Transmission Electron Microscope
HT7800 Series

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Application data

Life science

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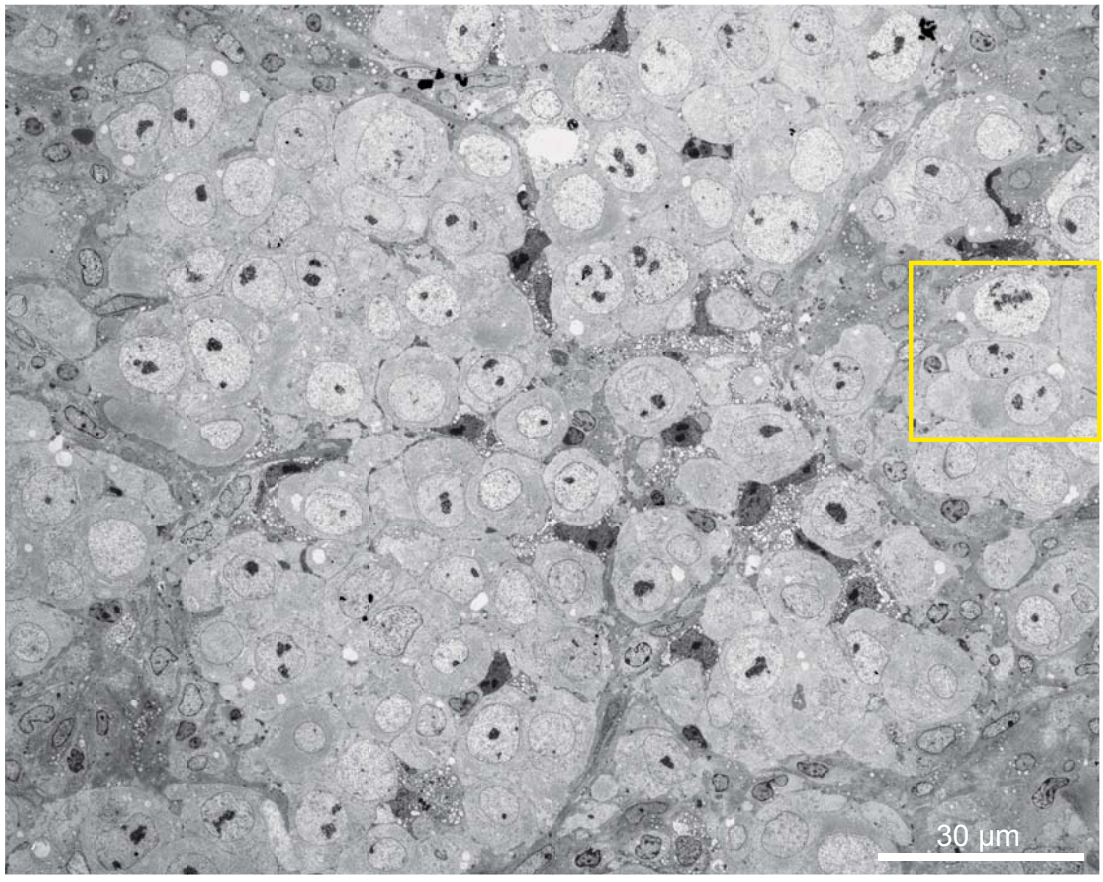
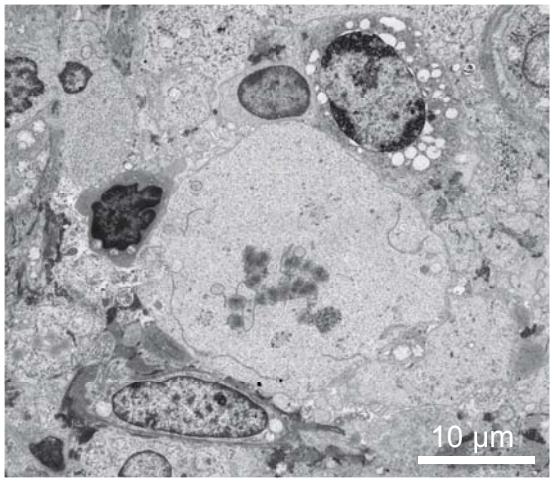
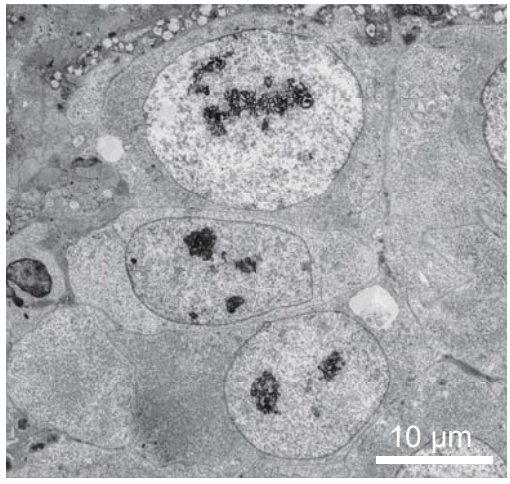
Pathological Tissue – Dysgerminoma –

The below images are of a resin embedded ultrathin section of a dysgerminoma. The top example is a single, seamlessly stitched image comprising of 16 individual tiled images – 4 vertical and 4 horizontal. The highly pixel-dense image covers an extensive area of 0.3 mm x 0.3 mm field of view that would otherwise not be obtainable with a single image. The high-resolution image allows many cells to be observed, as well as masses of tumor cells exhibiting chromatin aggregation. The bottom images are digitally enlarged to highlight the tumor cells, including cells that appear to be in prometaphase, which have lost their nuclear membranes and are exhibiting chromatin aggregation (bottom left). Additionally, when the area inside the yellow box is enlarged and examined, various cellular characteristics of dysgerminomas can be observed. One of them is the large percentage of the cells that are occupied by nuclei (bottom right). Therefore, it is possible to utilize highly detailed TEM images of pathological tissues to confirm cellular characteristics and/or status of a disease.



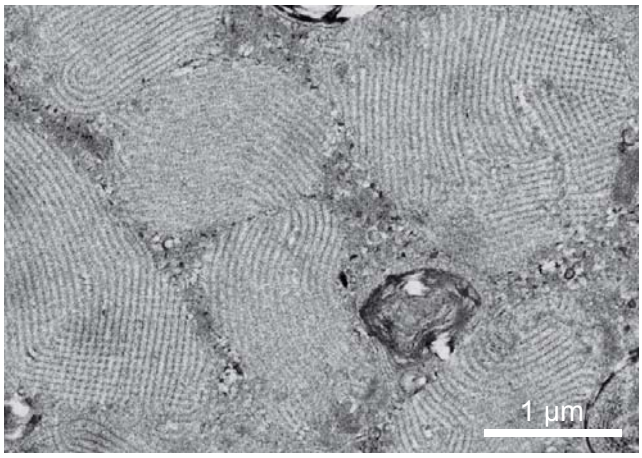
(Stitching photo image comprising 4 vertical and 4 horizontal, a total of 16 images)



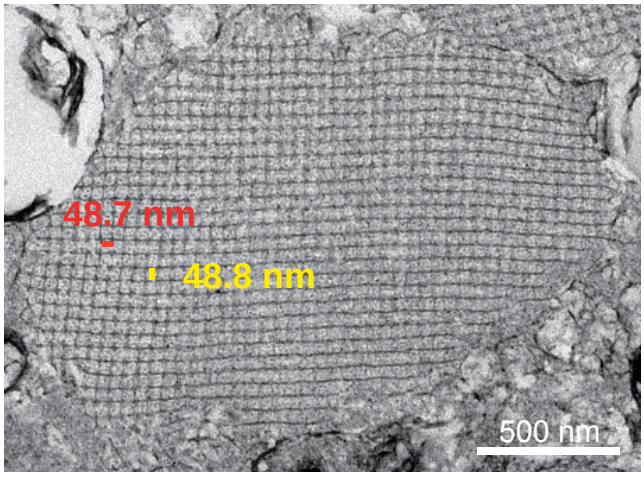


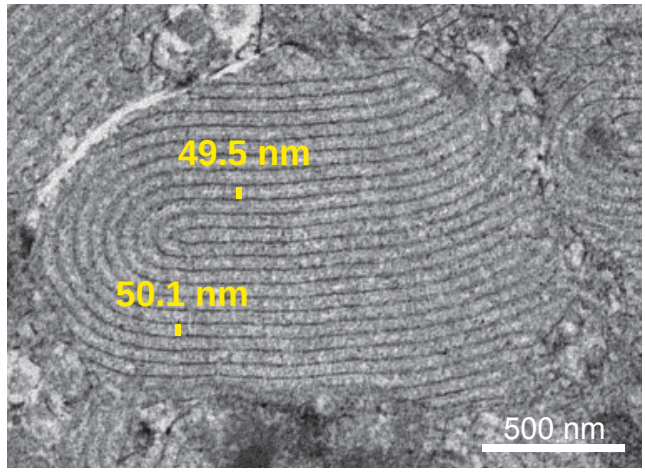
Specimen:
A human dysgerminoma,
Acceleration voltage: 80 kV,
Specimen courtesy:
Koichi Kawamura,
Waseda Research Institute
for Science and Engineering

Pathological Tissue – Rat Alveolar Epithelium –



The below TEM images are of a resin-embedded ultrathin section of a rat alveolar epithelium. Irregular myelin-like structures are distributed within the epithelial cells.
The bottom images highlight that this myelin-like structure has a periodicity of around 50 nm. These sequential surface-active substances, known as surfactants, form a thin cover over the alveolar surface. As a result, these surfactants weaken the alveolar surface tension, allowing the alveoli to easily expand.





Pseudocolor Display Function

Each instrument in the HT7800 series comes standard with the image processing software EMIP-EX. This powerful software package is equipped with various effective functionalities.
One function in particular is the Color Fitting function.
This feature turns typical grayscale TEM images into a pseudo color display.
The image to the right, illustrates that the cursor can be moved to change the color tone of the image, making it easier to see the periodic structure of the alveolar structure.

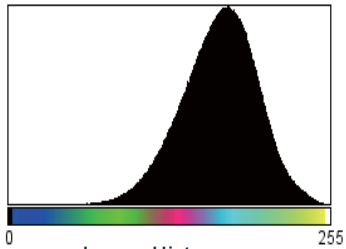
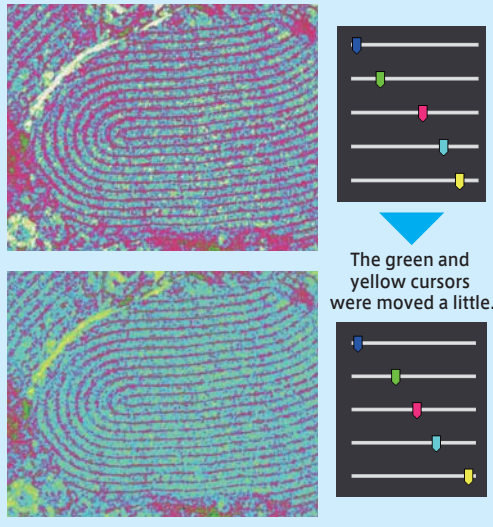


Image Histogram

The color tone of the image can be changed by selecting any of the 5 colors (blue, green, magenta, light blue, and yellow), and moving the cursor for each.



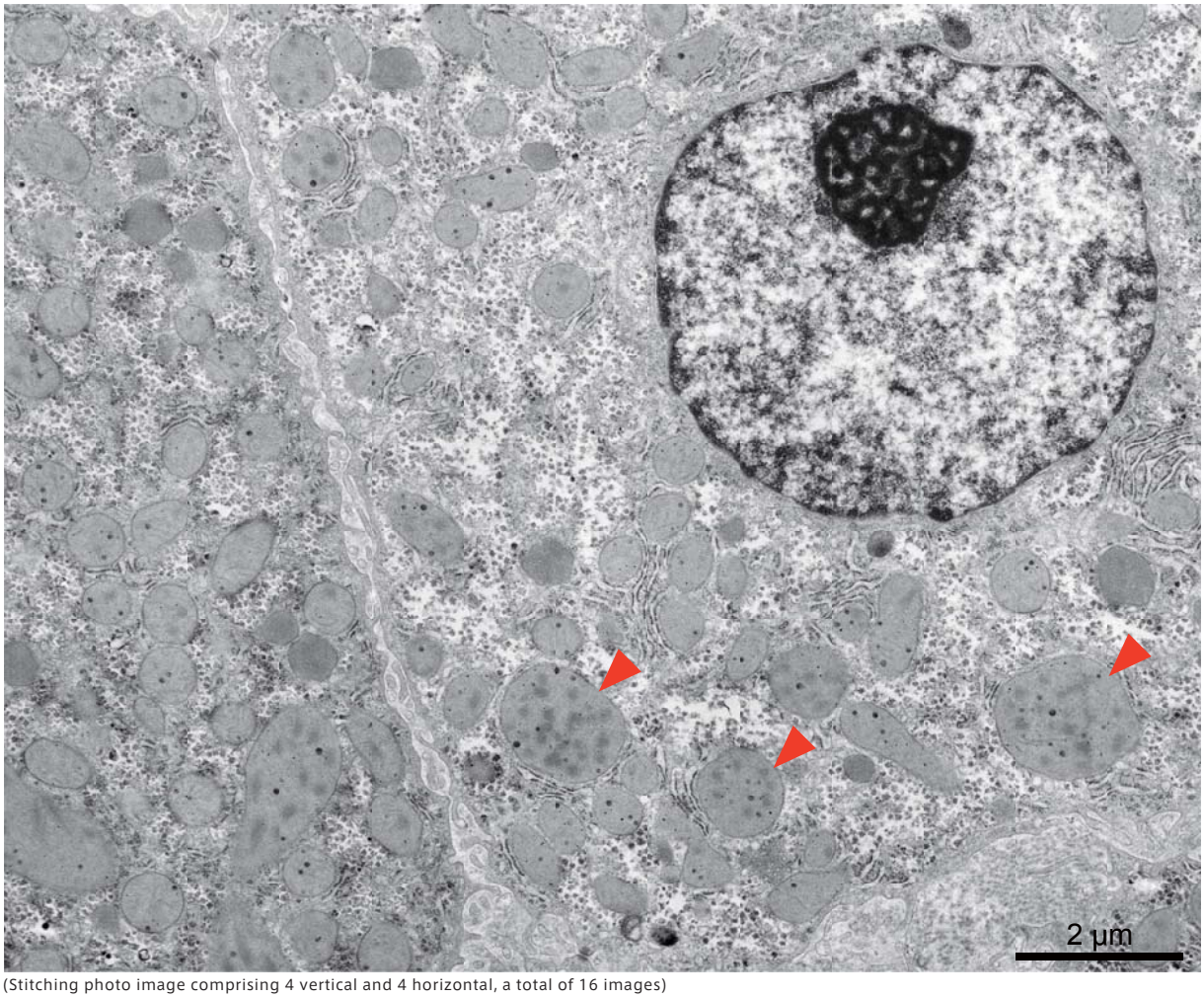
The green and yellow cursors were moved a little.

The color tone adjustment cursor settings and the color tone of the resulting image.

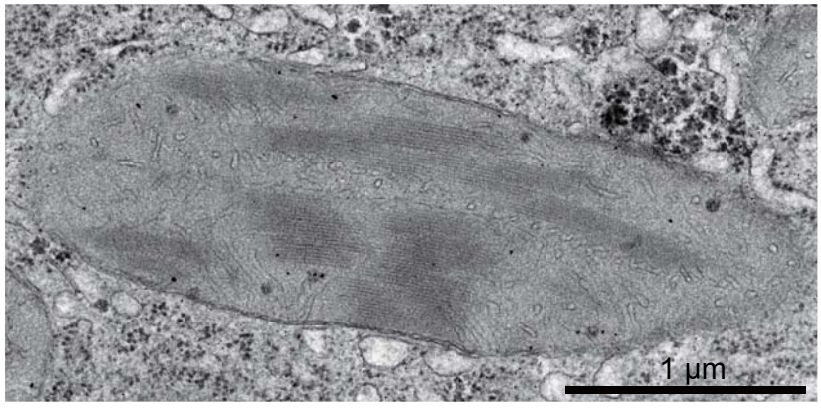
Specimen: a rat alveolar epithelium, Acceleration voltage:100 kV
Specimen courtesy: Kinji Ishida, Iwate Medical University

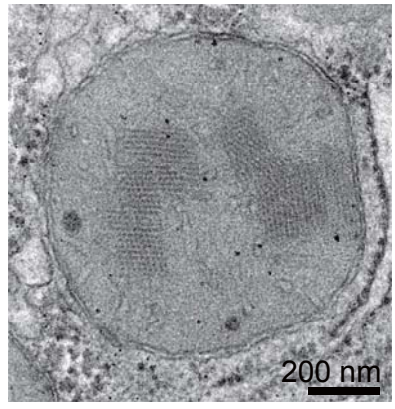
Pathological Tissue – Liver Disease –

This is an image of non-alcoholic steatohepatitis. The top image is a stitched image of a hepatocyte in which the cell nucleus is seen at high contrast, and many mitochondria are distributed around the nucleus. Some of the mitochondria (shown by the red arrows) have internal structures with high electron density. When shown highly enlarged, as in the bottom image, the internal periodic structure can be seen. Depending on the orientation of the section, the multilayer structure (image at bottom left) or the lattice-like structure in the transverse field of view (image at bottom right) can be seen. Thus, it is possible to observe the entire cell in detail, down to the detailed structure of the organelles.



(Stitching photo image comprising 4 vertical and 4 horizontal, a total of 16 images)

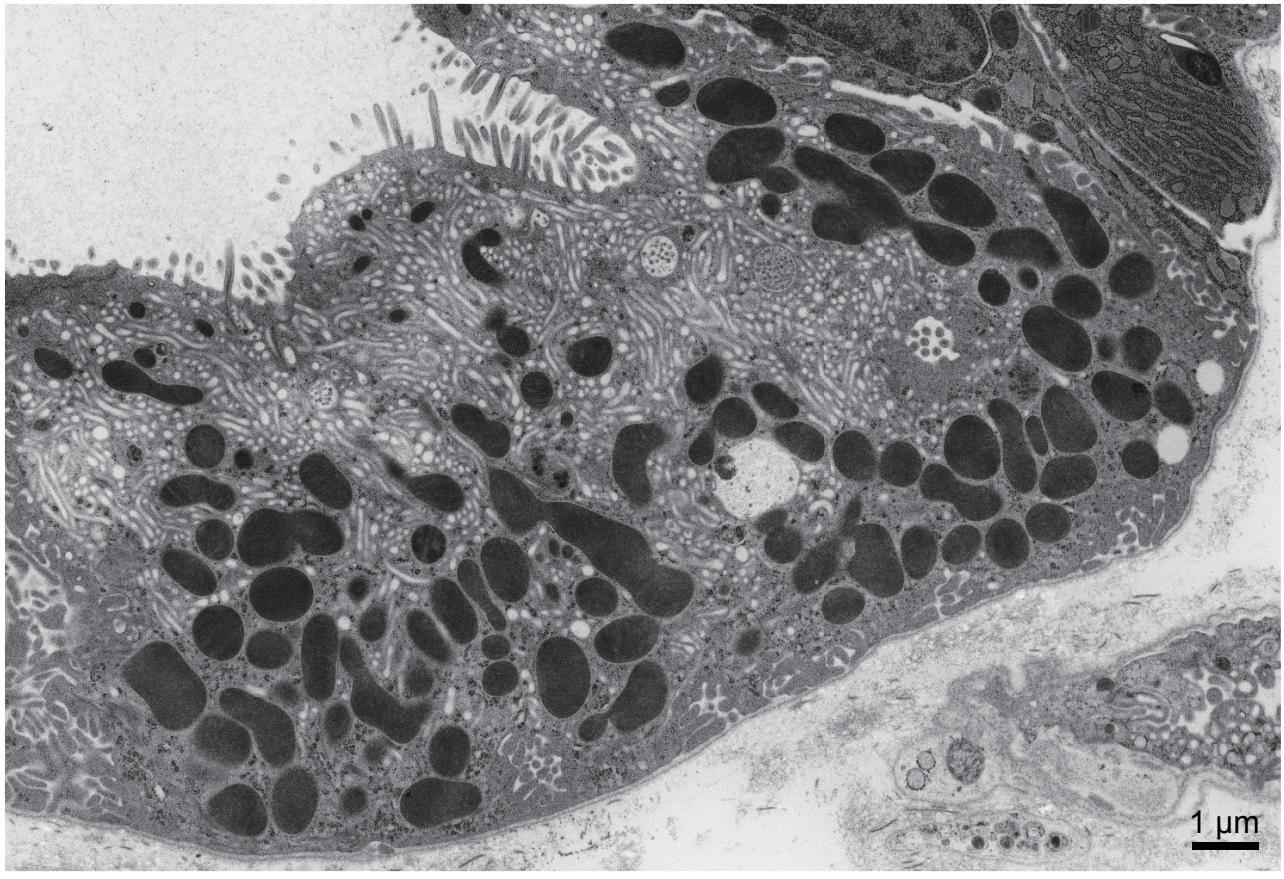




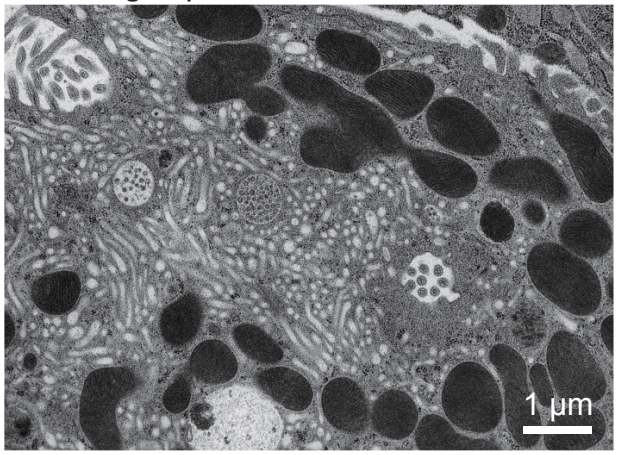
Specimen: human liver (non-alcoholic steatohepatitis), Acceleration voltage: 80 kV
Specimen courtesy: Kinji Ishida, Iwate Medical University

Ultrafine Patterns – Rat Stomach Mucosa –

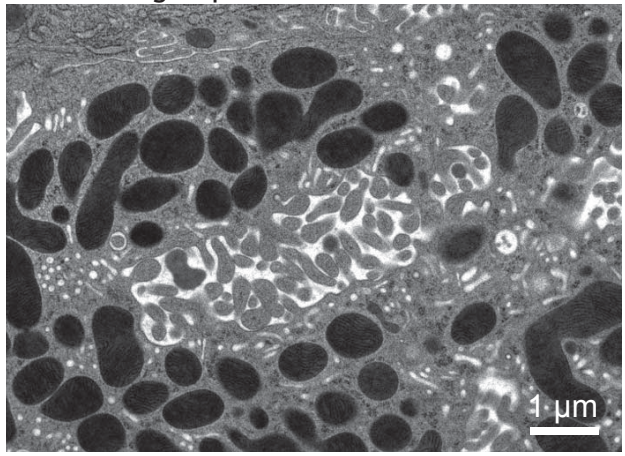
The below images are of ultrathin sections of rat stomach mucosa that were first high-pressure frozen, then resin embedded. The top image is a single, seamlessly stitched image comprising of 42 individual tiled images – 7 vertical and 6 horizontal. The highly pixel-dense image of a stomach mucosa with an inhibited gastric acid secretion covers an extensive area of 16 um x 22 um field of view. The high-resolution, high-contrast image makes it possible to see the distribution of fundic gland parietal cell tubular vesicles and mitochondria at low magnifications. When a portion of the image is digitally enlarged, multiple tubular vesicles can be seen in the fundic gland parietal cells (bottom left, inhibited group). Additionally, when gastric acid secretion was stimulated, tubular vesicles with high electron density can be seen (bottom right; stimulated group).



Inhibited group



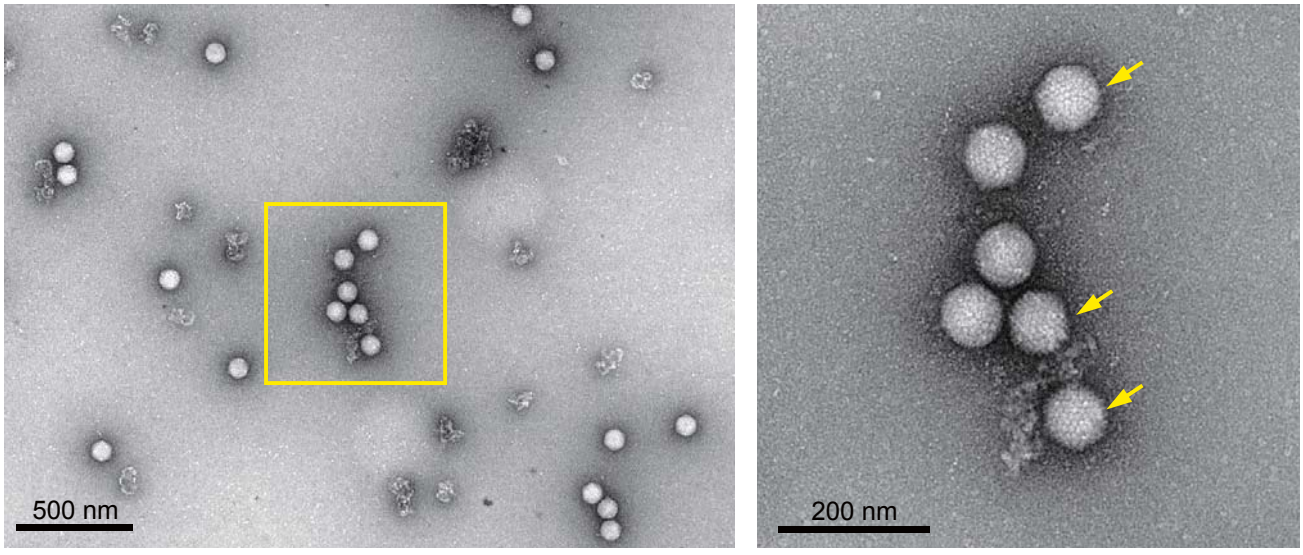
Stimulated group



Specimen: Rat Stomach Mucosa (Stitching photo image comprising 7 vertical and 6 horizontal, a total of 42 images), Acceleration voltage: 100 kV
Specimen courtesy: Prof. Akira Sawaguchi, Faculty of Medicine, University of Miyazaki

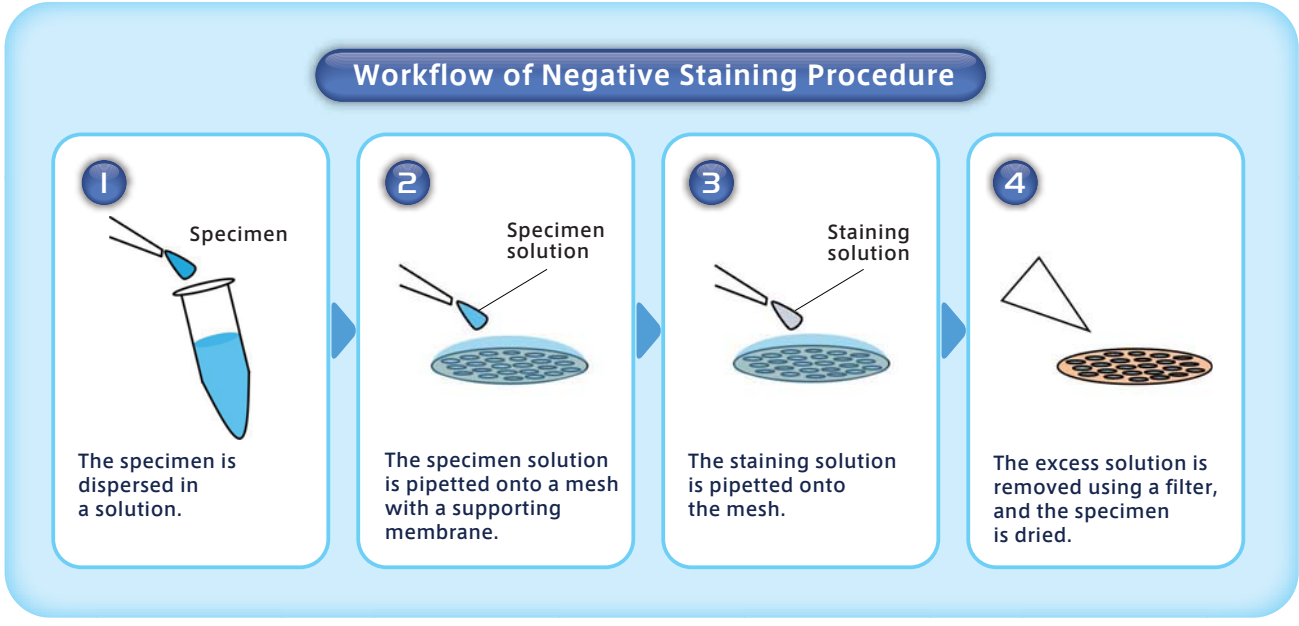
Negative staining – Adenovirus –

In the below application example, negative-stained adenoviruses, which are spherical particles of about 80 nm in diameter, are observed with high contrast. The yellow-framed area on the left TEM image has been digitally enlarged about 3 times to show detail on right. The surface structures of the adenoviruses (arrows) are explicitly observed. As shown in this example, it is possible to acquire high-pixel, high-definition TEM images of negative stained viruses.



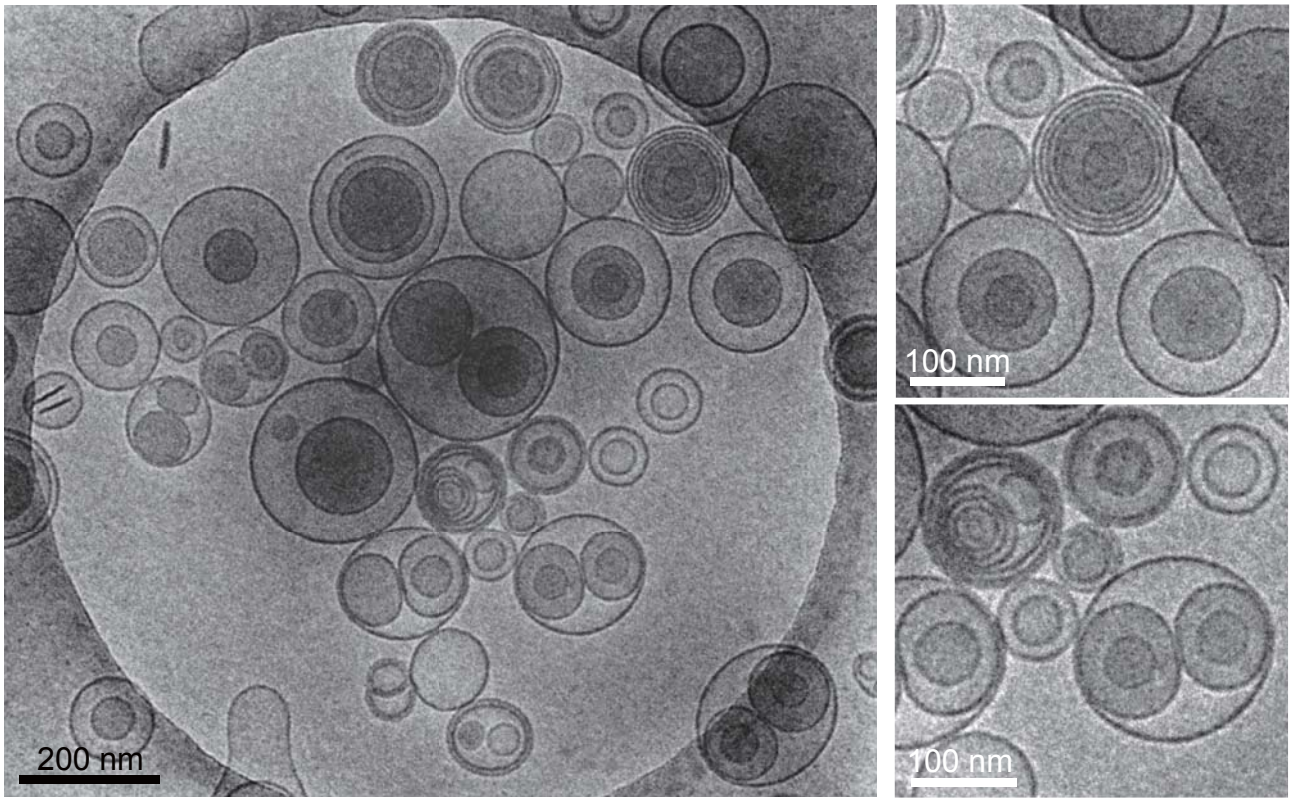
Specimen: Adenovirus,
Acceleration voltage: 120 kV

Negative staining is an effective method for enhancing the contrast of numerous microbial structures, such as virus spikes, bacterial flagella, or proteins, without disrupting cellular morphology. Heavy metals with high atomic numbers, such as uranyl acetate (UA), are commonly used to darkly stain the background and reveal the structural detail of the specimen due to the embellished contrast.



Cryo-transfer Image – Liposomes –

The below TEM images are of liposomes under cryogenic conditions of -175 ℃ (-283 °F). Such observations are possible by using the cryo-transfer technique with a cryo-transfer holder. This keeps the specimen close to liquid nitrogen temperatures in order to stay in its prepared state – vitreous ice-embedded via rapid freezing. One of the main benefits of cryo fixation is that the ultrastructural preservation is kept close to, or at, its native state. This is highlighted in the image below which clearly reveals the shape and the membrane structure of the liposomes. Additionally, adoption of a reduced electron beam, known as low-dose conditions, improves TEM images for electron-beam-sensitive materials such as the cryo specimens.



Specimen: Ice-embedded liposomes, Acceleration voltage: 120 kV, Temperature: -175 ℃

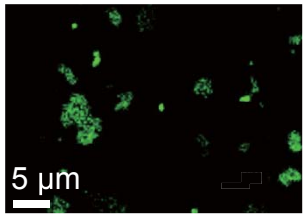
The rapid freezing method: How close ultrastructural preservation is kept close to its native state depends on many variables – one of the leading variables being the speed that cellular processes come to a halt. The method of rapid freezing gets its name by instantaneously fixating the specimen immersing it right into a coolant (e.g., liquid propane or liquid ethane). The resultant specimen is embedded in a thin layer of amorphous, vitrified ice which preserves the ultrastructural integrity.

Workflow of Rapid Freezing Procedure

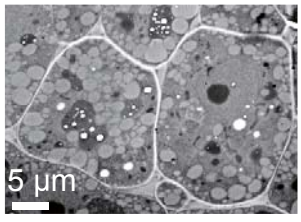
1. The mesh is picked up with tweezers and placed in the automated immersion freezing chamber.
2. The specimen solution is pipetted onto the grid.
3. Filter paper is used to absorb excess solution, and the specimen is then immediately immersed in liquid ethane and frozen.
4. Once frozen, the mesh is set into the cooled cryo-transfer holder and imaged by TEM.

Correlative light and electron microscopy (CLEM) – Peroxisome –

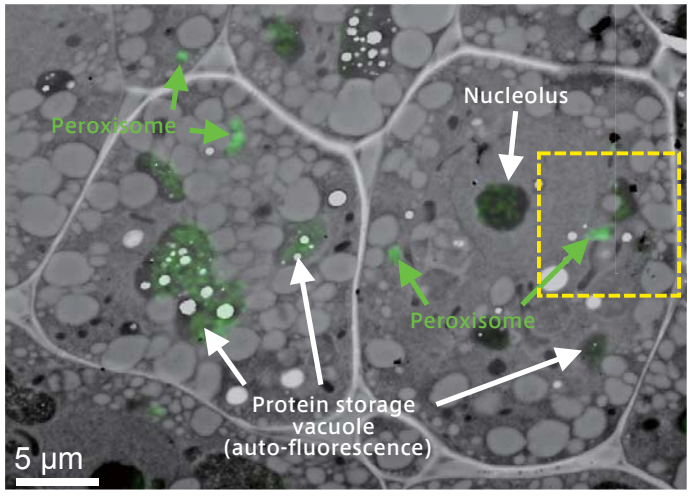
Correlative light and electron microscopy (CLEM) is an analytical method in which an optical microscope and an electron microscope are used to observe the same field of view, and then correlate the images. Below, we show a correlated image of GFP-labeled peroxisomes in the cotyledon of *Arabidopsis thaliana* during germination. By correlating the confocal laser scanning microscope (CLSM) and TEM images, it was possible to confirm the detailed structure and location of peroxisomes. In addition, HT7800 uses Hitachi's MirrorCLEM, a correlative light and electron microscopy system software that enables simple and rapid correlative imaging at the same location.



CLSM image



TEM image



Correlative image of the CLSM image and TEM image.

Enlarged yellow dotted line

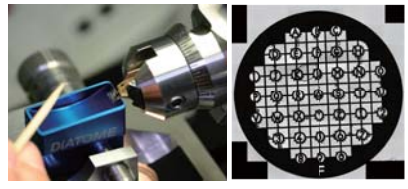
Specimen: Cotyledons of Arabidopsis thaliana,
Acceleration voltage: 120 kV,
Specimen courtesy:
Shoji Mano, National Institute for Basic Biology
Kiminori Toyooka, RIKEN CSRS

Workflow for Performing a TEM-CLEM Analysis

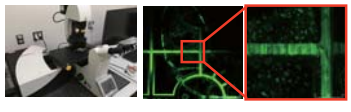
1 Fixation & Dehydration

2 Resin Embedding

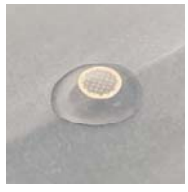
3 Sectioning



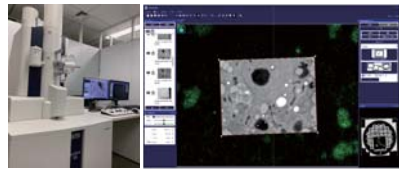
4 CLSM Observation



5 Electron Staining



6 CLEM Observation



Reference: [1] Toyooka K and Shinozaki-Narikawa N.: Microscopy, 68, 417-421 (2019)
[2] Mano S et al., :. Plant and Cell Physiology, 43, 3, 331–341

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＊Screen shows simulated image.

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