MONASH University
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Electron microscopy of cells and proteins in water and ice

Chikara Sato, PhD

National Institute of Industrial Science and Technology (AIST), Japan

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Abstract

Electron microscopy (EM) is now widely applied to the structure determination of proteins, and to the observation of macromolecular complexes in cells and tissues. We have applied transmission EM (TEM) single particle analysis (SPA) to determine the structures of successfully purified protein complexes, including ion channels, receptors and microtubules (MT). For in-solution observation of cells in natural aqueous environment, we have developed atmospheric Scanning EM (ASEM), realizing observation of water-immersed cells [1, 2]. The resolution of ASEM was 8 nm near SiN membrane [1]. ASEM realizes high throughput immuno-EM of cells without hydrophobic treatment, and makes it possible to observe intracellular supermolecular-complex formations. After the fixation of cells, axonal segmentation mechanism in primary culture neurons (Figure 1) [3] and accumulation of calcium sensor STIM-1 proteins in response to Ca^{2+} store depletion (Figure 2) [4] were visualized. Immuno-electron microscopy of megakaryocyte [5], bacteria [3], mycoplasma [6], rubella virus [3], ES cell [5] and immuno-dendritic cell [5] were successfully performed in solution using ASEM. Moreover, protein micro-crystals [7] and dynamic phenomena including electrochemistry [8] were imaged with ASEM. Using ASEM and SPA, we aim to merge molecular and cellular level studies for comprehensive understanding of cells.

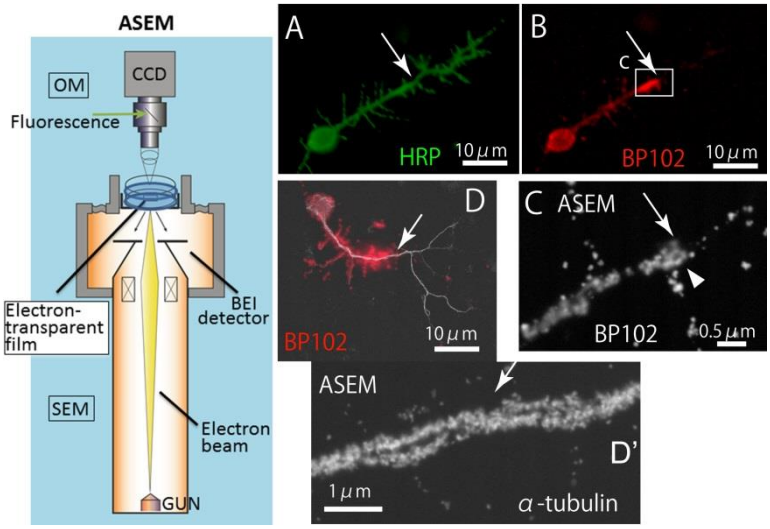

Figure 1: Configuration of the ASEM (left). ASEM enables light-electron correlative microscopy of intra-axonal boundary of primary-cultured *Drosophila* neurons immersed in aqueous solution. HRP distributed over the neuron (right), but BP102 distributed partly. The boundary formation might be related to the special structure of microtubules and its associated proteins.

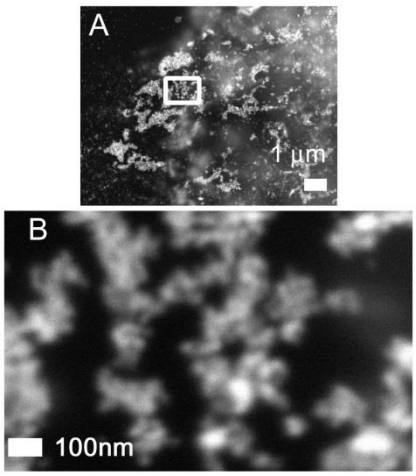

Figure 2: Gathering of Ca^{2+} sensor protein STIM1 on ER in response to Ca^{2+} store depletion. A, Immuno gold-labeled STIM. B, Higher magnification of the square in A at x100,000.

References

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Convenor: Dr Amelia Liu
Email: amelia.liu@monash.edu
Tel: 9905 58789

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