APPLICATION NOTE

A1 R/AX R Confocal Microscope

High-Definition Imaging of Mouse Neuromuscular Junction Using a Resonant Scanner

Since a resonant scanner can perform confocal imaging with higher temporal resolution than a galvano scanner, it is used in many cases to acquire life phenomena occurring at high speeds. In contrast, because the resonant scanner of the new generation AX R confocal microscope system supports up to 2K x 2K acquisition, it can be used for a wide range of purposes, from high-speed imaging to high-resolution imaging. This application note introduces examples of the structure of a neuromuscular junction in a mouse captured by high-definition 3D imaging using a resonant scanner.

Keywords: high speed XY-Z, high resolution, large FOV

High Resolution Imaging of Neuromuscular Junction

In a neuromuscular junction (NMJ), the neurotransmitter acetylcholine is released from the nerve endings of motor neurons and received by acetylcholine receptors present in muscle cells, inducing their depolarization and contraction. Motor neurons extend into the interstices of the muscle fiber structure like the growing branches of a tree (Fig. 1A, 1B). Images of nerve endings at the tips of motor neurons, which adhere to the muscle cells as if grasping them, and the acetylcholine receptors distribute around the site were acquired (Fig. 1C).

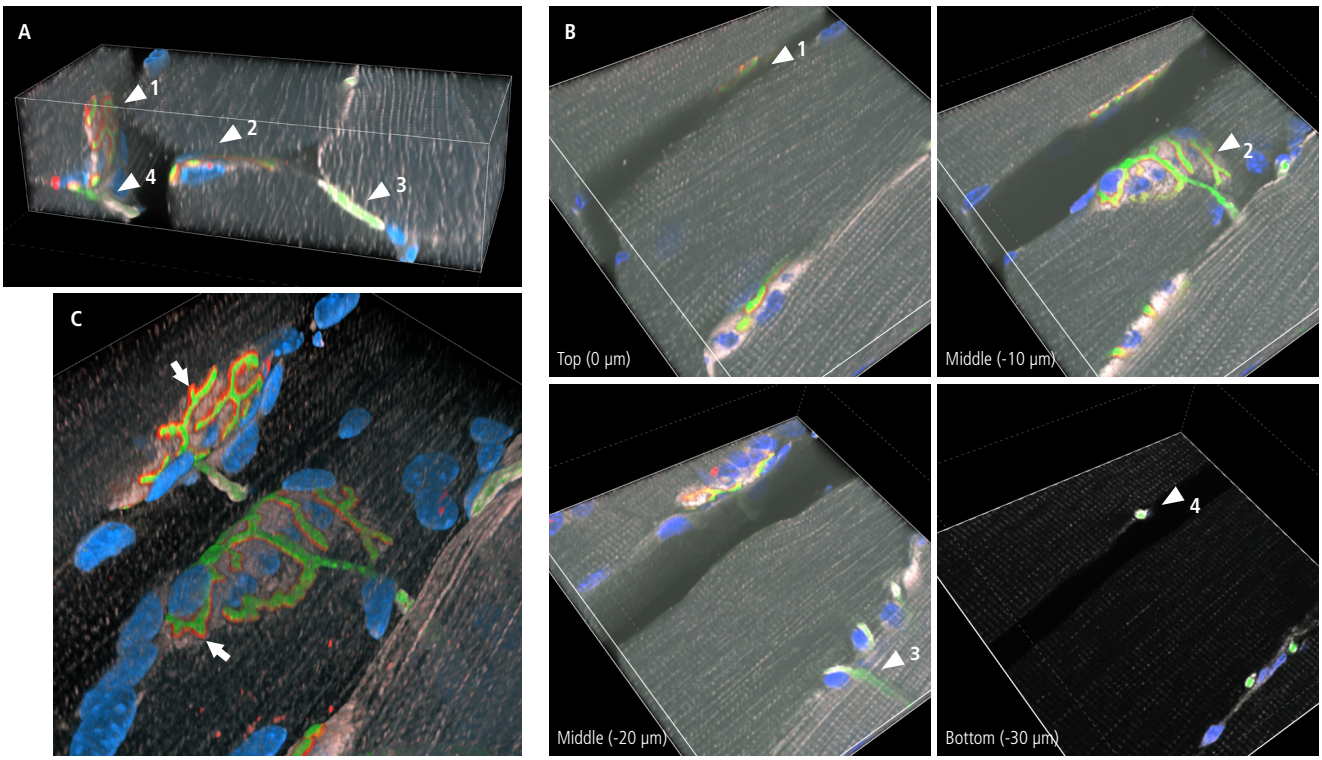

Fig. 1: High Resolution 3D Imaging of Neuromuscular Junction (NMJ) in a Mouse

(A) Three-dimensional display of NMJ taken with a resonant scanner. Neurons (green) extend into the interstices of muscle fibers.

(B) Three-dimensional image (A) displayed at different depths (Top 0 µm, Middle -10 µm, Middle -20 µm, Bottom -30 µm). Points indicated with white arrows in (A) are located in different Z positions.

(C) Nerve endings adhering to muscle cells. The white arrows indicate the distribution of acetylcholine receptors (red) surrounding the neurons (green).

DAPI: cell nucleus (blue), GFP: neurons (green), Alexa 555: acetylcholine receptor (red), CMDR: cell membrane (gray)

Objective: CFI Plan Apochromat Lambda S 25xC Sil

Not Only High Resolution, but Large 2K Field-of-view Imaging

The muscle tissue observed in this example is several millimeters in size, and NMJs are widely distributed. Since the NMJs extend three-dimensionally, it is also necessary to capture a deep area in the Z direction to find the desired part with a microscope. Since a 10x objective can capture a large field of view of close to 1.8 mm at one time, the entire muscle tissue can be captured with just a few tiled images (Fig. 2A). Furthermore, since the resonant scanner can acquire Z-stack images in a short time while maintaining a large field of view, it is easy to find the observation target (Fig. 2B). In addition, a confocal zoom makes higher resolution observation possible. The resonant scanner of the AX R supports up to 2K imaging, enabling image acquisition of an area four times larger than a conventional model at the same XY resolution. To obtain the same pixel resolution, a 5x zoom is required in a 1K image with a conventional model, but a 2.5x zoom is sufficient for 2K images with the AX R, allowing image acquisition of a larger area. This makes it possible to acquire not only the entire image of the neuromuscular junction, but also the structure around it. In short, 2K is very effective not only for high-resolution imaging, but also for wide-field observation of large samples. (Fig. 2C, 2D).

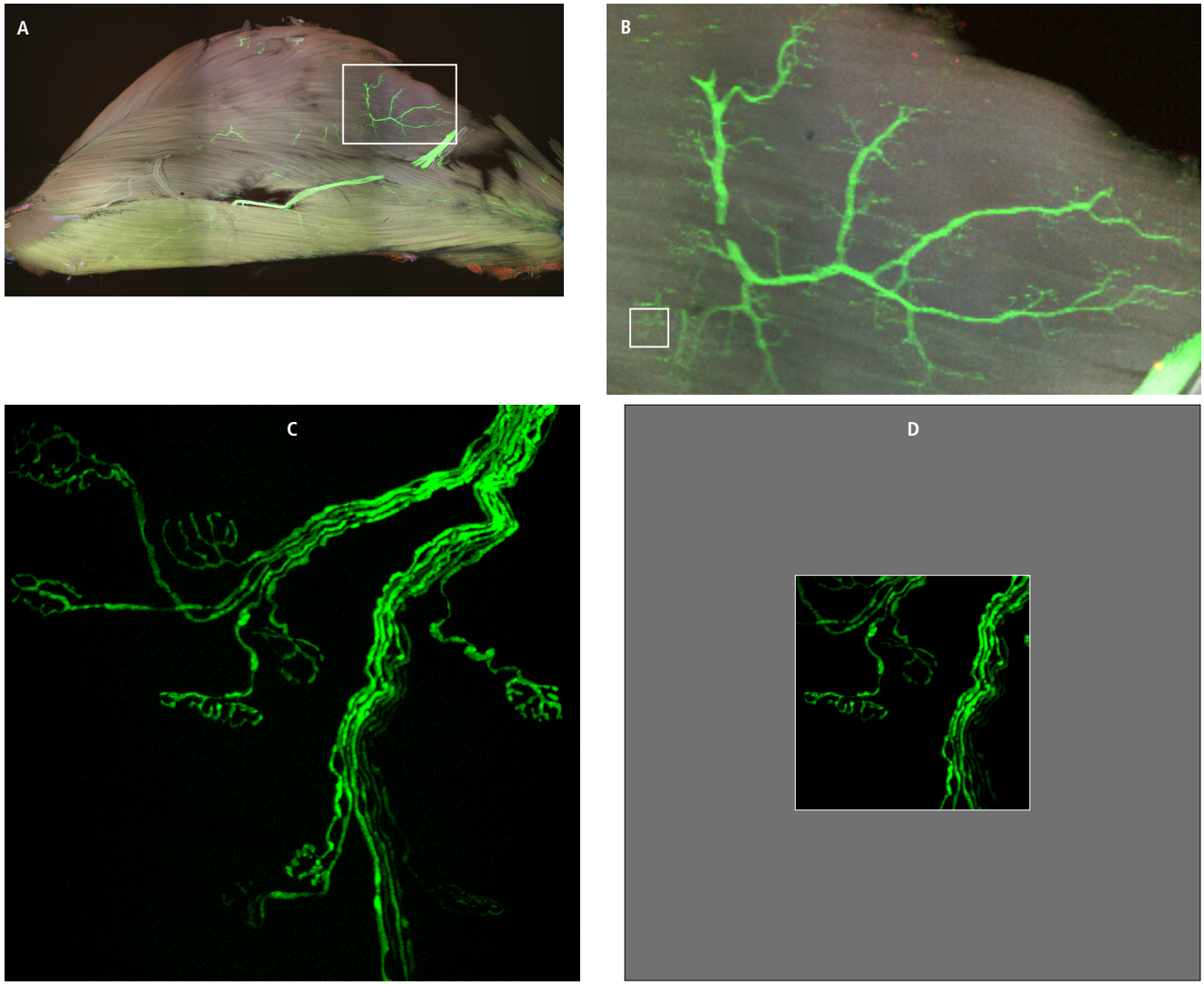

Fig. 2: Large Field-of-view Imaging Using a Resonant Scanner

(A) Entire view of mouse muscle tissue. Using a resonant scanner, 6 images were tiled during Z-stack imaging. Image resolution: 1024 x 1024 pixels. MIP display of captured Z-stack images. Objective: CFI Plan Apochromat Lambda 10X

(B) Enlarged display of the white frame in (A). The NMJ extends over a wide area in three dimensions within the muscle tissue.

(C) The area indicated by the white frame in (B) was imaged by confocal zooming using a 25X objective so as to obtain Nyquist resolution (0.123 µm/pixel). Using the 2K resonant scanner, an area of 0.25 mm^2 can be observed.

(D) Using the 1K resonant scanner, an area of 0.12 mm^2 can be observed.

Results and Summary

This application note has introduced examples of muscular tissue observation using a resonant scanner. Pixel resolution up to 2K is selectable for the new generation AX R confocal microscope system, which can be used for analyses of morphologies and localizations in observation areas with multiple differing structures, such as NMJs. Also, the 2K resonant scanner of the AX R is highly suited to observation of large samples, since it can acquire wide-field images of areas four times larger than that of a conventional 1K resonant scanner with the same pixel resolution using a confocal zoom.

Product Information

AX/AX R Confocal Microscope

These microscopes achieve high resolution images of 8K x 8K pixels, which is four times that of conventional models.
A large FOV with a diagonal of 25 mm allows acquisition of a large area of samples in a single scan, reducing phototoxicity. The AX R's resonant scanner achieves a high resolution of 2K x 2K pixels, allowing acquisition of live sample dynamics with high-speed imaging of up to 720 fps (2048 x 16 pixels).