APPLICATION NOTE

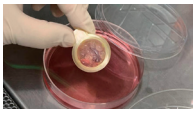
A1R HD25 Confocal Microscope

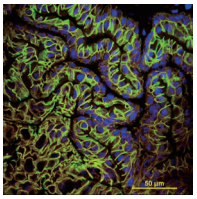
3D Imaging of Intestinal Organoid

To perform high resolution observation of the deep regions of specimens during confocal imaging, selecting the objective is very important. This application note, prepared with the cooperation of Dr. Hidenori Akutsu and Dr. Tomoyuki Kawasaki of the Department of Reproductive Medicine, Center for Regenerative Medicine, National Center for Child Health and Development, will introduce an example of 3D imaging of a small intestine organoid using two different objectives, comparing the difference in image quality between them.

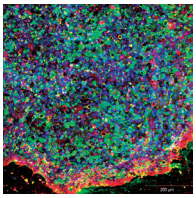
About the research

Akutsu et al. produced an intestinal organoid from human pluripotent stem cells which is utilized as a bio-model applied to research into and drug discovery for pediatric gastrointestinal disorders. The organoid is a "miniaturized organ" that possesses multiple cell types that exist in the target organ or tissue as well as their functions. Akutsu et al. were successful in producing by a unique method the intestinal organoid (mini-gut) that has a complex tissue structure and functionality similar to the human small intestine. This made possible the observation of human small intestinal tissue development and search for pathogeneses of illnesses, which up till then had been very difficult.





Mini-gut with intestinal mucosal epithelial tissue



Human ES cells

Experiment overview

Spherical aberrations, due to a discrepancy between the refractive index of the specimen or the encapsulant and the refractive index of the immersion liquid of the objective, may cause a decrease in contrast, image elongation, or other types of image quality deterioration when observing the deep regions of a specimen. In this experiment, the same location in the intestinal organoid was photographed with an objective that uses a silicone oil having a refractive index close to that of the encapsulant as the immersion liquid, and an objective that does not require an immersion liquid, and the resulting images were compared.

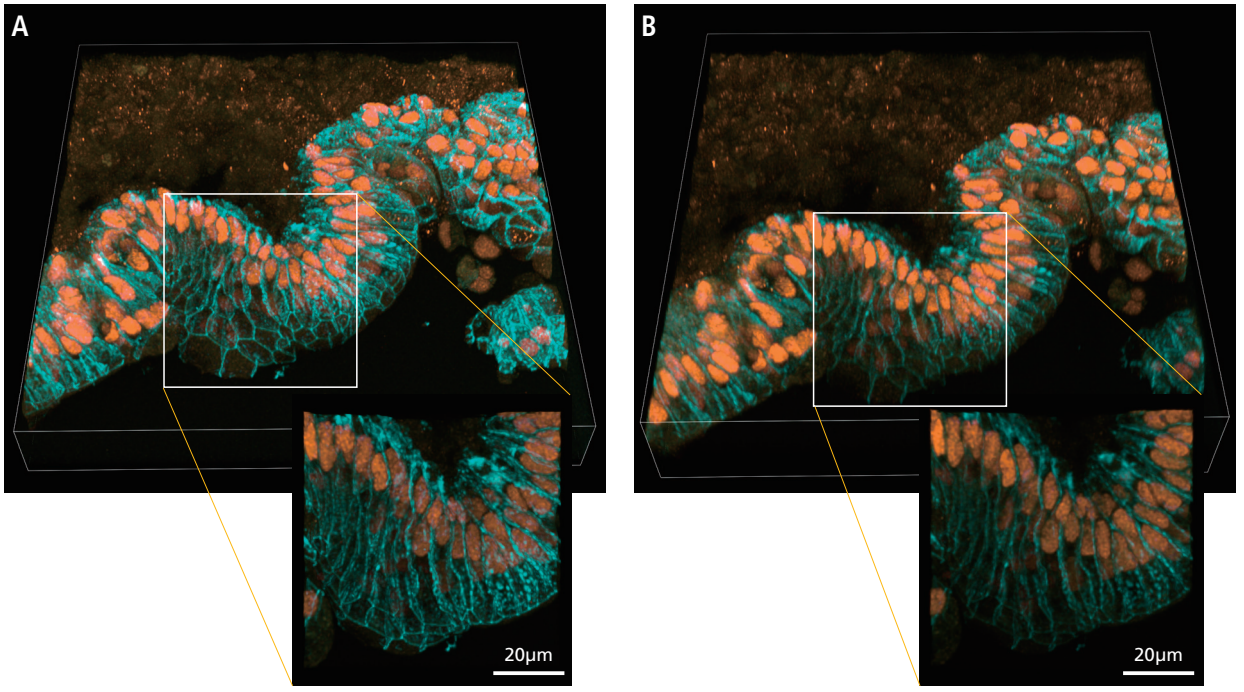


Fig. 1: Confocal 3D imaging of intestinal organoid

Z-stack images that captured a thickness of 30.5µm with two types of 40X objectives. Enlarged images cropped to the respective white frames are shown at the lower right.
The image acquired with the silicone immersion objective (Image A: CFI Plan Apochromat Lambda S 40XC Sil/ NA 1.25) is brighter than the image acquired with the dry objective (Image B: CFI Plan Apochromat Lambda 40XC/ NA 0.95) and enables high resolution observation of the structure and distribution of E-cadherin.
Cyan: Alexa488 (E-cadherin); Orange: Alexa546 (CDX2)

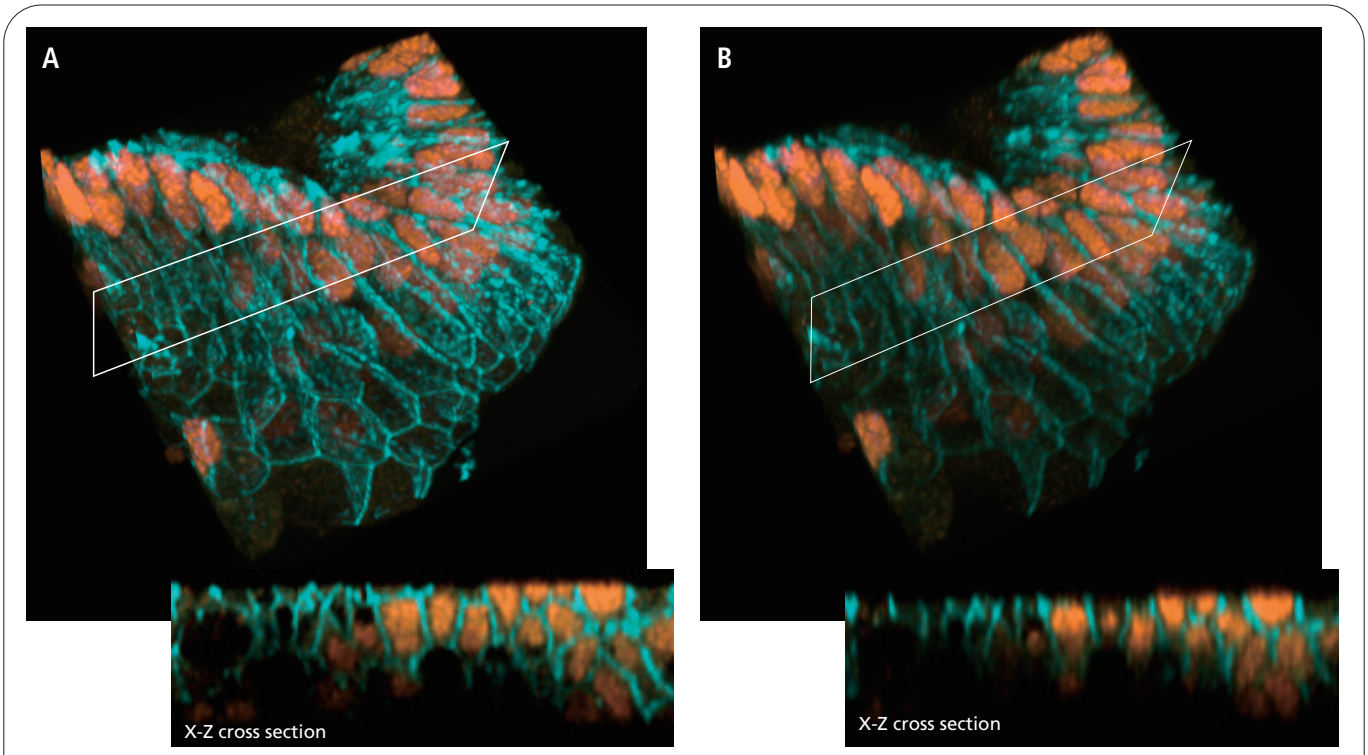


Fig. 2: Cross sectional images of intestinal organoids

3D display of Z stack images (Image A and B). The XZ cross section images indicated by the respective white frames are shown at the lower right.
The image acquired with the silicone immersion objective (Image A: CFI Plan Apochromat Lambda S 40XC Sil/ NA 1.25) is brighter down to the deeper region than the image acquired with the dry objective (Image B: CFI Plan Apochromat Lambda 40XC/ NA 0.95), and enables clear observation of the 3D structure of E-cadherin and the layered structure of the cells.

Summary

The CFI Plan Apochromat Lambda S 40XC Sil objective has a high numerical aperture among objectives of the same magnification, and is suitable for high resolution imaging. The localization of proteins in the tissue structure could be observed more clearly compared to the dry objective.
In addition, it was possible to observe the higher-order structure of intestinal organoids brightly and clearly down to the deep region by selecting a silicone immersion objective whose immersion liquid had a refractive index close to that of the encapsulant. Thus, when imaging, it is important to use the appropriate objective for the purpose of the experiment.

It has been suggested that the disturbance of intracellular localization of a specific protein is a trigger for some congenital bowel diseases. Research on unexplained intestinal diseases and development of cures are expected to progress through the utilization of high-resolution confocal imaging of disease organoids.

Acknowledgement

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Product Information

A1R HD25 Confocal Microscope

This is a confocal microscope with the widest field of view (25 mm) in the industry. It enables a large area to be observed and photographed at once, resulting in more efficient observation of large specimens.

CFI Plan Apochromat Lambda S 40XC Sil

This is a high numerical aperture objective suitable for multicolor imaging using a confocal microscope, and corrects chromatic aberration over a wide wavelength range, from the visible to the near infrared.
It enables the acquisition of high resolution images down to the deep regions of a specimen by using silicone oil that has a refractive index close to that of biological specimens as the immersion liquid.