History of the Electron Microscope in Cell Biology

Barry R Masters, *Massachusetts Institute of Technology, Cambridge, Massachusetts, USA*

In the years following World War II there was an explosion in the biological sciences with the rapid emergence of cell biology, molecular biology and biophysics. These events were affected by the development and use of new technologies for cellular fractionation and imaging, specifically the electron microscope, which provided a resolution, that is, unobtainable with light microscopes. Electron microscopes made visible the fine structure of cells and their organelles, the structure of viruses. Now cryo-electron microscopy is emerging as a key tool to visualize and localize the proteins in an entire cell, the organization of actin filaments in the cytoskeleton, and molecular complexes such as nuclear pores.

Advanced article

Article Contents

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Introduction

New developments in microscopic-imaging techniques aided and accelerated progress in the life sciences (Lemmerich and Spring, 1980). In a separate article, how new types of optical microscopes, specimen preparation techniques and molecular probes helped to advance our understanding of cell biology and medicine are described (Masters, 2008). **See also**: History of the Optical Microscope in Cell Biology and Medicine

This article describes the development of various types of electron microscopes and their effect on cell biology. The following questions are answered in this article: What limits the resolution of an optical microscope, and how does the electron microscope yield images with higher resolution? What are the physical principles of electron microscopes? What controversies in neurobiology were resolved by electron microscopy? What is the ultrastructure of cells and microbes that electron microscopy made visible? And finally, what are the new frontiers of electron microscopy such as cyro-electron microscopy that are being successfully applied to cell and structural biology?

Resolution and Its Limits in a Microscope

A microscope provides both enhanced resolution and increased magnification of the specimen as compared to the image observed with the naked eye, but these enhancements are limited due to the physical principles of each type of microscope. One definition of resolution is the ability to resolve two point sources of equal brightness (to see two illuminated points separated in space as two points). Owing to the finite aperture of any physical lens, a point source of light is not seen as a point of light but as the diffraction pattern of the instrument aperture; that diffraction pattern is called the Airy disk (Airy, 1835). **See also**: Light Microscopy – Brightfield and Darkfield Illumination

In 1896, Lord Rayleigh gave an estimate of the minimal resolvable distance between two equally bright light sources that are imaged in a diffraction-limited system (Rayleigh, 1896). He explained and defined his concept of resolution (Rayleigh resolution) that is based on assumed properties of the human visual system as follows: the two points of light are just resolvable if the central maximum of the diffraction pattern of one light source coincides with the first zero (first dark ring) of the diffraction pattern generated by the second light source.

The image of a point source of light, imaged with a lens of finite diameter, is not a point, but a diffraction pattern. The diffraction of light causes the image of a point source of light to be imaged as a bright central disk that is surrounded by a number of much fainter concentric rings of light. The diffraction pattern of a perfect optical system without light aberrations is a bright disk of light known as the Airy disk, surrounded by a dark ring, and the dark ring is surrounded by a ring of light that is much less intense than the central disk.

Optical microscopes are limited in resolution by the wavelength of light and the finite aperture of microscope objectives. Ernst Abbe who worked at Carl Zeiss in Jena, Germany showed that light diffraction limits the resolution of a light microscope to approximately one-half the wavelength of light or approximately 200–300 nm (Abbe, 1873; Masters, 2007). Abbe demonstrated that the resolution of an optical microscope is a function of the numerical aperture of the microscope objective and the wavelength of the incident light (see the section on Glossary). This diffraction limit of resolution for an optical microscope that is

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illuminated with visible light is known as the Abbe limit of resolution. **See also**: History of the Optical Microscope in Cell Biology and Medicine

To improve the resolution of an optical microscope either the numerical aperture of the objective must be increased, or the wavelength of the illumination must be decreased. The electron microscope is based on the latter technique; decreasing the wavelength of the illumination. A beam of electrons in the electron microscope replaces the beam of light in the optical microscope. Since the wavelengths of electrons (1–2 Å) are much smaller than the wavelengths of visible light, a microscope constructed to focus electrons should have a much higher resolution as compared to a light microscope. **See also**: Electron Microscopy; Far-field Light Microscopy

Early Development of the Electron Microscope

The ability to generate an electron beam, and then to deflect and focus such a beam with the use of electrostatic and magnetic coils or lenses, culminated in the invention of the cathode ray tube (Braun, 1897). In the early 1900s, the nexus of both the theoretical basis of the wave properties of electrons and the experimental conformation of electron diffraction, together with new electromagnetic lens design, contributed to the development of the electron microscope as a practical tool (**Table 1**).

The development of the electron microscope depended on both an understanding of electron optics and on correcting or minimizing the aberrations caused by the magnetic lenses. In 1931 in Berlin, Knoll and Ruska constructed a two-stage transmission electron microscope (TEM) with magnetic lenses with a magnification of 13 × (Knoll and Ruska, 1932). A wire mesh was used as the object (Ruska, 1980, 1986).

Two years later in 1933, Ernst Ruska improved their TEM and demonstrated its capability to surpass the resolving power of the light microscope (Ruska, 1980). Ruska built a TEM with three magnetic lenses, one of the lenses acted as a condenser, and the other two lenses, the objective and the projection lens, magnified the specimen onto the viewing screen. Thin metal films and cotton fibres

Table 1 The development of the electron microscope based on advances in electron optics

1855	Heinrich Geissler invented the first vacuum tube that was later called the Geissler tube
1859	Julius Plucker experimented with cathode rays and was the first to characterize their motion in a magnetic field
1878	Sir William Crookes modified the Geissler tube and confirmed the existence of cathode rays by displaying them in his Crookes tube
1891	George Johnstone Stoney coined the term 'electron'
1896	Kristian Birkeland focused electrons with a magnetic field
1897	Karl Ferdinand Braun developed the cathode ray tube (Braunsche Röhre). The Braun tube is the ancestor of the oscilloscope and the television picture tube
1924/1925	Louis Victor de Broglie developed his wave theory for electrons. In 1924, De Broglie postulated that all moving particles also have wave properties
1926	Hans Busch (Jena) developed the mathematical and experimental foundation of geometrical electron optics, that is, image formation of an object subjected to incident electron rays and with the use of magnetic lenses. He was the pioneer of electron optics
1927	Clinton Joseph Davisson and Lester Halbert Germer (USA) demonstrated the wave properties of electrons by demonstrating electron diffraction from crystals
1927	George Paget Thompson demonstrated the diffraction pattern produced by electrons and showed that the de Broglie waves could be deflected by a magnetic field
1929–1931	Max Knoll and Ernst Ruska developed the two-stage electron microscope by the use of the electron optic equations of Hans Busch
1929	Vladimir Kosma Zworykin invented the kinescope, which is a type of cathode ray tube for his television system
1932	Bodo von Borries and Ernst Ruska applied for a patent on a magnetic pole lens electron microscope
1933	Ernst Ruska described a new type of magnetic lens with a short focal length and published the work in his doctoral thesis
1937	Manfred von Ardenne invented the scanning electron microscope with great depth of focus and a high resolution (Raster-Elektronenmikroskop)
1938	Manfred von Ardenne constructed the first scanning electron microscope and the first scanning transmission electron microscope
1939	Hans Mahl constructed the first transmission electron microscope with two electrostatic lenses
1940	Manfred von Ardenne constructed an electron microscope with a resolution of 3 nm
1951	Erwin Wilhelm Müller, who worked at the University of Pennsylvania (Philadelphia), developed the field emission electron microscope and obtained images of the arrangement of atoms on the surface of the electrode tip (atomic resolution)

were used as specimens. A camera that was located outside the vacuum of the TEM could photograph the magnified image on the viewing screen. Each lens in the microscope was based on the design of von Borries and had iron pole pieces (von Borries, 1940). The microscope's total magnification was 12 000 ×, the rotating specimen stage could hold 8 specimens and the accelerating voltage was 75 kV. This instrument was called the 'Übermikroskop' since its resolution was 50 nm which exceeded that of contemporary light microscopes. The first commercial TEM was manufactured in the UK (Mulvey, 1989).

Transmission and Scanning Electron Microscopes

There are two varieties of electron microscopes: TEM and scanning electron microscopes (SEM). It is important to understand how images are formed in each type of microscope as well as their unique limitations and applications (Slayter and Slayter, 1992; Williams and Carter, 1996). Both microscopes operate in a high vacuum in order that molecules in the air do not scatter the beam of electrons.

In the TEM a beam of electrons is incident on an ultra thin specimen. Some of the electrons are absorbed, some are scattered and some are transmitted. The basic design of a TEM consists of the following components: the source of electrons is the electron gun, a condenser lens focuses the electron beam onto the specimen located in the specimen plane, an objective lens and two projector lenses that focus the transmitted electron beam onto a fluorescent screen, and the observer or a charge-coupled device (CCD) camera records the image (Slayter and Slayter, 1992). The resolving power and the image quality is dependent on the objective lens and its aberrations. The intermediate and the projector lens provide a wide range of magnifications; commercial instruments can achieve magnifications of up to 1 000 000 ×.

Another implementation of the electron microscope is the SEM (von Ardenne, 1938, 1940; McMullan, 1988, 1989). Earlier in 1935, Max Knoll developed an SEM that scanned a focused beam of electrons on a sample surface by magnetic deflection. The contrast of the image was due to the scattered electrons in various parts of the sample (different chemical composition). However, it was Manfred von Ardenne in 1938 who developed an SEM with submicroscopic resolution. In the United States, Vladimir K Zworykin and his research team working at the Radio Corporation of America developed the first working SEM. The magnification in an SEM can range from 25 × to 250 000 × (Zworykin *et al*., 1942).

What is the principle of the SEM? When the primary electron beam is incident on the specimen several processes can occur: there can be backscattered electron, secondary electrons, Auger electrons, X-rays, unscattered electrons, elastically scattered electrons and inelastically scattered electrons (Hawkes and Spence, 2007). Many SEMs can produce a spot size of the electron beam on the sample of 5–10 nm. The image contrast may be due to surface morphology or chemical composition or both.

The most common imaging mode for SEM collects low-energy secondary electrons ejected by the specimen atoms by ineleastic scattering interactions with the incident beam electrons (Hawkes and Spence, 2007). Because of their low energy these electrons originate within a few nanometers of the specimen surface. Alternatively, image contrast can be obtained from the backscattered electrons, which are high-energy electrons from the incident electron beam. They are backscattered out of the specimen interaction volume by elastic scattering due to interaction with the atoms of the specimen. Heavy atoms (high atomic number) backscatter more strongly than light atoms and they appear brighter in the image. The spatial resolution of the SEM is a function of the size of the electron spot, and that depends on both the wavelength of the electrons and the optical components that form the electron beam.

A great advantage of the SEM is the large depth of focus; defined as the height variations of the specimen that are simultaneously in focus. At a magnification of 1000 ×, the SEM is capable of a depth of focus that is 100 times larger than that of an optical microscope. In the life sciences, especially in cell biological applications, SEM is not as popular as TEM; yet there are applications in which SEM excels. Some examples include the surface morphology of biomaterials, implants, contact lenses, artificial skin and the study of the surface topology and shape of cells, bacteria and viruses.

Effect of the Electron Microscope in Neurobiology

When scientists in disparate fields work on a common problem there are sometimes unexpected results that arise due to the diversity of practices as well as novel insights that develop from interdisciplinary thinking and experimental approaches. For example, towards the end of the nineteenth century, the combined efforts of a group of anatomists, embryologists and physiologists worked to improve our understanding of the nervous system (Bennett, 2001). The leaders of these efforts were Santiago Ramón y Cajal, Wilhelm His and Charles Sherrington. Their combined efforts resulted in the Neuron Theory that conceptualized a nervous system composed of discrete nerve cells that communicated with each other through synapses.

Earlier there were opposing theories of the organization of the nervous system. In Italy, Camillo Golgi posited a nervous system composed of an interconnected reticulum (Golgi, 1898, 1906). Although Golgi observed free nerve endings, he incorrectly concluded the smaller nerve terminations were submicroscopic, and therefore they were not resolved with his light microscope. This conclusion was in agreement with Golgi's concept of an interconnected reticulum (Mazzarello and Bentivoglio, 1998).

Golgi in 1898 discovered the Golgi complex in neural tissue that was sectioned after prolonged immersion in a solution of osmium tetroxide and rubidium bichromate. Golgi used the term internal reticular apparatus (see the section on Glossary).

Other neuroanatomists such as Wilhelm His proposed that the nervous system was composed of discrete, discontinuous nerve cells. Santiago Ramón y Cajal integrated the light microscope with various staining methods: those developed by Camillo Golgi, and later using Paul Ehrlich's methylene blue stain, as well as developing new staining techniques himself. Cajal's works provided evidence for a neuron organization composed of discrete nerve cells (Cajal, 1906). He also postulated his law of dynamic polarization, in which transmission between nerve fibres is unidirectional (Cajal, 1906). Cajal used specimens from embryos and young animals from a wide range of species; therefore, the neurons were more sparse and shorter.

The word 'neuron' is due to Wilhelm von Waldeyer, a professor of anatomy in Berlin. He conceived of the nervous system made up of nerve units (neurons) that are anatomically and genetically independent of each other and composed of three parts: the body, the fibre and the terminal branches. The word 'dendrite' was introduced by Wilhelm His. Rudolf von Kölliker introduced the term 'axon'. The word 'synapse' was introduced by Charles Sherrington to describe the gap between neurons and between neurons and the muscles that they innervate (Bennett, 2001).

At the end of the World War II, commercial electron microscopes became available in Europe and in the United States, and these improved instruments together with improved specimen preparation methods (fixing, staining, embedding and sectioning) enabled the visualization of both the synaptic gaps and the synaptic vesicles that contained the purported neurotransmitters.

Finally, the neurophysiology based on both electrical and chemical studies could be reconciled with the morphology of the nervous system as visualized with the electron microscope (Bennett, 2001; Robinson, 2001). In 1954, Stanford Palay who visited George Palade at the Rockefeller Institute published images from the rat cerebellum that showed a 200 Å gap between the pre- and the postsynaptic cells: that gap was later termed the 'synaptic cleft'. Also in 1954, Eduardo DeRobertis and Stanley Bennett in Seattle described gaps of 100–150 Å between pre- and postsynaptic cells of frog sympathetic ganglia and the earthworm nerve cord (Bennett, 2001).

At the same time neurobiologists identified vesicles in the presynaptic terminals of neurons (Santini, 1975). In 1954, DeRobertis and Stanley Bennett described their electron micrographs that showed vesicles in the presynaptic terminals of diameter 200–500 Å. They named these vesicles 'synaptic vesicles', and posited that they may contain neurotransmitters. At the same time, Stanford L Palay identified synaptic vesicles (300–500 Å) and mitochondria in electron micrographs of presynaptic terminals (Bennett, 2001). In TEM micrographs the myelin sheath of nerves appears as a multilayered concentric organization. In summary, the use of the TEM visualized both the synapse and synaptic vesicles. These structures could not be visualized with light microscopes. **Figure 1** shows an electron micrograph of a nerve fibre and a synaptic junction. **See also**: Neurons; Synaptic Vesicle Traffic

Early Application of the Electron Microscope to Cell Biology

Before the development of the electron microscope and its application to the imaging of cells and viruses their fine structure was not accessible to biologists. The limited resolution of optical microscopes precluded their visualization.

This situation dramatically changed 50 years ago, when Albert Claude, Keith Porter and Ernest Fullam published the first picture of an intact cell taken with an electron microscope. The first electron micrograph of an intact cell was published in March 1945, when Keith R Porter, Albert Claude, and Ernest F Fullam published their seminal paper: 'A Study of Tissue Culture Cells by Electron Microscopy', in *The Journal of Experimental Medicine* (Porter *et al*., 1945). The cell was a cultured fibroblast originating from a chick embryo, which Porter grew on a polyvinyl film, and which was subsequently transferred to a wire specimen grid. The cell was fixed with osmium tetroxide. Their TEM image magnified the specimen 1600 times and their first electron micrograph of a cell revealed the mitochondria, the Golgi apparatus and a structure that Porter later named the 'endoplasmic reticulum'. **Figure 2** shows an electron micrograph of cultured cells and their organelles. **See also**: Cell Structure; The Cell Nucleus

The electron microscope would eventually provide images with several hundred times the resolving power of the best light microscopes. The fine structure of cells was made visible through the pioneering work of Claude and Porter at the Rockefeller Institute. Later, George Palade, Christian de Duve, Philip Siekevitz and their colleagues combined electron microscopy with biochemistry and cell fractionation techniques to isolate and study these subcellular structures (Palade, 1974). **See also**: The Cell Nucleus

Modern Applications of the Electron Microscope in Biology

The development and the use of the electron microscope resulted in the advancement of our understanding of cell biology. In the course of a few decades of intense experimental work, the TEM revealed the fine structure of cells to biologists.

The cells were subjected to cellular fractionation combined with differential centrifugation and finally the fractions were imaged with TEM. Working at the Rockefeller University on these techniques were Albert Claude,

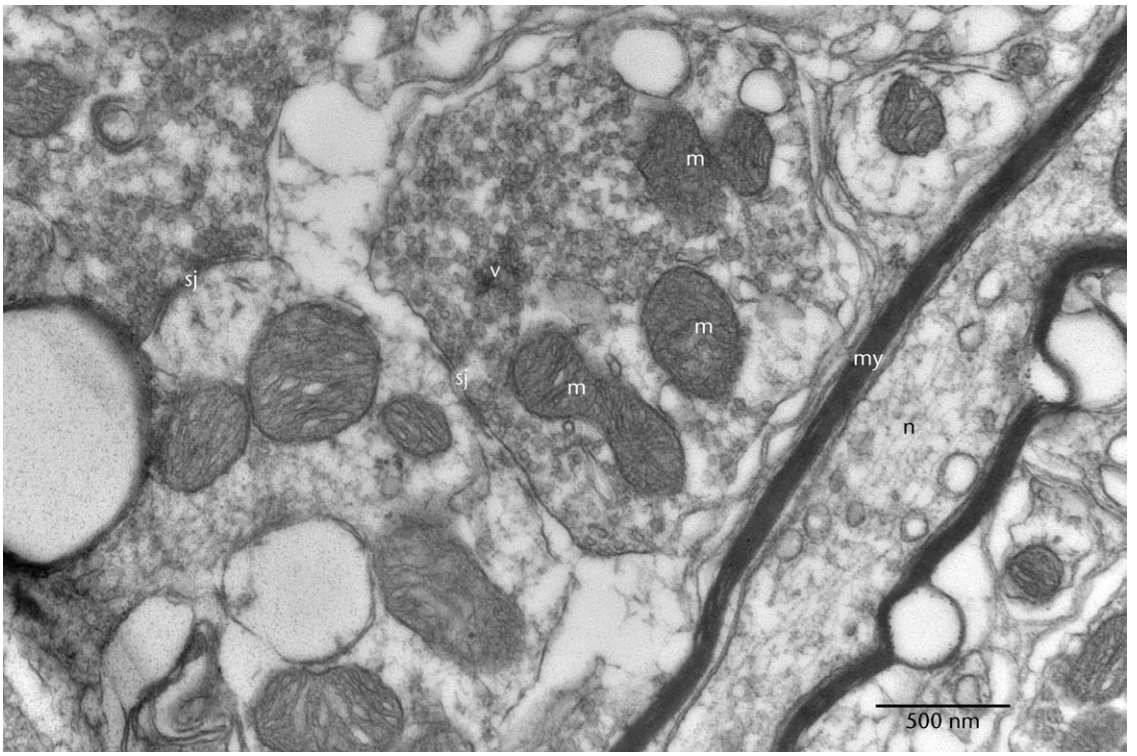

Figure 1 TEM of mouse cerebellum. v, neurotransport vesicles; m, mitochondria; my, myelin sheath; n, nerve fibre and sj, synaptic junction. Magnification bar equals 500 nm. Image obtained by Nicki Watson, Whitehead Institute, Massachusetts Institute of Technology and reproduced with permission.

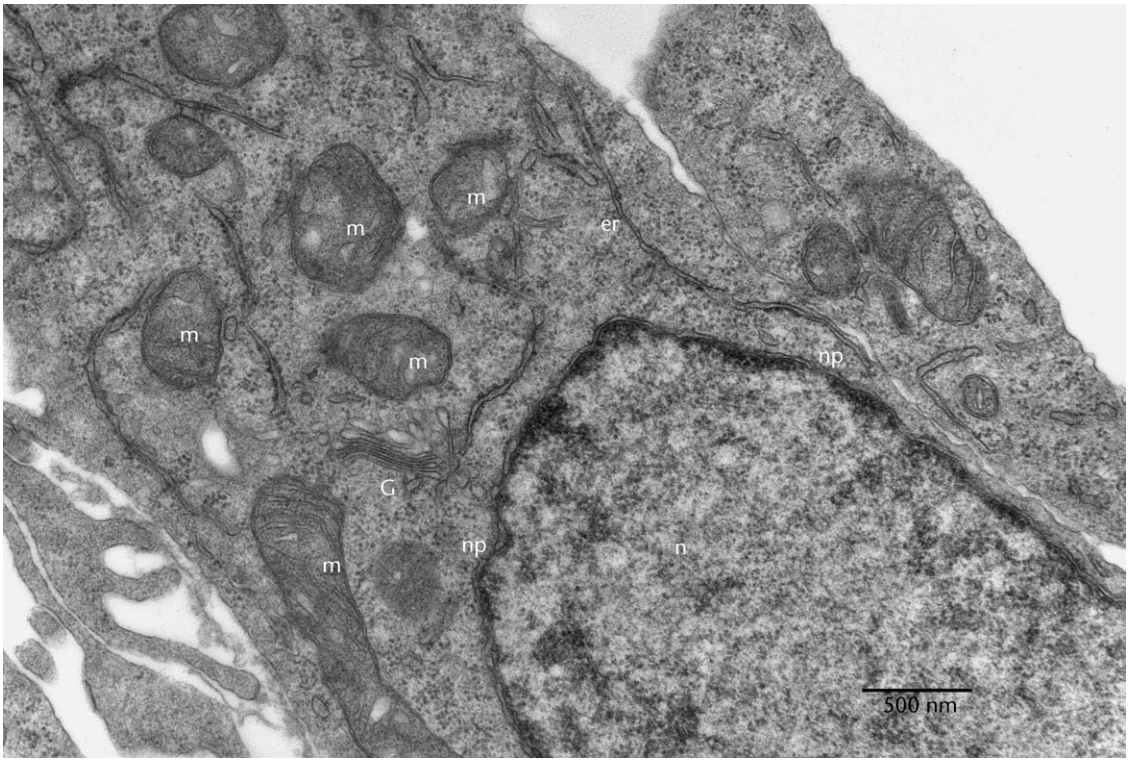

Figure 2 TEM of cultured cell line. Mouse 3T3 control cells. m, mitochondria; n, nucleus; G, Golgi complex; np, nuclear pores and er, endoplasmic reticulum. Magnification bar equals 500 nm. Image obtained by Nicki Watson, Whitehead Institute, Massachusetts Institute of Technology and reproduced with permission.

George E Palade, Paul Siekevitz, and Christian de Duve of Louvain, Belgium. Claude coined the term 'microsome' for the fraction that contained the ribosomes, ribonucleic acid (RNA) and the endoplasmic reticulum. **See also**: Ribosome Structure and Shape

A brief summary of the seminal events in cell biology that were made possible with the electron microscope is listed.

- Between 1932 and 1934, Ladislaus Marton (Brussels) built an electron microscope with three lenses and used it to form the first image of a biological specimen, a 15-μm thick specimen of the leaf of a sundew plant that was impregnated with osmium tetroxide.
- Between 1934 and 1935, Ruska's microscope was used to image unfixed biological specimens (the wings and legs of a housefly). Shortly after, Friedrich Krause imaged diatoms, epithelial cells and bacteria.
- In 1941, Manfred von Ardenne used the TEM to image myosin.
- In 1942, FO Schmitt, CE Hall and M Jakus obtained the first TEM images of collagen at MIT (Cambridge, MA).
- In 1943, Fritiof Sjöstrand who worked at the Karolinska Institute in Stockholm improved the ultramicrotome and used it to study TEM of skeletal muscle.
- In 1945, Keith Porter, Albert Claude and Ernest F Fullam used osmium tetroxide for the fixation and the staining of cells in tissue culture. George Palade earlier recommended the use of buffered osmium tetroxide to preserve cell and tissue ultrastructure.
- In 1947, Albert Claude, Keith R Porter and EG Pickels used the TEM to image the RNA sarcoma virus (RVS).
- Between 1948 and 1953, scientists developed the ultramicrotome; a microtome used in cutting sections 0.1-μm thick, or less, for electron microscopy.
- In 1951, AJ Dalton and MD Felix first observed the Golgi apparatus with the TEM. In the same year FS Sjöstrand and V Hanson published TEM micrographs of the Golgi apparatus. Since the detailed structure of the Golgi apparatus is not visible with the light microscope, it was the development of the TEM that finally permitted the visualization of this organelle as the cell's centre for the processing and the secretion of proteins.
- In 1952, George Palade used TEM to show the first images of the folds of the mitochondria membrane. In 1953, FS Sjöstrand used TEM to image the fine structure of the mitochondria and their double membranes. With the advent of improved staining techniques and thin sectioning both Palade and Sjöstrand independently described the internal structure of mitochondria. In many types of cells the mitochondria appear to be randomly oriented; however, in some cases they are oriented and located in proximity to other organelles; i.e. in cardiac muscle. Another example is the arrangement of mitochondria in the contractile component of the mammalian sperm tail.
- In 1953, HS Bennett and Keith R Porter used the TEM to image sectioned muscle. The sliding-filament theory of muscle contraction was published in 1955 based on the TEM studies of J Hanson and Hugh E Huxley.
- In 1954, Don W Fawcett and Keith R Porter used TEM to study the fine structure of ciliated epithelia.
- In 1954, C Morgan, SA Ellison, HM Rose and DH Moore used TEM to image the human simplex virus 1 (HSV-1). These viruses contain double-stranded deoxyribonucleic acid (DNA) within an icosahedral capsid.
- In 1955, Cecil Hall described negative staining of viruses with TEM. In this technique the stain is not absorbed by the specimen, but only stains the background and thus provides contrast. In 1956, Hugh Huxley made similar observations with tobacco mosaic virus (TMV). Subsequently in 1959, Sidney Brenner and Robert Horne described the technique of negative staining for high-resolution electron microscopy.
- In 1956, the group of de Harven and Bernhard published the first description of the vertebrate centriole with its nine parallel tubules.
- In 1959, SJ Singer introduced the use of electron-dense ferritin coupled with immunogloblins to identify specific antigen sites. This technique provided molecular specificity to visualize a particular protein in the specimen. Other important techniques with high specificity include immunogold labelling, immunoenzymatic labelling and high-resolution autoradiography. **See also**: Immunoelectron Microscopy
- In 1961, Hans Ris published the first TEM images of the ultrastructure of the animal chromosome and showed the elementary chromatin fibre at 1.3 nm resolution.
- The development of the TEM permitted the visualization of the fine structure of the cytoskeleton. The main components of the cytoskeleton include: microtubules (30 nm), microfilaments such as actin filaments (9.5 nm) and intermediate filaments (7.12 nm). In both the plant and the animal kingdoms microtubules and microfilaments are essential components of the cell and are involved in movement, cell division and contraction.
- In 1963, HS Slayter, JR Warner, A Rich and CE Hall used the TEM to visualize the polyribosomal structure.
- In 1968, David de Rosier and Aaron Klug produced the first three-dimensional (3D) reconstruction of a virus from electron micrographs.
- In 1981, BJ Poiesz, FW Ruscetti, MS Reitz, VS Kalyahazaman and RC Gallo used the TEM to characterize a new Retrovirus (HTLV) in primary cultured cells of a patient with Sezary's T-cell leukaemia.

Figure 3 shows an electron micrograph of mouse skeletal muscle.

Cryo-electron Microscopy

A major problem of using electron microscopy with biological specimens is their sensitivity to radiation from the electron beam. This critical problem is counterbalanced by the high resolution attainable with the electron microscope. These problems were ameliorated by new techniques

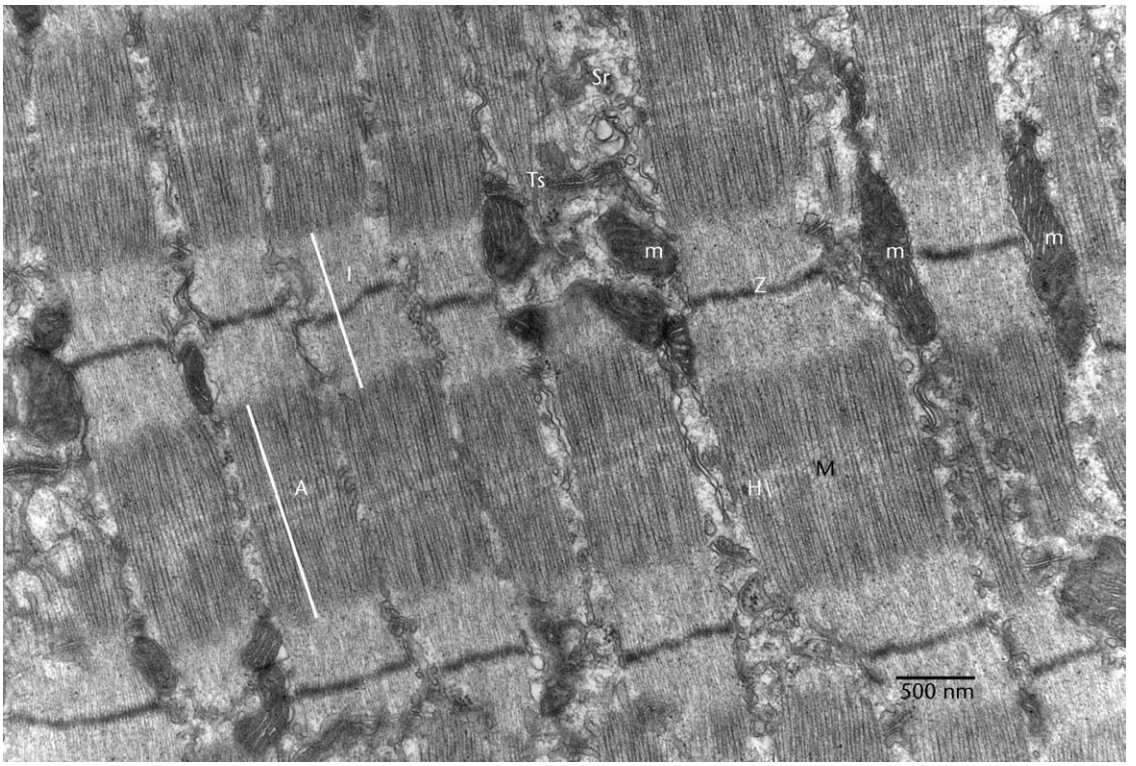

Figure 3 TEM of mouse skeletal muscle. A, A-band; M, M-line; Z, Z-disk; I, I-band; H, H-band; m, mitochondria; Sr, sarcoplasmic reticulum and Ts, T-system. Magnification bar equals 500 nm. Image obtained by Nicki Watson, Whitehead Institute, Massachusetts Institute of Technology and reproduced with permission.

for dehydration and water-substitution methods, and electron beam damage was mitigated by staining with heavy metals (Glaeser, 2008).

A breakthrough was achieved in 1981 when Dubochet and McDowall introduced the cryotechnique as a means to improve specimen preservation (Dubochet and McDowall, 1981). The biological specimen is embedded in water or a buffer solution by rapidly freezing to the temperature of liquid nitrogen (90 K). The liquid water is converted to an amorphous state, without crystallization, and avoids cellular damage due to ice crystal formation; this technique permitted biological samples to be investigated in their native state without the introduction of artifacts, thereby simplifying image interpretation (see Baumeister). **See also**: Electron Cryomicroscopy and Three-dimensional Computer Reconstruction of Biological Molecules

Owing to the large depth of focus of the TEM, the images that are obtained are two-dimensional (2D) projections of the specimen. To obtain the correct 3D structure a large number of 2D images obtained at various tilt angles are required, together with computer reconstruction techniques.

Modern cryo-electron microscopy is an important tool in structural biology and is based on three techniques to achieve 3D imaging: single-particle electron microscopy (cryo-EM), cryo-electron tomography (cryo-ET) and cryo-electron crystallography (cryo-EC). These methods are based on the fact that the parallel projection of a 3D specimen is equivalent to a slice in the 3D Fourier space of the object. To form the total 3D reconstruction it is necessary to obtain different slices in the Fourier space; the specimen is rotated and the back-projection method is typically used to form the 3D specimen. The sum of all the projections, properly weighted, represents the density distribution of the original specimen and is called the tomogram. Alternatively, for single-particle analysis, identical copies of the specimen occur in many different orientations, and these images are used to reconstruct the tomogram. The technique of cryo-EC can be used to determine the 3D structures of macromolecular assemblies.

At present, cryo-ET is the only 3D imaging method that can image cells and their organelles in their native state at molecular resolution (Gruska *et al.*, 2008; Nickell *et al.*, 2007; Sartori *et al.*, 2007). Important applications of cryo-ET include the 3D structure of the proteasome that is an ubiquitous macromolecular assembly designed for the controlled proteolysis of either abnormal or short-lived regulatory proteins; the 70S ribosome which is a molecular machine responsible for protein synthesis (Ortiz *et al.*, 2006) and the 3D molecular mapping of all the proteins in an entire cell.

Concluding Remarks

To investigate the structure and the function of cells it is necessary to integrate the imaging techniques of a variety of

microscopes, from optical microscopes to electron microscopes to cover a wide range of resolutions. The integration of microscopes, specimen preparation techniques and digital image processing can yield the 3D structure of a cell and its molecular components. The implementation of these interdisciplinary approaches to cell imaging should result in new advances in our understanding of cell biology. **See also**: Actin and Actin Filaments; Bacterial Ribosomes; Electron Cryomicroscopy; Intermediate Filaments; Tubulin and Microtubules

Glossary

Cryo-electron microscopy A technique in which unstained and fully hydrated biological specimens are embedded in vitreous ice, maintained at liquid nitrogen temperatures, and imaged in a TEM.

Cytoskeleton The supermolecular protein complexes that form the mechanical scaffold for the cytoplasm of a cell. It consists of actin filaments, intermediate filaments and microtubules.

De Broglie's hypothesis In 1924 de Broglie proposed that material particles such as electrons possess wave properties. For material particles, $p = \frac{h}{\lambda}$, which is the de Broglie relation, where p is the momentum of the particle, h the Planck's constant and λ the de Broglie wavelength of the particle. Davisson and Germer, and simultaneously GP Thompson, demonstrated the wave properties of electrons in 1926; thereby confirming de Broglie's ideas.

Freeze fracture A method to prepare specimens for electron microscopy. It involves the freezing, fracturing and sublimation of water from the fractured surfaces, and the subsequent shadowing of the specimen with metal. It is used to image the two faces of bilayer membranes.

Golgi apparatus This is a major compartment of the secretary membrane system in cells. It functions to process glycoproteins and to sort molecules in the lumen and lipid bilayer.

Intermediate Filaments These are a family of cytoplasmic proteins (one type called the lamins occurs in the nucleus) with an average diameter of 10 nm that are important for cell adhesion. Unlike microtubules and microfilaments the intermediate filaments lack polarity; the two ends of each filament are identical.

Microfilaments (actin filaments) These are the thinnest filaments (7 nm diameter) of the cytoskeleton and are located in the cytoplasm of all eukaryotic cells. They exist in either bundles or in networks and are very dynamic structures.

Microtubule These are relatively rigid cylindrical polymers of α- and β-tubulin that serve as conductor paths for motor proteins such as kinesins and dyneins.

Negative staining A contrast method to prepare specimens for electron microscopy in which the specimen is dried in a drop of heavy metal salts.

Numerical aperture The numerical aperture (NA) of a microscope objective is defined as $NA = n\sin\theta$, where n is the index of refraction of the medium between the lens and the specimen (1.0 for air, 1.33 for water and 1.56 for oil) and θ the half-angle of the maximum cone of light that enters or exits the lens.

Ribosome A complex of ribosomal RNAs together with other proteins that catalyse the synthesis of polypeptides.

Synaptic vesicle Small vesicles that contain neurotransmitters and are concentrated in the presynaptic endings of neurons.

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