Micron 104 (2018) 21–25

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Tutorial

Electron tomography of whole cultured cells using novel transmission electron imaging technique

Taiga Okumura[a,1], Minami Shoji[a], Akiko Hisada[a,*], Yusuke Ominami[b], Sukehiro Ito[b], Tatsuo Ushiki[c], Masato Nakajima[c], Takashi Ohshima[a]

[a] Hitachi, Ltd., Research & Development Group, 2520, Akanuma, Hatoyama, Saitama, 350-0395 Japan
[b] Hitachi High-Technologies Corporation, 882, Ichige, Hitachinaka-shi, Ibaraki, 312-8504 Japan
[c] Graduate School of Medical and Dental Sciences, Niigata University, 757, Asahimachidori-1, Chuo-ku, Niigata-shi, Niigata, 951-8510, Japan

ARTICLE INFO

Keywords:
Plate-transmission electron microscopy
Electron tomography
Whole-cell imaging
Scanning electron microscope
Scintillator plate

ABSTRACT

Since a three-dimensional (3D) cellular ultrastructure is significant for biological functions, it has been investigated using various electron microscopic techniques. Although transmission electron microscopy (TEM)-based techniques are traditionally used, cells must be embedded in resin and sliced into ultrathin sections in sample preparation processes. Block-face observation using a scanning electron microscope (SEM) has also been recently applied to 3D observation of cellular components, but this is a destructive inspection and does not allow re-examination. Therefore, we developed electron tomography using a transmission electron imaging technique called Plate-TEM. With Plate-TEM, the cells cultured directly on a scintillator plate are inserted into a conventional SEM equipped with a Plate-TEM observation system, and their internal structures are observed by detecting scintillation light produced by electrons passing through the cells. This technology has the following four advantages. First, the cells cultured on the plate can be observed at electron-microscopic resolution since they remain on the plate. Second, both surface and internal information can be obtained simultaneously by using electron- and photo-detectors, respectively, because a Plate-TEM detector is installed in an SEM. Third, the cells on the scintillator plate can also be inspected using light microscopy because the plate has transparent features. Finally, correlative observation with other techniques, such as conventional TEM, is possible after Plate-TEM observation because Plate-TEM is a non-destructive analysis technique. We also designed a sample stage to tilt the samples for tomography with Plate-TEM, by which 3D organization of cellular structures can be visualized as a whole cell. In the present study, Mm2T cells were investigated using our tomography system, resulting in 3D visualization of cell organelles such as mitochondria, lipid droplets, and microvilli. Correlative observations with various imaging techniques were also conducted by successive observations with light microscopy, SEM, Plate-TEM, and conventional TEM. Consequently, the Plate-TEM tomography technique encourages understanding of cellular structures at high resolution, which can contribute to cellular biological research.

1. Introduction

Biological functions crucially depend on the three-dimensional (3D) structure and arrangement of organelles and protein complexes inside cells. Accordingly, the 3D cellular structure has been widely examined with a variety of techniques that use photons, electrons, and X-rays (Helmstaedter et al., 2008; Micheva and Smith, 2007; Schneider et al., 2010). Among them, light microscopy is advantageous because living cells can be observed with a live-imaging technique, but the objects that can be visualized are only labeled proteins. To understand the functions of proteins, it is necessary to reveal the spatial relationships between proteins and surrounding organelles. Electron microscopy, instead of light microscopy, plays a prominent role in visualizing the subcellular structure at high resolution (Baumeister et al., 1999; Frank, 1995). However, specimens that can be observed using electron microscopy are resin-embedded cells. Therefore, the cells on cultivation dishes must be separated from the dishes before resin-embedding. Such processes are time-consuming and may affect cellular structures. In this study, we

Abbreviations: 3D, three-dimensional; TEM, transmission electron microscopy; SEM, scanning electron microscope; SSTEM, serial section TEM; SBF-SEM, serial block face-SEM; Plate-TEM, plate-transmission electron microscopy; GSO, gadolinium silicon oxide; YAG, yttrium aluminum garnet; SEs, secondary electrons; BSEs, backscattered electrons

* Corresponding author.
E-mail address: akiko.hisada.uf@hitachi.com (A. Hisada).

[1] Present address: Department of Earth and Planetary Science, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033 Japan.

http://dx.doi.org/10.1016/j.micron.2017.10.006
Received 24 July 2017; Received in revised form 13 October 2017; Accepted 13 October 2017
Available online 16 October 2017

developed a 3D observation technique of whole cells without separating from substrates such as cultivation dishes.

Traditionally, transmission electron microscopy (TEM) is used for ultrastructural investigation of cellular components. Although conventional TEM is used to investigate ultrathin sections, namely one slice of samples, various electron microscopic techniques have been developed for understanding 3D structural information. For example, serial section TEM (SSTEM) is a well-established technique for examining the 3D structures of cellular components (Harris et al., 2006). With this technique, ultrathin (60–80 nm thick) sections of resin-embedded samples are serially prepared with an ultramicrotome, and 3D reconstruction is carried out by integrating the TEM images of serial ultrathin sections. However, it is difficult even for experienced operators to collect the serial sections onto a TEM grid of 3 mm in diameter without losing sections and generating artifacts such as holes, folds, shrinkage, and stretching. Recently, serial sections have also been investigated using scanning electron microscopy (SEM), even though SEM does not reach the spatial resolution of TEM (Micheva and Smith, 2007; Wacker and Schroeder, 2013). In this case, the serial sections are put onto a solid substrate, such as glass or silicon, which is easier than transferring the sections to a small TEM grid. Nevertheless, the processes for handling and aligning the serial sections are still necessary. To address these difficulties, an automatic tape-collecting ultramicrotome was recently developed to automatically collect the serial sections on a carbon-coated tape for subsequent SEM observation (Hayworth et al., 2006; Schalek et al., 2011; Tapia et al., 2012). In addition, software that semi-automatically aligns the serial-sectional images has been commercialized and used in some studies (Eberle et al., 2015).

An alternative 3D visualization technique is electron tomography, in which a relatively thick (a few hundred nanometers thick) section is analyzed using TEM, and 3D reconstruction is created from a tilt-series of TEM images in the same way as with computed tomography (Hoenger and McIntosh, 2009; West et al., 2011). However, electron tomography is not applicable when a large volume needs to be reconstructed because the section thickness should be below 0.5 μm for maintaining electron-transparency. To overcome this limitation, serial section electron tomography was introduced (Soto et al., 1994). Relatively thick sections are serially collected, and 3D data acquired from each section using electron tomography are integrated. Thus, this technique can reduce the number of necessary sections but takes a long time because electron tomography must be conducted for each section. Though high-voltage TEM enables the investigation of much thicker (a few microns) sections, distortion of sections caused by the high electron dose needs to be carefully monitored (Martone et al., 2000; Murata et al., 2014).

Recently, two novel techniques for 3D reconstruction by SEM have attracted attention (Peddie and Collinson, 2014). One is serial block face-SEM (SBF-SEM), by which the surface of a resin-embedded sample is cut repeatedly using an ultramicrotome inside an SEM chamber, and the new successive block faces are observed using SEM (Briggman and Denk, 2006; Denk and Horstmann, 2004; Helmstaedter et al., 2008). The laborious process for making sections can be entirely omitted, and the *z*-resolution reaches a few dozen nanometers, though it is limited by section thickness. On the other hand, focused ion beam-SEM (FIB-SEM) possesses higher *z*-resolution because it uses a focused ion beam instead of an ultramicrotome to mill the sample surfaces (Knott et al., 2008; Wei et al., 2012). Although the analyzable area is smaller with FIB-SEM than with SBF-SEM, the *z*-resolution is as good as 10 nm. However, both techniques are completely destructive and do not allow re-examination and correlative inspections with other techniques such as light microscopy.

In this study, we developed the plate-transmission electron microscopy (Plate-TEM) technique, in which samples placed onto a plate are observed using a transmission electron mode of SEM (Ominami et al., 2014a, 2014b). The electron tomography using this technique, namely Plate-TEM tomography, enables us to explore 3D structures of whole cells without destructive sectioning processes. Furthermore, this technique can enable correlative examination using electron and light microscopies and contribute to the understanding of the relationships between cellular structures and functions.

2. Materials and methods

2.1. Sample preparation

The Mm2T cells (the cell line derived from the thymus of the Indian muntjac, purchased from Riken Cell Bank, Riken) were cultivated on plates consisting of transparent scintillating crystals such as Ce-doped gadolinium silicon oxide (GSO) and Ce-doped yttrium aluminum garnet (YAG). They were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 30 min. The samples were postfixed with 1% osmium tetroxide in the same buffer solution for 1 h at 4 °C and rinsed several times with distilled water. After postfixation, they were dehydrated with a series of graded ethanol. For Plate-TEM observation, the dehydrated samples were dried in a critical point dryer (HCP-2, Hitachi) using CO_2, and coated with carbon by using a vacuum evaporator (VE-2030, Vacuum Device) to avoid specimen charging. After Plate-TEM observation, the samples were embedded in epoxy resin, and the scintillator plates were removed from the resin block. The resulting sample blocks were sectioned with an ultramicrotome (MT6000, DuPont) to the thickness of 60–80 nm and doubly stained with uranyl acetate and lead citrate for TEM observation.

2.2. Data acquisition and processing

Plate-TEM and SEM images were acquired using an S-4200 scanning electron microscope (Hitachi) operated at accelerating voltages of 6 and 3 kV, respectively. We newly fabricated Plate-TEM system equipped with a sample stage to tilt samples. Its experimental setup is described in the results section in detail. Using this tilt-stage, a tilt-series of SEM images was acquired in the range of ± 45° for obtaining the surface information of samples. A tilt-series of Plate-TEM images for tomography was also collected in the range of ± 60° with an increment angle of 2°. Subsequently, image alignment and 3D reconstruction were carried out with IMOD (Kremer et al., 1996) and image visualization was carried out with Avizo 6.0.0 (Mercury Computer Systems). After Plate-TEM examination, ultrathin sections were prepared, as mentioned above, and observed using an H-7100 transmission electron microscope (Hitachi) operated at an accelerating voltage of 75 kV.

3. Results

3.1. Experimental setup

The principle of Plate-TEM is shown in Fig. 1A. Biological samples are directly placed onto a transparent plate consisting of scintillating crystals such as GSO (Takagi and Fukazawa, 1983) and YAG (Autrata et al., 1978). The samples are inserted into a conventional scanning electron microscope and irradiated with an incident electron beam. The plate emits scintillation light (photons) produced by electrons that pass through the samples. Some of the incident electrons are backscattered and absorbed by dense materials inside the samples, which means that the quantity of penetrating electrons and emitted photons varies with the density of the samples. The photons emitted from the underside of the plate are detected using a photodetector. Thus, Plate-TEM enables transmission electron imaging of whole cells without the sample-sectioning processes. In addition, sample surface information can be obtained simultaneously by detecting secondary and/or backscattered electrons (SEs/BSEs).

To conduct electron tomography using Plate-TEM, we fabricated a sample stage for tilting the samples. The design of the equipment for Plate-TEM tomography is shown in Fig. 1B. The new tilt-stage was