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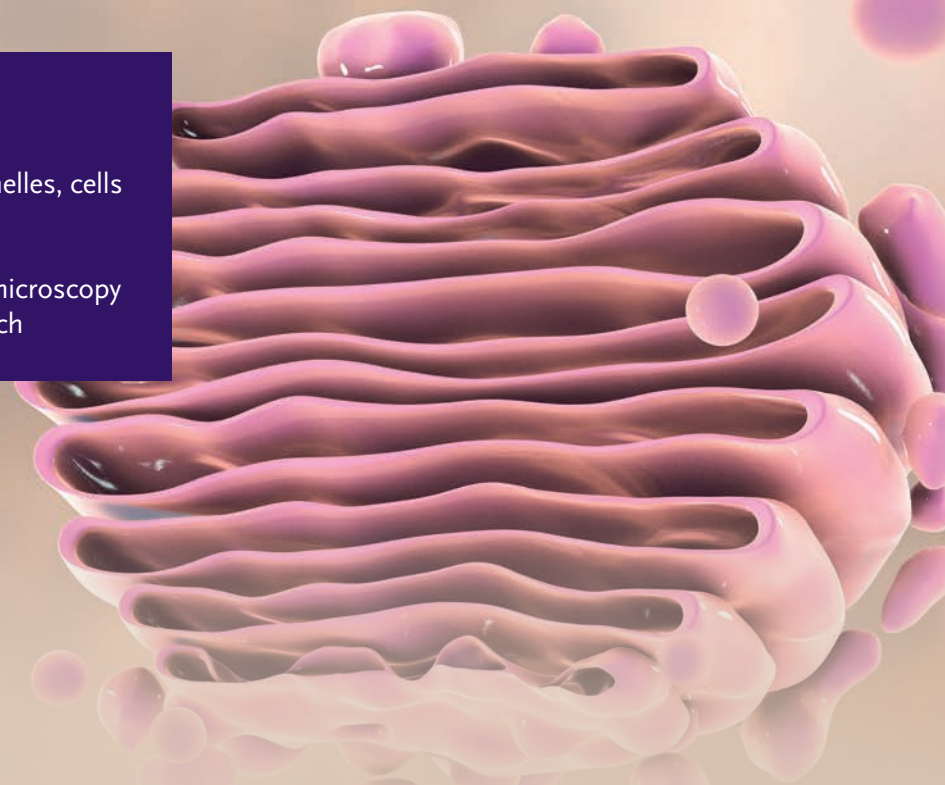
Learning and developing scanning electron microscopy (SEM) techniques for biological research

Daisuke Koga

Impact Objectives

- Reveal the 3D architecture of cell organelles, cells and tissues
- Learn and develop scanning electron microscopy (SEM) techniques for biological research



The extra dimension

Dr Daisuke Koga shares his passion for developing a new electron microscopy technique and talks about its broad applications

Can you outline your background as a specialist in scanning electron microscopy (SEM) for biomedicine?

I think that learning electron microscopy techniques is very important because I am passionate about the quality of electron micrographs. The main theme of my research is to reveal the 3D architecture of cell organelles, cells and tissues. To achieve this, I have learned and developed many SEM techniques for biological research: the osmium maceration method for observing membranous cell organelles; connective tissue digestion method for observing cellular structure hidden by connective tissues such as glands, blood capillaries and peripheral nerves; alkali-water maceration method for observing connective tissues; and the vascular casting method for revealing the vascular system. We recently established the novel 3D-SEM technique (serial section SEM and 3D reconstruction method) for analysing the 3D shape of cell organelles.

I am primarily interested in the morphology of the Golgi apparatus as well as SEM techniques for biomedicine. I have studied the 3D ultrastructure of membranous cell organelles by SEM of osmium macerated tissues since I was graduate student. In the process of the study, I have imaged many cell organelles, including the Golgi, endoplasmic reticulum (ER) and mitochondria in different cells and learned instrumental skills and principles in SEM.

How important is 3D reconstruction of consecutive SEM images for the analysis of biological specimens?

Morphological studies on biological specimens have been mainly performed by light microscopy and transmission electron microscopy (TEM) of a single thin or ultrathin section. However, it is difficult to understand the 3D architecture of tissues, cells and organelles by these approaches, because they have a complicated 3D structure. Therefore, serial section SEM is a powerful approach to imaging the 3D structure of biological specimens. One advantage is that consecutive sections on the glass microscope slide can be stored semi-permanently and imaged repeatedly by SEM. Serial section SEM also allows the observation of an extensive area of resin-embedded specimens. Several different resins can also be used as specimen embedding materials for serial section SEM, depending on the purpose of the study. Recently, I have succeeded in developing a novel serial section SEM technique incorporating immunostaining. The combination approach is very important for demonstrating the localisation of molecules within 3D reconstructed images. These are the advantages compared to related 3D-SEM techniques such as focused ion beam (FIB)-SEM and serial block-face (SBF)-SEM.

I think that serial section SEM is not only suitable for the analysis of the 3D structure of cells, tissues and cell organelles such as the Golgi apparatus, nucleus and mitochondria, but also the connectome. Consequently, serial section SEM (i.e. array tomography which was introduced by Micheva and Smith) is attracting worldwide attention in the field of microscopy. In the future, many researchers will learn serial section SEM technique and reports using this imaging technique will be published in scientific journals.

Can you talk about some of the technical expertise of your team members and academic partners in this work?

Satoshi Kusumi (Kagoshima University, Japan) is a chief academic partner. We have cooperated to develop or establish SEM techniques, including the combination method between the osmium maceration method and immunofluorescence (correlative light and scanning electron microscopy), SEM imaging of sections, serial section SEM technique and so on. We are both highly skilled in a lot of electron microscopy techniques, including ultramicrotomy and SEM techniques. Now, we are focusing on the correlative light and electron microscopy (CLEM) method, a novel imaging technique expected to bridge the resolution gap between light and electron microscopy images and relationship between the molecular distribution and ultrastructure of the target molecules/organelles. ●

Seeing in 3D

*Scientists at **Asahikawa Medical University** have developed a new electron microscopy technique that allows for the imaging of organelles in 3D*

Electron microscopy (EM) is a sophisticated imaging technique invented in the 1930s that can capture images of extremely small structures. Instead of using light as in standard microscopy, EM uses a beam of electrons. Electrons have a shorter wavelength than photons and therefore can report smaller structures than light can.

Dr Daisuke Koga of Asahikawa Medical University, Japan, is a world-leading expert in EM. He explains that electron microscopes can now focus on examining everything from relatively large structures such as tissues and whole cells down to individual protein structures. This makes the technique extraordinarily flexible and therefore useful to a wide range of biological fields, and experts in the technique are continually developing novel methods to examine an even wider range of biological features.

Through his extensive research efforts, Koga has developed a novel EM technique capable of constructing 3D images of the Golgi apparatus. This organelle has attracted the attention of morphologists for a long time because of its mysterious and beautiful structure. The morphological studies of the Golgi have been mainly performed by TEM. Although TEM of a single ultrathin section provides the ultrastructure of the Golgi, the 3D shape of this organelle remains uncleared. 'The Golgi apparatus is responsible for protein modification and trafficking,' he explains. The Golgi takes freshly synthesised proteins from the ER and adds additional features such as phosphoryl groups and sugars that confer on additional functions on the protein.' He says that it also packages proteins that need to be secreted out or even for storage. These functions are diverse and extremely important for normal cell operations.

3D printed model of the Golgi apparatus in a gonadotorope in the anterior pituitary.

MAKING 3D MODELS

Koga has now established serial section SEM that has the power to accurately reconstruct 3D images of various subcellular structures. His success arose from improvements in signal detection systems of SEM, which allowed for the imaging of ultrastructure information from tissue sections embedded in resins. He has combined these instrumental improvements with a unique sample preparation technique. By slicing hundreds of extremely thin sections of samples embedded in resin and imaging the regions of interest, the 3D structure of subcellular components can be revealed by 3D reconstruction of consecutive SEM images. 'I have revealed the entire 3D shape of the Golgi apparatus in different cells such as the epithelial principal cells in epididymis, pancreatic acinar cells, parotid acinar cells and cerebellar Purkinje cells using the serial section SEM and 3D reconstruction method,' outlines Koga. He has printed 3D models of the Golgi apparatus to clarify the 3D configuration of this organelle. Now, he is trying to reveal the 3D morphological diversity of the Golgi in the anterior pituitary tissue which comprises several kinds of endocrine cells (somatotrope, mammotrope, gonadotrope, thyrotrope and corticotrope). 'Going forward, I would love to discover the full 3D shape of the Golgi apparatus in cells throughout the body.'

RESOLVING POWER

Koga's creation of novel SEM methods makes imaging a wide range of important cellular and subcellular structures possible. Other important organelles are an obvious target, including mitochondria (the powerhouse of the cell) and the ER (the factory of proteins and lipids). 'Revealing these structures will play a crucial role in understanding how these organelles function and how they act in the 3D space,' notes Koga. 'Studying mutations and unusual examples of these structures could also reveal interesting pathologies only truly visible in 3D.' In short, Koga's techniques will add much-needed rich detail to our understanding of cells and organelles, building on both our biological and medical knowledge, as many diseases affect the functioning of organelles. ●

> *'Revealing these structures will play a crucial role in understanding how these organelles function and how they act in the 3D space'*

Project Insights

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BIO

Dr Daisuke Koga is Associate Professor at Department of Microscopic Anatomy and Cell Biology, Asahikawa Medical University, Japan. His research interest is to elucidate the 3D architecture of the Golgi apparatus *in vivo*. He is a specialist in SEM and recently established a novel 3D-SEM technique. By utilising this new method, he has demonstrated the entire 3D shape of the Golgi in various kinds of cells. Koga was awarded the Encouragement Award of the Japanese Association of Anatomist (2015), Encouragement Award of the Japanese Society of Microscopy (2016) and 'Microscopy Paper Award' (2018).

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