

## New Scanning Electron Microscope Capable of Observing Cells in Solution

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Optical microscope in combination with fluorescent stain is a powerful device for real time observation of cell macro-organelles. However, its resolution is limited upto 100 nm level, which is insufficient for observing smaller subcellular structures. Scanning Electron Microscope (SEM) is a powerful tool to obtain high-resolution image, however, the sample should be in vacuum. Recently, the capsule for liquid SEM imaging was developed. The film of polyimide [1] or silicon nitride (SiN) [2] was set as a window of an air-filled capsule facing the cells inside, and located in the sample chamber of SEM. The capsule is closed system, however, is limited in capacity to 15  $\mu$ l, and does not allow for prolonged cultivation or external drug administration.

To overcome these difficulties, we have developed a brand-new Atmospheric SEM (ASEM) with open culture system (Fig. 1). SEM had to be redesigned to inverted organization: the gun is set at the bottom and the SiN airlock-window, which equips culture dish (ASEM Dish) at the top. Although the thickness of SiN film is only 10 - 100 nm, the film endures 1 atmospheric pressure gap and is transparent to electrons. The ASEM Dish alone, when filled with medium of a few ml, can be used for prolonged cultivation of cells in CO<sub>2</sub> incubator. After fixation of the cultured cells, the dish is loaded onto the ASEM. Electron beam is projected upward through the SiN film to the cells, and the electrons backscattered through the film are captured by a detector positioned below for SEM imaging. Therefore, the enormous preprocessing including dehydration for the electron microscope observation is not required. Above the dish, optical microscope with immersion lens is set to realize quasi-simultaneous observation.

The high throughput and high resolution was demonstrated as the most vital aspects of the system. COS7 cells cultured in an ASEM Dish were fixed with glutaraldehyde. Their endoplasmic reticula (ER) were stained with a fluorescent dye for optical microscopy, and further stained with a heavy metal dye for SEM. It took about one minute to complete this relatively easy process. Locations of the ERs (green) were identified with the optical microscope, and the same area of view was photographed in the ASEM (Fig. 2). The ASEM obtained clear images at a magnification of x20,000, while the maximum magnification of the optical microscope was about x1,000. SEM had also achieved a resolution of 8 nm with a different sample of WGA-gold (15 nm) labeling. These results demonstrate that the ASEM is able to quickly observe the microstructure of cells in solution, which is difficult to achieve with optical microscopy. The other vital aspect is open ASEM Dish which is applicable to drug administration. After drug administration, their effects on cells can be observed using the optical microscope. At the decisive moment, the cells are fixed, stained and can be observe with the SEM.

The ASEM will also work as an immunoelectron microscope, enabling labeling of specific proteins by fluorescence/gold and acquisition of high resolution SEM images. This will facilitate detailed structural studies currently impracticable with optical microscopy, and will open doors for clinical studies and drug discovery/development.

References

[1] S. Thiberge, O. Zik, and E. Mosesa, Rev. Sci. Instrum. 75 (2004) 2280-2289.  
 [2] <http://www.2spi.com/catalog/instruments/silicon-nitride-membrane-window-grids-slot-square.html>.

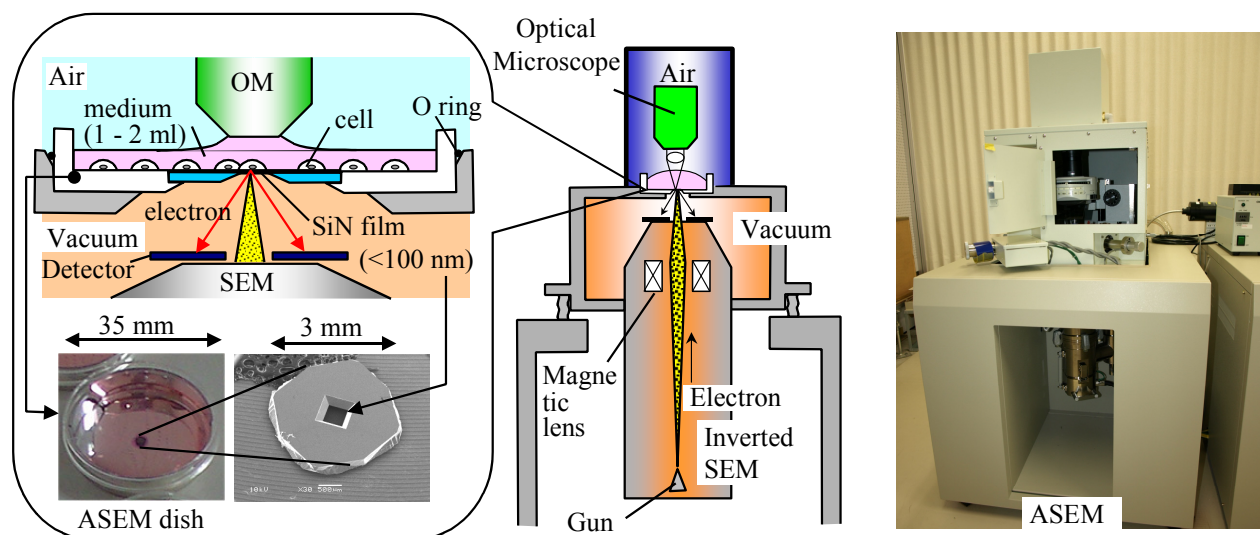


Fig. 1. Configuration of the ASEM. An optical microscope is arranged opposite an inverted SEM with the specimen dish between. The optical microscope is used to identify the cell organelle in solution using fluorescence. The dish, where cells can be cultured, features a SiN film window at the bottom. The film, while thin enough to transmit an electron beam, maintains atmospheric pressure. The electron beam is projected onto cells through the film, and backscattered electrons emitted from the cells are detected for the acquisition of high resolution SEM images.

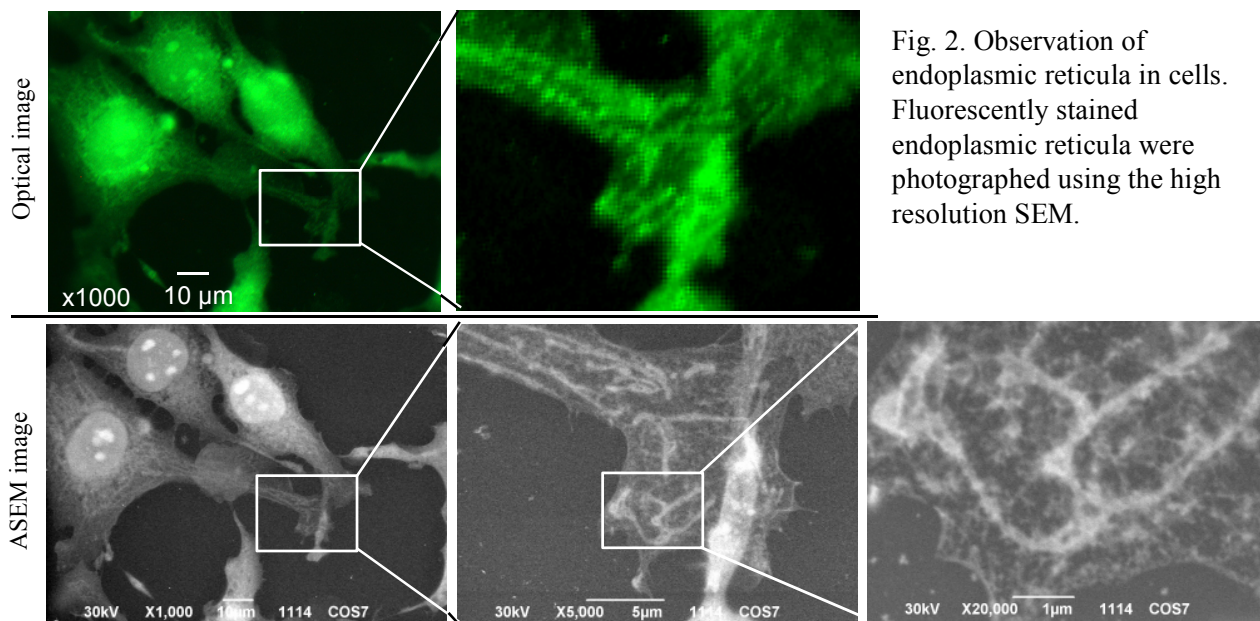


Fig. 2. Observation of endoplasmic reticula in cells. Fluorescently stained endoplasmic reticula were photographed using the high resolution SEM.