electrons [1]; however, the best images achieved to date with biological specimens are at a much lower resolution because radiation damage limits the useful information that can be obtained [2]. Nevertheless, by the use of effective averaging techniques, a variety of 3D structures have been determined at resolutions in the range of ~3–30 Å, which allows interpretation of complex structures in terms of their underlying tertiary, secondary and primary structural elements [3–8]. The great challenge for electron-based imaging methods, however, is the determination of the molecular architectures of complex biological assemblies, such as whole cells and organelles that do not lend themselves to averaging. The best approach that is currently available for 3D imaging of such assemblies is to record a series of images of the object of interest over a range of orientations and to computationally combine the information to reconstruct the 3D architecture of the object [9,10]. An ultimate, long-term, goal of such electron tomographic analyses is to be able to routinely interpret the spatial arrangement of the constituent proteins, solutes and ions of subcellular structures at the highest possible resolution. This is a field in which one can safely predict that the most exciting advances are yet to come, and in this review I highlight recent progress in selected areas.

Tomography of stained specimens

One way to mitigate the effects of specimen damage caused by electron irradiation is to infiltrate biological specimens with salts that contain heavy metals, such as uranyl acetate and osmium tetroxide, to make a 3D cast of the specimen that is preserved by embedment in a plastic resin. The 3D reconstruction of the envelope of the stained specimen that is obtained by this approach is valuable, despite the fact that it is a relatively coarse approximation of the native structure. Typically, one can easily identify simple structural features, such as membrane boundaries, or protein complexes, such as ribosomes, which can be differentially stained relative to their immediate cellular neighbors. Examples in which such approaches have been successfully used include studies in many laboratories of the architecture and the connectivity of internal membranes such as the Golgi apparatus or multivesicular bodies [11•,12–15]. Such work on the Golgi has led to a stimulating debate as to whether the Golgi should be considered to be a stack of separated membranes with small vesicles that mediate traffic across the Golgi [16] or whether the membranes of the various subcompartments are, in fact, physically connected [11•,17•]. Evidence has been presented in favor of both theories, but it seems probable that both mechanisms, and possibly other as yet undiscovered modes, are operative in cells. Furthermore, there is little doubt that the organization of internal cellular membranes is highly dynamic and that the arrangement and the connectivity of different intracellular membranes will vary significantly between different cell types and even when the same cell is examined during different states of differentiation or activation [11•].

There is extensive literature on the nuances of specimen preparation that can be used for this type of tomographic imaging at room temperature. Some favor the use of aldehyde reagents followed by treatment with osmium tetroxide, which fixes and preserves the specimen in a specific 3D conformation by chemical cross-linking. Because of the potential formation of artifacts [18] in the few minutes it takes for the fixative to take effect, other methods favor the use of high-pressure freezing followed by the gradual exchange of the aqueous solvent with organic solvents that contain dissolved osmium salts at low temperatures [19]. Yet another variation of this method includes chemical fixation followed by rapid freezing and subsequent immunolabeling. Following this, the samples are stained with uranyl acetate [20]. Specimens that are prepared by the procedures described above are usually imaged at room temperature at relatively high electron doses (total doses of ~1000 electrons/Å² or more) to collect data for the generation of a tomographic reconstruction. These harsher specimen preparation and

imaging conditions are not a serious concern as specimens prepared in this way are used primarily to yield information on the membrane connectivity of cells and organelles or for recognition of the approximate cellular location of large and easily-stained objects. In principle, immunolabels that are attached to electron-dense particles such as gold can further complement this approach to provide information about the spatial localization of small proteins [21]. However, this strategy has limited utility for the spatial mapping of proteins in cells, mainly because it is very difficult to obtain quantitative immunolabeling, even under the most optimal conditions. An alternative strategy for the 3D mapping of individual proteins utilizes genetically attached tags that serve the dual purpose of being detectable by fluorescence imaging as well as by electron microscopy as they provide a site for the collection of electron-dense deposits [22]. Although this method also does not guarantee quantitative labeling, the potential of correlative imaging is a powerful feature.

Proteins or protein complexes that are sufficiently electron dense to be detectable in tomograms on the basis of their own intrinsic properties present an especially interesting opportunity for spatial localization studies. The oligomeric protein ferritin is a good example because there is a cluster of ~3000 iron atoms at the core of each molecule of ferritin. Zhang *et al.* [23] have recently taken advantage of this feature of ferritin to determine its spatial localization in axons obtained from the brains of mice that have knockouts of the genes for iron regulatory proteins IRP1 and IRP2 (Figure 2). Previous optical microscopic studies [24] had suggested that there is substantial accumulation of ferritin inside the axonal regions of these mice (Figure 2 inset). However, 3D mapping of the distribution of ferritin within the presumptive axonal space showed that ferritin is only detected in the invaginated regions in which the neighboring oligodendrocyte cells had penetrated the axonal interior, but could not be found in the axonal milieu itself. This study presents an example in which the additional resolution afforded by electron tomography prevented a potentially erroneous conclusion that could have been drawn from studies at optical resolution alone.

Cryo-electron tomography

The common feature of methods that utilize tomography at room temperature and at high electron doses is that the 3D images that are obtained primarily represent the structure that arises from differential partitioning of the stain. To obtain structural information about protein complexes under conditions that are directly relevant to the native state of the cell, it is necessary to record images at very low electron doses (typically <20 electrons/Å² for the entire tilt series) in unstained biological specimens that are rapidly vitrified at liquid nitrogen temperatures. These cryo-electron tomographic approaches are technically more challenging than the studies at room temperature, but have become possible owing to the major advances that have been made in computerized data collection [3].

Over the past two years, efforts to generate tomographic reconstructions of a variety of viruses, prokaryotic and eukaryotic cells have been reported [25,26•,27,28]. For example, tomographic reconstructions on the edge of plunge-frozen *Dictyostelium* cells deposited on copper grids allowed Medalia *et al.* [25] to visualize the network of actin filaments and to identify large molecular complexes that are present in the cytoplasm. Similar studies on isolated *Dictyostelium* nuclei allowed visualization of aspects of the 3D architecture of nuclear pore complexes that are present at the nuclear membrane [29]. Despite these exciting advances, a fundamental limitation to studies of whole-cell specimens is that useful images can only be recorded from the thinnest regions of the cell (typically <1 μm) or from partially lysed cells in which some or all of the cytoplasm has leaked out. In particular, low levels of lysis of the internal content might not be easily detected, but this will need to be taken into consideration when making deductions (based on tomography) of the extent of crowding [30] or of other aspects related to the spatial architecture of the cytoplasm.

Vitreous sectioning

A logical approach to the challenge of imaging interior regions of cells and tissues is to generate thin vitreous sections from cells that have been frozen rapidly enough to minimize damage caused by the formation of ice crystals (Figure 3a). The generation of thin sections at temperatures low enough to minimize perturbation of the vitrified state of cells and tissues was first demonstrated over three decades ago by Christensen [31] and was subsequently pioneered for cell biological applications by Dubochet and co-workers over the past two decades [32, 33•]. The past two years have seen especially rapid progress in vitreous sectioning of a variety of specimens such as bacteria [34], yeast [33•] and skin cells [35]. Although the reason that these specimen varieties have been so successful is not yet completely clear, it is conceivable that it correlates with the lower free-water content in bacterial and skin cells in comparison with the majority of mammalian cell-types. To date, the best spatial resolutions have been achieved in bacterial cells (Figures 3b,c); these images can be good enough to visualise the two leaflets of a single lipid bilayer [36].

De-noising and segmentation

As techniques for data collection and tomogram reconstruction are becoming more streamlined, the development of methods to analyze the enormous amounts of information in these tomograms remains a major challenge. Electron tomograms are intrinsically noisy; this problem is especially acute for data recorded from radiation-sensitive specimens at low electron doses. Efforts to establish tools for quantitative interpretation of tomograms are in an early stage of development [37], but are beginning to be applied to a range of biological problems, as reviewed recently by Frangakis and Forster [38]. The general strategy for information extraction is to improve the signal as much as possible relative to the noise; it is clear that the development of automated strategies for segmenting and mining specific features in tomograms, such as filamentous structures [39] or membranes boundaries, will be an especially important area of focus for the future. It is reasonable to expect that these advances will also drive the development of objective criteria to describe the information content in tomograms in quantitative terms [40].

Alternative methods for three-dimensional reconstruction

As discussed in previous sections, electron tomography provides a practical approach to understand the internal organization of cells and organelles. By contrast, scanning electron microscopic techniques have historically provided complementary information about surface structures. Two novel approaches in which a scanning electron microscope can be adapted to investigate the 3D architecture of cells have recently been reported, which are of interest in this context. In one approach, the biological specimen is stained and embedded in a plastic block and is then sectioned in a microtome that is located inside the imaging chamber of a scanning electron microscope [41•]. A scanning electron beam records the image of the block after each section has been removed, which is then used to generate a series of images of the specimen at intervals of ~70 nm. In the second approach, biological specimens are sectioned electronically using a focused ion beam inside the chamber of a dual-beam microscope. A scanning electron beam then records images from the sectioned specimen [42]. This process can be iterated to sample the 3D structure at intervals of ~100 nm, as illustrated in Figure 4. Two unique features of the dual-beam imaging approach are that it can be applied with ease to both plastic-embedded and vitrified specimens and that the focused ion beam can be used to carve out sections of a precisely controlled thickness for subsequent imaging by electron tomography. Nevertheless, both methods have the potential to be automated to generate images of large cellular volumes and are likely to be used more widely for imaging large cells and tissues in the future.

Conclusions

Electron tomography is rapidly becoming an indispensable tool in the arsenal of structural and cell biologists to bridge the information gap between X-ray crystallographic and optical microscopic methods to describe cellular structure. Impressive advances in technology are being made, especially in the areas of technological innovation and automation in data collection and processing, and it might not be long before the level of automation catches up with the levels already attained in more mature technologies such as X-ray crystallography, NMR spectroscopy and confocal microscopy. There can be little doubt that in this era of integrative biology, 3D electron microscopy can, and will, make a vital contribution to the understanding of cellular function.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. Batson PE, Dellby N, Krivanek OL. Sub-angstrom resolution using aberration corrected electron optics. *Nature* 2002;418:617–620. [PubMed: 12167855]
2. Henderson R. Image contrast in high-resolution electron microscopy of biological macromolecules: TMV in ice. *Ultramicroscopy* 1992;46:1–18. [PubMed: 1481269]
3. Baumeister W. From proteomic inventory to architecture. *FEBS Lett* 2005;579:933–937. [PubMed: 15680977]
4. Subramaniam S, Milne JL. Three-dimensional electron microscopy at molecular resolution. *Annu Rev Biophys Biomol Struct* 2004;33:141–155. [PubMed: 15139808]
5. Orlova EV, Saibil HR. Structure determination of macromolecular assemblies by single-particle analysis of cryo-electron micrographs. *Curr Opin Struct Biol* 2004;14:584–590. [PubMed: 15465319]
6. Rossmann MG, Morais MC, Leiman PG, Zhang W. Combining X-ray crystallography and electron microscopy. *Structure (Camb)* 2005;13:355–362. [PubMed: 15766536]
7. Ludtke SJ, Chen DH, Song JL, Chuang DT, Chiu W. Seeing GroEL at 6 Å resolution by single particle electron cryomicroscopy. *Structure (Camb)* 2004;12:1129–1136. [PubMed: 15242589]
8. Borgnia MJ, Shi D, Zhang P, Milne JL. Visualization of α -helical features in a density map constructed using 9 molecular images of the 1.8 MDa icosahedral core of pyruvate dehydrogenase. *J Struct Biol* 2004;147:136–145. [PubMed: 15193642]
9. Hart RG. Electron microscopy of unstained biological material: the polytropic montage. *Science* 1968;159:1464–1467. [PubMed: 4183952]
10. De Rosier DJ, Klug A. Reconstruction of three-dimensional structures from electron micrographs. *Nature* 1968;217:130–134.
- 11•. Marsh BJ, Volkmann N, McIntosh JR, Howell KE. Direct continuities between cisternae at different levels of the Golgi complex in glucose-stimulated mouse islet beta cells. *Proc Natl Acad Sci USA* 2004;101:5565–5570. [PubMed: 15064406] The authors use dual-axis electron tomography to examine the architecture of the Golgi apparatus in plastic-embedded sections that are obtained from freeze-substituted murine cells. They present evidence for the presence of multiple types of direct connections between Golgi cisternae and suggest these connections could provide a continuous lumen to facilitate the rapid transit of newly synthesized secretory proteins (see also [17•]).
12. Murk JL, Lebbink MN, Humbel BM, Geerts WJ, Griffith JM, Langenberg DM, Verreck FA, Verkleij AJ, Koster AJ, Geuze HJ, et al. 3-D Structure of multilaminar lysosomes in antigen presenting cells reveals trapping of MHC II on the internal membranes. *Traffic* 2004;5:936–945. [PubMed: 15522096]

13. Murk JL, Humbel BM, Ziese U, Griffith JM, Posthuma G, Slot JW, Koster AJ, Verkleij AJ, Geuze HJ, Kleijmeer MJ. Endosomal compartmentalization in three dimensions: implications for membrane fusion. *Proc Natl Acad Sci USA* 2003;100:13332–13337. [PubMed: 14597718]
14. Mironov AA, Mironov AA Jr, Bezoussenko GV, Trucco A, Lupetti P, Smith JD, Geerts WJ, Koster AJ, Burger KN, Martone ME, et al. ER-to-Golgi carriers arise through direct en bloc protrusion and multistage maturation of specialized ER exit domains. *Dev Cell* 2003;5:583–594. [PubMed: 14536060]
15. Segui-Simarro JM, Austin JR II, White EA, Staehelin LA. Electron tomographic analysis of somatic cell plate formation in meristematic cells of *Arabidopsis* preserved by high-pressure freezing. *Plant Cell* 2004;16:836–856. [PubMed: 15020749]
16. Marsh BJ, Mastronarde DN, Buttle KF, Howell KE, McIntosh JR. Organellar relationships in the Golgi region of the pancreatic beta cell line, HIT-T15, visualized by high resolution electron tomography. *Proc Natl Acad Sci USA* 2001;98:2399–2406. [PubMed: 11226251]
17. Trucco A, Polishchuk RS, Martella O, Di Pentima A, Fusella A, Di Giandomenico D, San Pietro E, Bezoussenko GV, Polishchuk EV, Baldassarre M, et al. Secretory traffic triggers the formation of tubular continuities across Golgi subcompartments. *Nat Cell Biol* 2004;6:1071–1081. [PubMed: 15502824] Using tomographic approaches, as in [11], the authors study the architecture of the secretory pathway, and provide evidence for the presence of intercisternal tubules under selected conditions. Their results especially highlight the dynamic nature of the connections between compartments in the Golgi apparatus.
18. Murk JL, Posthuma G, Koster AJ, Geuze HJ, Verkleij AJ, Kleijmeer MJ, Humbel BM. Influence of aldehyde fixation on the morphology of endosomes and lysosomes: quantitative analysis and electron tomography. *J Microsc* 2003;212:81–90. [PubMed: 14516365]
19. McDonald K, Morphew MK. Improved preservation of ultrastructure in difficult-to-fix organisms by high pressure freezing and freeze substitution: I. *Drosophila melanogaster* and *Strongylocentrotus purpuratus* embryos. *Microsc Res Tech* 1993;24:465–473. [PubMed: 8490232]
20. Peters PJ, Hunziker W. Subcellular localization of Rab17 by cryo-immunogold electron microscopy in epithelial cells grown on polycarbonate filters. *Methods Enzymol* 2001;329:210–225. [PubMed: 11210537]
21. Lefman J, Zhang P, Hirai T, Weis RM, Juliani J, Bliss D, Kessel M, Bos E, Peters PJ, Subramaniam S. Three-dimensional electron microscopic imaging of membrane invaginations in *Escherichia coli* overproducing the chemotaxis receptor Tsr. *J Bacteriol* 2004;186:5052–5061. [PubMed: 15262942]
22. Gaietta G, Deerinck TJ, Adams SR, Bouwer J, Tour O, Laird DW, Sosinsky GE, Tsien RY, Ellisman MH. Multicolor and electron microscopic imaging of connexin trafficking. *Science* 2002;296:503–507. [PubMed: 11964472]
23. Zhang P, Land W, Lee S, Juliani J, Lefman J, Smith S, Germain D, Kessel M, Leapman R, Rouault TA, et al. Electron tomography of degenerating neurons in mice with abnormal regulation of iron metabolism. *J Struct Biol* 2005;150in press
24. LaVaute T, Smith S, Cooperman S, Iwai K, Land W, Meyron-Holtz E, Drake SK, Miller G, Abu-Asab M, Tsokos M, et al. Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. *Nat Genet* 2001;27:209–214. [PubMed: 11175792]
25. Medalia O, Weber I, Frangakis AS, Nicastro D, Gerisch G, Baumeister W. Macromolecular architecture in eukaryotic cells visualized by cryoelectron tomography. *Science* 2002;298:1209–1213. [PubMed: 12424373]
26. Forster F, Medalia O, Zauberman N, Baumeister W, Fass D. Retrovirus envelope protein complex structure in situ studied by cryo-electron tomography. *Proc Natl Acad Sci USA* 2005;102:4729–4734. [PubMed: 15774580] This paper demonstrates that 3D images of Moloney murine leukemia retrovirus particles can be obtained at a resolution high enough to identify the individual glycoprotein trimers that protrude from the surface of the virus. The authors show that the coherent averaging of a number of individual trimers computationally extracted from the tomogram results in a density map in which individual domains of the complex can be resolved.

27. Kurner J, Medalia O, Linaroudis AA, Baumeister W. New insights into the structural organization of eukaryotic and prokaryotic cytoskeletons using cryo-electron tomography. *Exp Cell Res* 2004;301:38–42. [PubMed: 15501443]
28. Grunewald K, Desai P, Winkler DC, Heymann JB, Belnap DM, Baumeister W, Steven AC. Three-dimensional structure of herpes simplex virus from cryo-electron tomography. *Science* 2003;302:1396–1398. [PubMed: 14631040]
29. Beck M, Forster F, Ecke M, Plitzko JM, Melchior F, Gerisch G, Baumeister W, Medalia O. Nuclear pore complex structure and dynamics revealed by cryoelectron tomography. *Science* 2004;306:1387–1390. [PubMed: 15514115]
30. Grunewald K, Medalia O, Gross A, Steven AC, Baumeister W. Prospects of electron cryotomography to visualize macromolecular complexes inside cellular compartments: implications of crowding. *Biophys Chem* 2003;100:577–591. [PubMed: 12646392]
31. Christensen AK. Frozen thin sections of fresh tissue for electron microscopy, with a description of pancreas and liver. *J Cell Biol* 1971;51:772–804. [PubMed: 4942776]
32. McDowall AW, Chang JJ, Freeman R, Lepault J, Walter CA, Dubochet J. Electron microscopy of frozen hydrated sections of vitreous ice and vitrified biological samples. *J Microsc* 1983;131:1–9. [PubMed: 6350598]
33. Al-Amoudi A, Studer D, Dubochet J. Cutting artefacts and cutting process in vitreous sections for cryo-electron microscopy. *J Struct Biol* 2005;150:109–121. [PubMed: 15797735] A thorough and detailed study that describes many of the challenges that are currently faced in the generation of vitreous sections of cells and tissues. Obtaining sections with minimal defects is an important step towards the goal of visualizing the interior of cells in molecular detail.
34. Matias VR, Al-Amoudi A, Dubochet J, Beveridge TJ. Cryo-transmission electron microscopy of frozen-hydrated sections of *Escherichia coli* and *Pseudomonas aeruginosa*. *J Bacteriol* 2003;185:6112–6118. [PubMed: 14526023]
35. Al-Amoudi A, Norlen LP, Dubochet J. Cryo-electron microscopy of vitreous sections of native biological cells and tissues. *J Struct Biol* 2004;148:131–135. [PubMed: 15363793]
36. Zhang P, Bos E, Heymann J, Gnaegi H, Kessel M, Peters PJ, Subramaniam S. Direct visualization of receptor arrays in frozen-hydrated sections and plunge-frozen specimens of *E. coli* engineered to overproduce the chemotaxis receptor Tsr. *J Microsc* 2004;216:76–83. [PubMed: 15369487]
37. Volkman N. A novel three-dimensional variant of the watershed transform for segmentation of electron density maps. *J Struct Biol* 2002;138:123–129. [PubMed: 12160708]
38. Frangakis AS, Forster F. Computational exploration of structural information from cryo-electron tomograms. *Curr Opin Struct Biol* 2004;14:325–331. [PubMed: 15193312]
39. Fernandez JJ, Li S. An improved algorithm for anisotropic nonlinear diffusion for denoising cryo-tomograms. *J Struct Biol* 2003;144:152–161. [PubMed: 14643218]
40. Penczek PA. Three-dimensional spectral signal-to-noise ratio for a class of reconstruction algorithms. *J Struct Biol* 2002;138:34–46. [PubMed: 12160699]
41. Denk W, Horstmann H. Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. *PLoS Biol* 2004;2:e329. [PubMed: 15514700] A strategy is presented for the reconstruction of large volumes of tissue by automated imaging of the face of a plastic-embedded tissue block that is being sectioned with an ultra-microtome situated within the chamber of a scanning electron microscope. It is shown that images from the block can be obtained at intervals of 50–70 nm, which suggests that it should be possible to reconstruct volumes that span several tens of microns.
42. Gestmann I, Hayles M, Shi D, Kumar G, Giannuzzi L, Lich B, Subramaniam S. Site-specific 3D imaging of cells and tissues using dualbeam technology. *Microsc and Microanal* 2004;10(Suppl 2): 1124–1125.

Figure 1.

The range of applications of key technologies that are used for imaging objects, from molecules to humans. The figure shows a schematic comparison on a single size scale of the relative sizes of various objects that are of interest in biology. At one end of the spectrum are small entities such as lipids, proteins and whole viruses, which are generally described in terms of their molecular weight. For larger entities, such as whole cells and organelles, it is conventional to describe their size in terms of linear dimensions such as length and width, which are typically expressed in micron units. For very large entities, such as whole organisms, sizes are frequently measured by the weight of a single item and are expressed in units of mass. In the unified scale shown here, the sizes of all objects are described in units of 'molecular weight'. Using this scale, one can make a more standardized quantitative estimate of the relative sizes across the biological spectrum, showing, for example, that the 'molecular weight' of an adult human is about 25 orders of magnitude greater than the molecular weight of an amino acid. The main purpose of this figure is to show that high-resolution electron microscopy bridges a very important technique gap in the size spectrum for subcellular imaging at resolutions between those allowed by X-ray crystallography and by light microscopy.

Figure 2.

Cartoon of an axon and an oligodendrocyte cell that shows a schematic of the cellular anatomy of axonal degeneration in transgenic mice that have knockouts of genes for the two iron regulatory proteins IRP1 and IRP2 (adapted from reference [23]). The small brown dots represent ferritin particles, the molecular structure of which is shown. The inset shows an actual image from such a region in the brain that was stained to visualize ferritin and then was recorded by optical microscopy. The optical microscopic investigations suggest that there is a large increase in ferritin in the presumptive axonal regions (thick diagonal brown line in the inset) of the IRP-knockout mice. However, electron tomographic studies reveal that the most of the excess ferritin is localized to regions in the interior of the axon that are generated by invaginations of neighbouring oligodendrocyte cells into the axon.

Figure 3.

Vitreous sectioning of unstained cells to visualize their internal detail using low cryo-dose electron microscopy (adapted from reference [36]). **(a)** Snapshot (courtesy of Helmut Gnaegi from Diatome Inc, Switzerland, and Erik Bos and Peter Peters, Netherlands Cancer Institute, Amsterdam) of a ribbon of thin vitreous sections (~30 nm thick) attached to a diamond knife immediately before transfer to a carbon-coated grid for imaging by an electron microscope. The ribbon of sections was derived by repetitive sectioning in an ultra-microtome of a pellet of bacterial cells frozen at high pressure inside a thin copper capillary. **(b)** Low-dose electron microscopic image from the interior of a 20 nm thick section such as the one shown in panel (a). The outlines of the inner and outer membranes can be easily visualized, as can the presence of dense particles with a diameter of ~20 nm. We interpret these to be ribosomes. **(c)** Magnified view of an image such as the one shown in panel (b), which demonstrates that the resolution of the images can be good enough to visualize the two leaflets of the 4 nm wide lipid bilayer in the inner and outer membranes.

Figure 4.

Volume rendering of the three-dimensional architecture of a dividing yeast cell (reference [42] and unpublished results from Ingo Gestmann, Mike Hayles, and Ben Lich from FEI Inc., Eindhoven, Jurgen Heymann, and Sriram Subramaniam from the National Cancer Institute, NIH). A focused ion beam was used to mill into a designated site in a pellet of yeast cells; the milled surface was then viewed with a scanning electron beam. Iteration of these two steps several times results in the generation of a series of surface maps of the specimen at regularly spaced intervals, which were then combined computationally to generate the surface map shown here. The roughly contoured internal region at the lower left portion of the cell represents a large intracellular vacuole, whereas the smoother central contour corresponds to the surface of the nucleus. The image of the cell is shown against an enhanced red/yellow background for visualization purposes only.