APPLICATION NOTE

AX R Confocal Microscope

High-speed, high-resolution 3D imaging using resonant scanner and Denoise.ai

Although resonant scanners are suitable for live cell imaging as they enable confocal imaging with high temporal resolution, in terms of image resolution, galvano scanners are superior. However, the resonant scanner of the AX R confocal microscope system can capture high-resolution images at high speed because it achieves 2K pixel resolution. Moreover, using Denoise.ai, the AI module of NIS-Elements imaging software, can remove shot noise generated by resonant scanning. As this can shorten exposure times and reduce photobleaching, it is also effective for capturing images of fixed samples. This application note introduces examples of acquisition of high-speed, high definition images using a resonant scanner together with Denoise.ai.

High-speed Z-stack imaging using a resonant scanner

At neuromuscular junctions (NMJ), the neurotransmitter acetylcholine is secreted from the nerve endings of motor neurons. Depolarization of muscle cells and muscle contraction is induced when the acetylcholine receptors in muscle cells receive the acetylcholine. Using a high-speed resonant scanner, the structure of a NMJ and the filamentous structure inherent to muscles called a sarcomere could be observed over a short period of time (Figure 1).

A resonant scanner can capture images at higher speeds than a galvano scanner. Therefore, when the image shown in Fig. 1 was acquired by Z stacking with 1x averaging using a galvano scanner, it took 9 minutes and 53 seconds, which is approximately 1.6 times longer than the acquisition time of a resonant scanner. The resonant scanner of the AX R supports up to 2K resolution, enabling high-quality images equivalent to those of a galvano scanner to be captured in a short time. Moreover, the shot noise inherent to a resonant scanner could be removed using Denoise.ai, resulting in high-definition images (Figure 2).

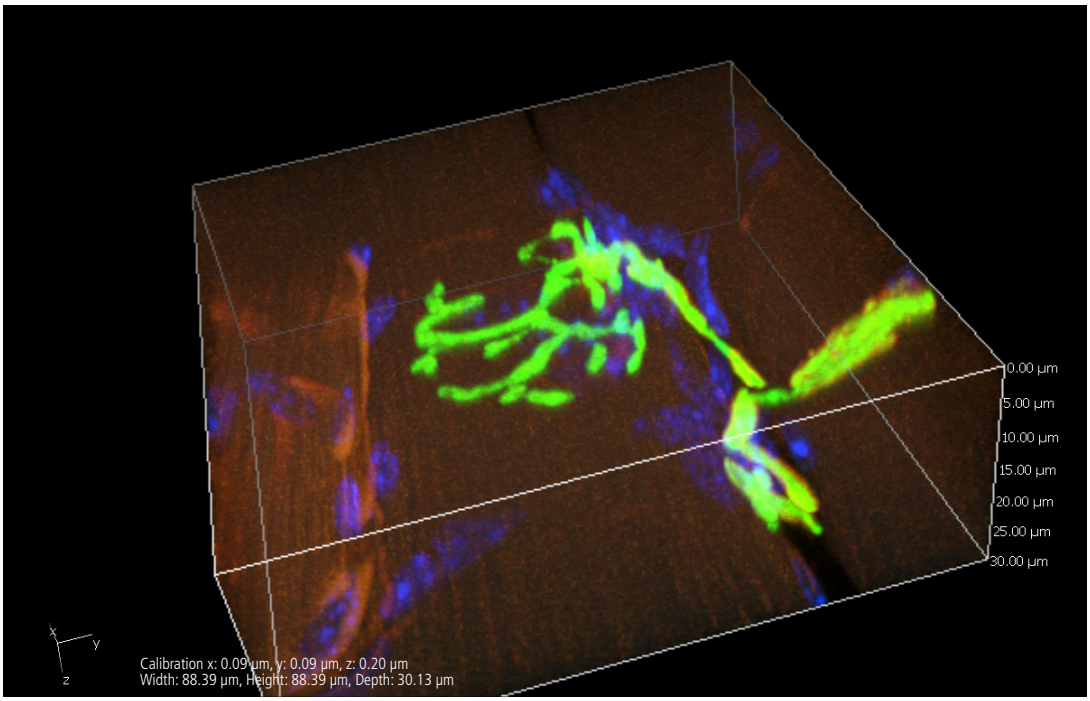

Figure 1. NMJ captured by high-speed imaging using resonant scanner

Sample: neuromuscular junction (NMJ) of mouse
DAPI: nucleus (blue), GFP: motor neuron (green), Alexa 555: acetylcholine receptor (orange), CMDR, cell membrane (red)
Scanner: resonant, Averaging: 8x (2.1 seconds per frame), Resolution: 1K x 1K pixels
Z-stack acquisition time: 6 minutes 15 seconds, Z depth: 30.127 µm, Z-step: 0.205 µm, 148 images captured, Channel: minimal crosstalk, Scan mode: bidirectional scan
Objective: CFI Plan Apochromat Lambda S 40XC Sil

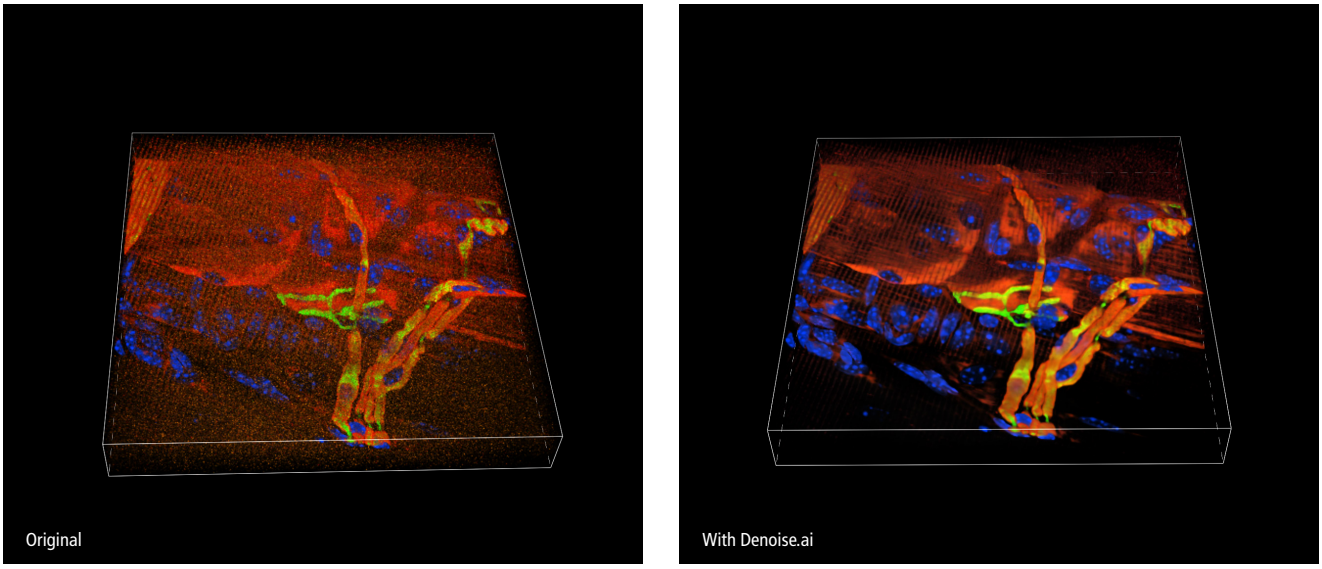

Figure 2. NMJ captured by resonant scanner (left) and example after processing by Denoise.ai (right)
Scanner: resonant, Averaging: none, Resolution: 1K x 1K pixels,
Z-stack acquisition time: 1 minute 35 seconds, Z depth: 30 µm, Z-step: 0.343 µm, 89 images captured, Channel: minimal crosstalk, Scan mode: bidirectional scan
Objective: CFI Plan Apochromat Lambda S 25XC Sil

Achieving noise reduction in resonant images by applying Denoise.ai

Denoise.ai is an AI module for microscopy that automatically removes shot noise inherent to resonant scanning. Using Denoise.ai in combination with a resonant scanner enables acquisition of low-noise images without executing an averaging process. Improvement of the resolution of the resonant scanner incorporated in the AX R and the development of Denoise.ai enabled the acquisition of high-resolution images with suppressed noise in a shorter time while reducing photobleaching (Figure 2). This is very effective for 3D imaging of large samples such as biological tissue slices using a resonant scanner.

Summary

As a resonant scanner enables high-speed imaging, it can obtain images in a shorter time than a galvano scanner, streamlining the imaging process. Additionally, using Denoise.ai can remove shot noise that is generated during resonant scanning, resulting in clearer images.

Product information

AX/AX R Confocal Microscope

These microscopes achieve high resolution images of 8K x 8K pixels, four times that of conventional models. A large FOV with a diagonal of 25 mm allows acquisition of large areas 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 at high speeds of up to 720 fps (2048 x 16 pixels).