APPLICATION NOTE

AX Confocal Microscope

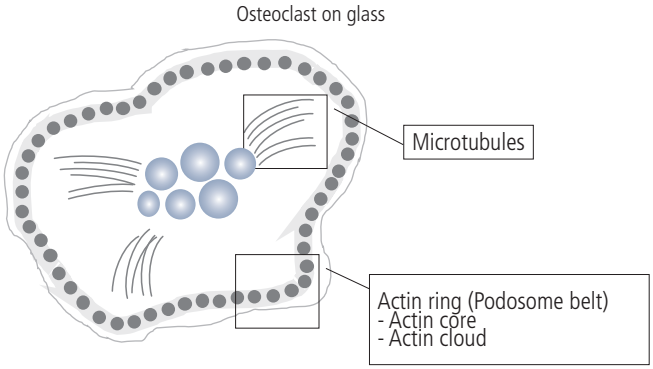
Wide FOV, High-Resolution Confocal Imaging of Podosomes in Osteoclasts

~ Macro and micro observation ~

Dr. Tadahiro Iimura, Dr. Ji-Won Lee, et al. of the Department of Pharmacology, Faculty and Graduate School of Dental Medicine, Hokkaido University have been studying lifelong changes in bones and joints from the viewpoint of skeletal development, maintenance of homeostasis, aging, and pharmacology. There is a ring structure called the "actin ring" on the adhesive surface between osteoclasts and bone, and electron microscope level resolution was required up until now in order to observe the microstructure of "podosomes", which are the components of this actin ring. This application note introduces examples of osteoclast macro-observation and podosome micro-observation using the new generation AX confocal microscope, and quantitative analysis thereof.

Research Background

The actin ring has a ring-like structure wherein pseudopodia (podosomes) accumulate with actin fiber bundles as their axis (figure on right).
In previous studies, Dr. Iimura and Dr. Lee have shown that podosomes can be quantitatively observed with optical microscope resolution when using a super-resolution microscope. Furthermore, they revealed that chemokine receptors (CCR5) are present in osteoclasts that destroy and absorb bone, and that these are involved in the regulation of osteoclast function.
In osteoclasts of CCR5-deficient mice, and in human bone cells treated with the CCR5-antagonist Maraviroc, the formation of actin rings, which are important for the adhesion and motility of osteoclasts, is inhibited and the bone resorption activity of osteoclasts is reduced (see the reference).



8K ultra-high pixel count enables micro observation using a low magnification objective

Previously, high-magnification objectives were essential for micro observation, and low-magnification objectives could not meet the resolution requirements (optical resolution and pixel count). With 8K pixels, the pixel count problem has been solved, and individual podosomes can now be observed with low-magnification objectives, enabling quantitative evaluation (Fig. 1).

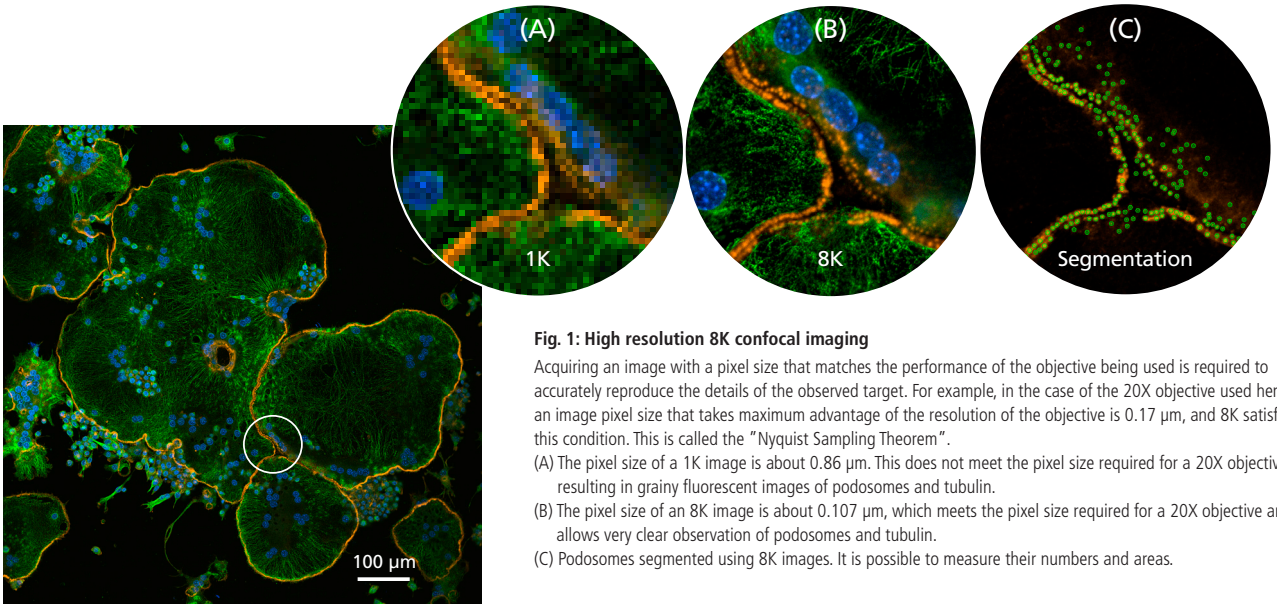


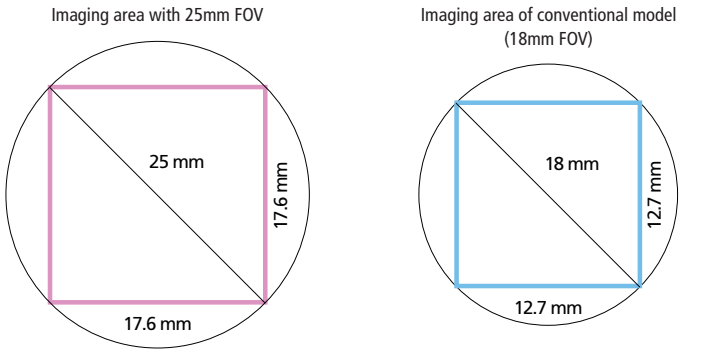
Fig. 1: High resolution 8K confocal imaging

Acquiring an image with a pixel size that matches the performance of the objective being used is required to accurately reproduce the details of the observed target. For example, in the case of the 20X objective used here, an image pixel size that takes maximum advantage of the resolution of the objective is 0.17 µm, and 8K satisfies this condition. This is called the "Nyquist Sampling Theorem".
(A) The pixel size of a 1K image is about 0.86 µm. This does not meet the pixel size required for a 20X objective, resulting in grainy fluorescent images of podosomes and tubulin.
(B) The pixel size of an 8K image is about 0.107 µm, which meets the pixel size required for a 20X objective and allows very clear observation of podosomes and tubulin.
(C) Podosomes segmented using 8K images. It is possible to measure their numbers and areas.

Capturing large osteoclasts in one shot

Low-magnification objectives have a large observation field, and macro-observation of large osteoclasts of hundreds of microns is possible when such objectives are combined with the large field of view (25mm FOV) of the AX confocal microscope (Fig. 2A, Fig. 2B). While high-magnification objectives have high resolution, they have a small observation field of view that cuts off part of the osteoclast (Fig. 2C).

(A) When a 1X objective is used



What is FOV number?

The field of view (FOV) number is a value that represents the size of a field of view. The actual imaging field (diameter) is called the actual field of view, and is calculated as follows: Actual field of view (mm) = FOV ÷ objective magnification ÷ zooming magnification. For example, when an image is acquired with 25mm FOV, 1X objective and 1X zoom, the formula is 25÷1÷1=25, meaning the actual field of view is 25 mm in diameter (17.6 mm square) (figures on left).

(B) When a 20X objective is used

(C) When a 100X objective is used

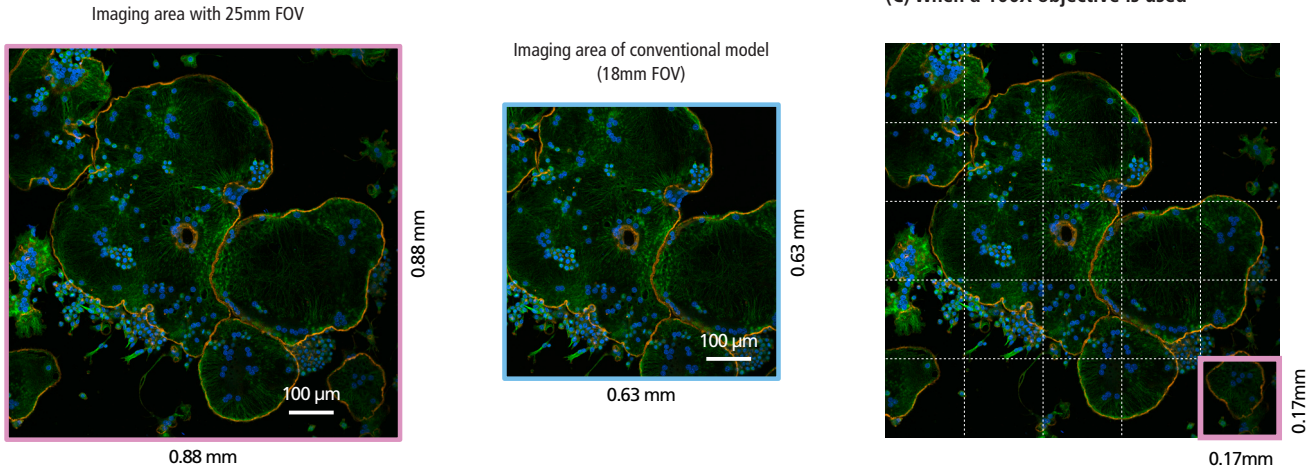


Fig. 2: Large field observation with 25mm FOV

(A) The new generation AX confocal microscope has a 25 mm diagonal observation field of view, which is approximately twice the area of a product with an 18 mm field of view.
(B) Images observed using a 20X objective. With a 25 mm field of view, large osteoclasts can be captured in a single shot. Meanwhile, in the case of 18 mm fields of view, the entire image of the osteoclasts does not fit in the observation field.
(C) In the case of a 100X objective, while high resolution observation is possible, the field of view that can be observed at one time is small. Image stitching is necessary to capture the entire image of the osteoclasts.

Result and Summary

There is a trade-off between high resolution and large field of view, making it difficult to achieve both micro- and macro-observations. In this study, using the new-generation AX confocal microscope with a large 25 mm field of view and an 8K ultra-high pixel count, it was possible to evaluate the formation of pseudopodia (podosomes) of osteoclasts comprehensively and quantitatively, while capturing the entire picture of osteoclasts using a low magnification objective.

Osteoclasts are target cells for therapeutic agents for bone and joint disorders, including osteoporosis. Due to the technological innovation of confocal microscopes, further contribution is expected for the analysis of pathological mechanisms and the evaluation of pharmacological action.

Reference

The HIV co-receptor CCR5 regulates osteoclast function.
Nature Communications 2017, DOI: 10.1038/s41467-017-02368-5

Product Information

AX Confocal Microscope

Achieves high resolution images of 8192 x 8192 pixels, which are four times that of conventional models. With a large diagonal field of view of 25 mm, wide areas of samples can be acquired at once, reducing phototoxicity. An automatic shading correction function enables acquisition of images with uniform brightness.