Special Contributions

Advanced Biomedical Transmission Electron Microscopy

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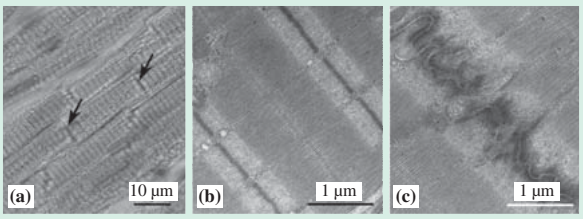
OVERVIEW: Transmission electron microscopy provides resolutions that greatly exceed what is possible on optical microscopes, and the post-genomic era of recent years has seen the emergence of new developments in the application of this technology to biomedical research. In addition to microstructural changes to cells in knockout organisms (in which a particular gene has been disabled), huge demand is emerging for verifying the microscopic structure of cells and tissue grown from iPS cells, a type of stem cell that has unlimited potential to differentiate into every other kind of cell. This article looks ahead to the next generation of microscopes to present the latest information about how, in response to these new developments, the most recent transmission electron microscopes have made dramatic advances in their roles as precision instruments that are simple and easy to use, incorporating functions for auto-focus, fully digital photography using CCD cameras, and image transmission via video conferencing system.

INTRODUCTION

HUMAN beings are very acquisitive animals who want to magnify objects that are too small to see with the naked eye and to break open those that are encased in something so as to expose them to view. It is this acquisitiveness that is behind the use of transmission electron microscopes in biomedical research to uncover the fine structures encased in cell membranes at a microscopic level. This article brings the perception of transmission electron microscopy up to date, using the HT7700 transmission electron microscope made by Hitachi High-Technologies Corporation as a basis for providing an introduction to this technology, which has evolved to meet the needs of biomedical research. The article delves into the origins of transmission electron microscopy, including its use in topical research into induced pluripotent stem (iPS) cells, and proposes a new generation of the technology that will satisfy the demands placed upon it.

ADVANTAGES OF TRANSMISSION ELECTRON MICROSCOPY WITH HIGH SPATIAL RESOLUTION

Optical microscopes can provide bright images of fluorescent markers. It is the high spatial resolution of electron microscopes, however, that is their most notable feature compared to optical microscopes, and this feature continues to provide unshakeable scientific evidence despite their being limited to monotone images. As a simple example, whereas it is possible to just make out the distinctive stripes and linear features of intercalated disks [shown by the arrows in Fig. 1 (a)] in myocardial fiber in the optical



H&E: hematoxylin and eosin

Fig. 1—Comparison Images Demonstrating the High Resolution of Electron Microscopes (Rat Heart Muscle).
Image (a) was taken by an optical microscope using H&E staining and a ×100 oil-immersed objective lens. The distinctive striped pattern of heart muscle and the linear shape of the intercalated disks are visible (shown by the arrows). Images (b) and (c) were taken by a transmission electron microscope using uranium and lead staining. Image (b) shows the regular arrangement of actin and myosin in the myocardial fiber, and image (c) clearly shows the microscopic structure of the intercalated disks, which perform an important function in the beating of the heart, with localized joints in the gap.

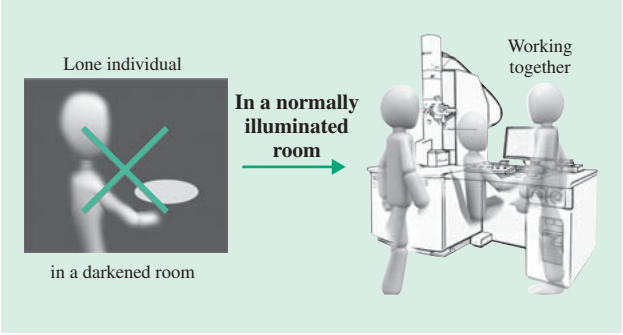


Fig. 2—Viewing Electron Microscope Images on a Monitor. Use of an electron microscope has changed from being something done by a lone individual in a darkened room to a collective activity in a normally illuminated room.

microscope image of a rat heart muscle, transmission electron microscope images enable more detailed examination, including showing the fibrous structure of the actin and myosin that make up the stripes [see Fig. 1 (b)] and the complex shape of an intercalated disk [see Fig. 1 (c)].

ADVANCED TRANSMISSION ELECTRON MICROSCOPIC OBSERVATION AND PHOTOGRAPHY

Simple Operation in Normally Illuminated Room Together with Colleagues

Using a fluorescent screen to display cell morphology imaged by an old-style transmission electron microscope using an electron beam requires the room to be dark. Transmission electron microscopes were typically installed in back rooms away from sunlight and tended to be used by a single individual shut away in a darkroom as the fluorescent screen on the other side of the observation window into the scope did not allow for group viewing. This image is now out of date. On the latest transmission electron microscopes, the image on the fluorescent screen is captured by a screen camera and displayed on a monitor, meaning it is no longer necessary to peer into a fluorescent screen in a darkroom. Instead, images can be viewed together with colleagues in a normally illuminated room (see Fig. 2). Nowadays, transmission electron microscopes are installed in rooms with glass windows fronting onto corridors so that people can see the microscope in action as they pass by.

Auto-focus that Works Like a Digital Camera

It is not just beginners who struggle with focusing and find themselves having to re-take photographs after discovering that the membrane structure in the developed image is indistinct. The latest transmission electron microscopes, by contrast, have made this a thing of the past, coming equipped with an auto-focus function that takes only a few seconds to focus the image automatically. In other words, electron microscope imaging can now be performed in much the same way as using a digital camera (see Fig. 3). The ability to view images and assess their quality immediately has also provided a step up in operational efficiency.

Digital Images that do not Need to be Developed and Smart Image Data Management

The elimination of film associated with the switch to digital imaging means it is no longer necessary for people to strain their eyes developing images under the safety light in a darkroom. Furthermore, image recording systems include a helpful captioning function that can be used to record experimental

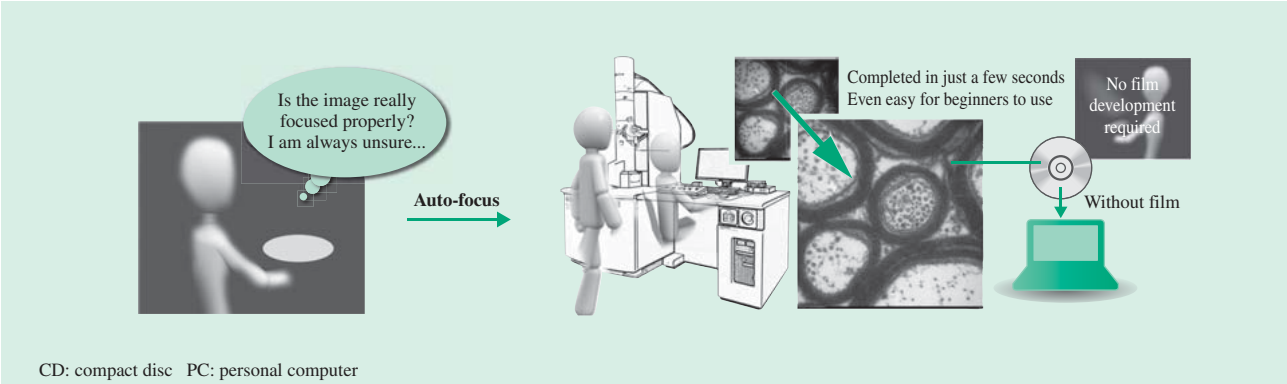


Fig. 3—Electron Microscope Image Acquisition Using Auto-focus and without Film. Images are stored on CD and can be viewed or saved on a PC.

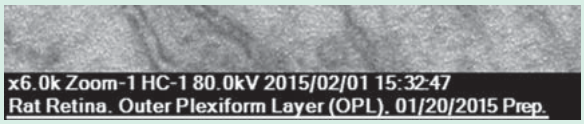
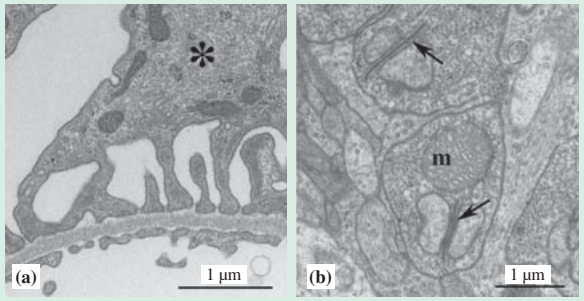


Fig. 4—Captioning Function.
The captioning function shown here is useful for recording and cataloging electron microscope images. Comments can be inserted as required in a field at the bottom of the image (white underlined text).



HC: high contrast CCD: charge-coupled device

Fig. 5—HC Mode and High-performance CCD Camera.
The high-performance CCD camera can capture adequate images using Reynolds lead staining rather than uranium staining. Image (a) shows a podocyte (indicated by the asterisk) in the glomerulus of a rat kidney. Image (b) shows the outer plexiform layer of a rat retina. A mitochondrion (indicated by the "m") and synaptic ribbon (indicated by the arrow) are clearly visible.

data, comments, and other information as required (see Fig. 4). Database management functions are also available to simplify the cataloging of large numbers of recorded images as well as search and retrieval.

High-performance CCD Camera that Eliminates the Inconvenience of Using Uranium Staining

The high-contrast (HC) mode of the compound objective lens in the HT7700 together with the high-quality imaging achieved using the high-performance charge-coupled device (CCD) camera provided as a standard feature mean it can achieve adequate contrast using lead staining only, thereby eliminating the need for the uranium staining that was once common practice (see Fig. 5). As uranium is a difficult material to obtain, being subject to strict conditions, including those relating to the storage of waste fluid after use, this improvement represents a major step toward encouraging applications for transmission electron microscopy.

Significant Boost in Efficiency Provided by Stage Memory Function and Three-Specimen Holder

The restricted field of view available with transmission electron microscopy means that it is not uncommon to lose track of where on the specimen cross section the microscope is looking and so to lose the ideal point of observation after it has been identified, like being lost in the woods because one can only see the trees. However, this too is now a thing of the past. The latest transmission electron microscopes such as the HT7700 are equipped with a stage memory function that records the movements of the specimen stage and eliminates the problem of losing the observation location (see Fig. 6). The microtrace function, meanwhile, which displays the sequence of specimen stage movements, can be used to see which parts of the specimen have or have not been viewed.

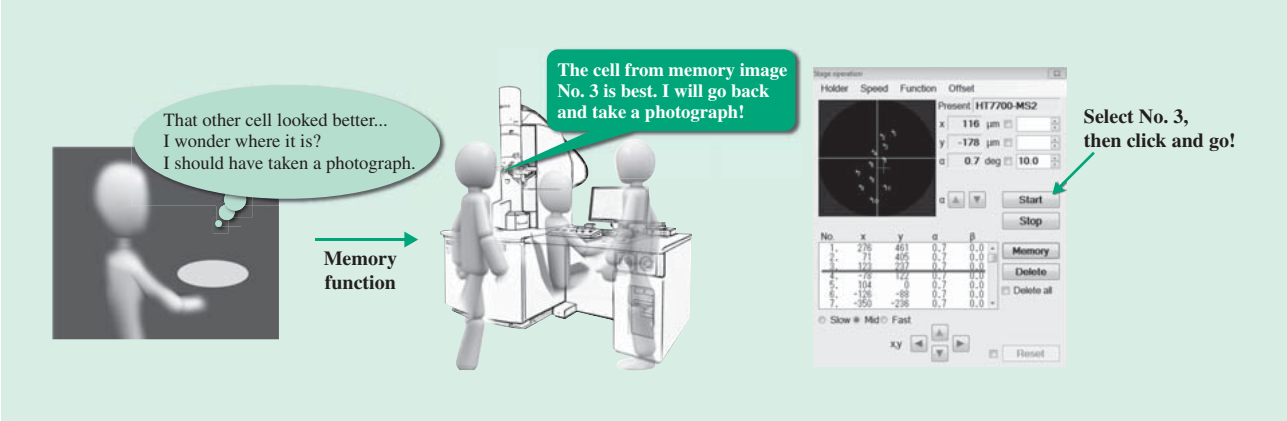


Fig. 6—Stage Memory Function.
The stage memory function avoids missing out on the best images.

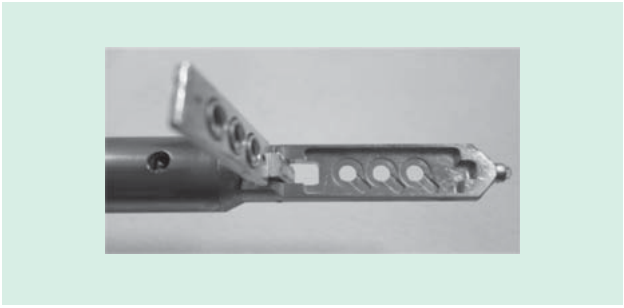


Fig. 7—Three Specimen Holder.
Three grids can be inserted at a time using the convenient three specimen holder. This significantly improves efficiency by reducing the amount of effort spent on swapping grids.

Transmission electron microscopy requires the insertion and removal of specimens from the microscope barrel, which is maintained in a state of vacuum to obtain the electron beam. Accordingly, operational efficiency can be significantly improved by combining the stage memory function with the three specimen holder shown in Fig. 7 (available as an option), which can be used to insert three specimen-containing grids at a time.

Powerful Support for Joint Research Provided by Electron Microscope Image Transmission and Videoconferencing System

An electron microscope image transmission system that uses the latest information technology can send electron microscope images acquired by the screen camera or high-performance CCD camera to designated recipients on the Internet. Used in conjunction with a videoconferencing system, this makes it possible to share electron microscope images in realtime while holding a discussion with a remotely located joint research team. The electron microscope image transmission system keeps tight control over information security. Transmission electron microscopy is set to move from a solitary person working on their own in a darkened room to an era in which people work with colleagues in a normally illuminated room and are able to share images with others located remotely (see Fig. 8).

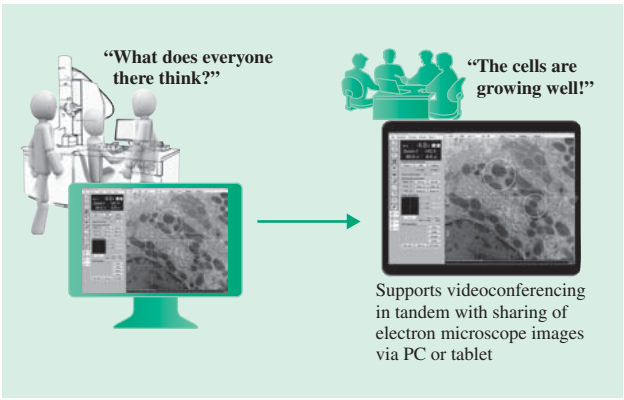


Fig. 8—Use with Videoconferencing System.
It is now possible to share images while holding discussions with a joint research team at a remote location.

SIMPLE, QUICK, AND RELIABLE —DRAMATICALLY IMPROVED SPECIMEN PREPARATION FOR TRANSMISSION ELECTRON MICROSCOPES—

A negative perception associated with transmission electron microscopy is that it requires complex and time-consuming specimen preparation, and while there are many cases in which it can be avoided, simple, quick, and reliable specimen preparation techniques have now been produced into which a wide variety of practices have been incorporated[(1)-(3)]. To enable specimens acquired in the early morning to be observed under a transmission electron microscope on the evening of the same day (see Fig. 9), workflows have started to be adopted in practice that involve the routine use of a transmission electron microscope for screening samples and then subjecting the selected samples to molecular biological analysis, an approach that was rejected in the past as impractical.

How many users have experience of repeatedly preparing and observing ultra-thin sections until a suitable cell is identified? This is another outdated practice. The way things are done now is to use an

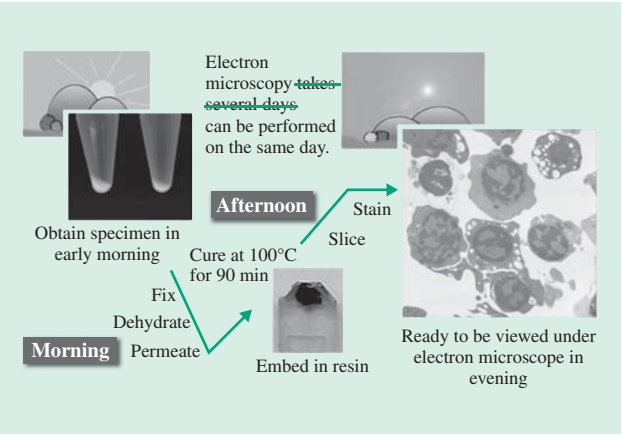


Fig. 9—Improved Specimen Preparation Technique.
It is now possible to collect a specimen in the early morning and view it under a transmission electron microscope that same evening.

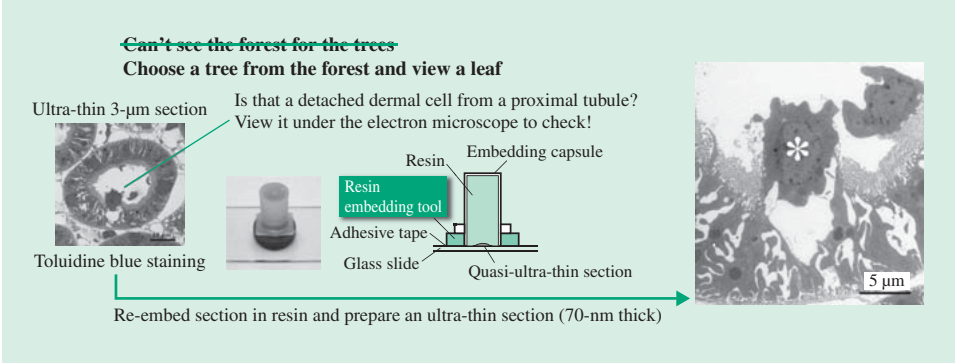


Fig. 10—Using an Optical Microscope to Select Ultra-thin Section for Viewing Under Transmission Electron Microscope.
It is possible to use an optical microscope to pinpoint a target cell and then study it under a transmission electron microscope.

optical microscope to screen quasi-ultra-thin sections, and then to re-embed them in resin and slice an ultra-thin section for viewing under a transmission electron microscope[2), (3)]. This is because it is easy to use a transmission electron microscope to image a cell once it has been identified as the target using the optical microscope (see Fig. 10).

PROPOSAL FOR NEXT GENERATION OF BIOMEDICAL TRANSMISSION ELECTRON MICROSCOPY—CONCLUSIONS—

Transmission electron microscopy, which provides resolutions that greatly exceed what is possible on optical microscopes, has made an incalculable contribution to biomedical research. During the burgeoning of research techniques for molecular biology in the 1990s, research based primarily on the electron microscope was derided as being outdated and old-fashioned, like scavenging for rice in a paddy field that has already been harvested. The author, who had a secret fascination with a certain medical cartoon character, will never forget having been told this upon swapping a scalpel for an electron microscope and choosing the path of anatomical research. The words were spoken by an older colleague who was a self-described molecular biologist.

A quarter century has passed since then. New developments have emerged as the world enters a post-genome-sequencing era. Transgenic organisms have been created as well as knockout organisms such as mice in which a particular gene has been disabled, and these have required comparative studies to look at what changes are present in the fine structure of cells and tissue. Furthermore, the development of iPS cells has given rise to huge demand for verifying the microscopic structure of cells and tissue grown from iPS cells, which can differentiate to form every other kind of cell.

One such example of this verification is the undertaking of basic research into the production of platelets from IPS cells designated in the Ministry of Education, Culture, Sports, Science and Technology's iPS Cell Research Roadmap, which is approaching the stage of large-scale production for clinical applications. In a paper on the establishment of this core technology[4], transmission electron microscopy made a major contribution, with the need for it made obvious by Fig. 11, in which the platelets are very small compared to the red blood cells and neutrophils and would appear as mere dots under an optical microscope. In contrast, the open canalicular system and secretory granules inside platelets are clearly visible using transmission electron microscopy [see Fig. 11 (b) and (c)]. The diameter of platelets is only in the range of 1 to 2 µm and transmission electron microscopy is essential for the detailed observation of their internal structure.

Currently, studies into clinical applications in areas such as regenerative medicine and drug development, based on using iPS cells to produce cells, tissue, and

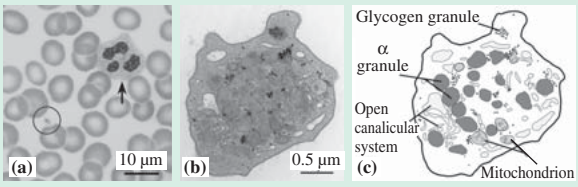


Fig. 11—Human Platelets.
Image (a) is a peripheral blood smear and was taken with an optical microscope using Giemsa staining. The small object indicated by the circle is a platelet, the arrow indicates a neutrophil, and the nearby circular cells are red blood cells. Image (b) is from a transmission electron microscope using lead staining and image (c) is a schematic diagram of the internal structure.

organs of many different types, are proceeding in parallel with the establishment of an all-Japan research regime. What was once equivalent to scavenging for rice in a paddy field that has already been harvested has now become a new era where there is a prospect of harvest (electron microscopy), with the ears of grain (cells induced to differentiate from iPS cells) laden with a new variety of rice (iPS cells) in a newly reclaimed paddy. It is a field where, rather than using scanning electron microscopes for imaging the surface of cells, it will be the use of transmission electron microscopes for imaging their interior that will play the major role. From specimen preparation to observation and image acquisition, this transmission electron microscopy is evolving into a precise analytical technique that is simple and convenient. The author would like to conclude this article by looking forward to electron microscopes raising the standard of the latest biomedical research and opening up new fields of research.

REFERENCES

(1) K. L. McDonald, "Rapid Embedding Methods into Epoxy and LR White Resins for Morphological and Immunological Analysis of Cryofixed Biological Specimens," Microsc Microanal. **20**, Issue 1, pp 152–163 (2014).
(2) A. Sawaguchi et al., "Capsule-supporting Ring: A New Device for Resin Embedding of Glass-mounted Specimens," J Microsc. **234** (2), pp. 113–117 (2009).
(3) N. Takahashi et al., "Targeting Ultrastuctural Observation of Biological Samples by Using Capsule-Supporting Ring," KENBIKYO **48**, No. 2, pp. 113–117 (2013) in Japanese.
(4) S. Nakamura et al., "Expandable Megakaryocyte Cell Lines Enable Clinically Applicable Generation of Platelets from Human Induced Pluripotent Stem Cells," Cell Stem Cell. **14**, Issue 4, pp. 535–548 (2014).

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