APPLICATION NOTE

AX/AX R Confocal Microscope

In vivo Confocal Imaging of Mouse Organs that Clearly Captures Fast Dynamics

The Research Group led by Professor Masaru Ishii (immunology and cell biology) at the Graduate School of Medicine and Frontier Biosciences, Osaka University, is studying the mechanism of immune cell movement *in vivo*, by visualizing cell motility using the microscopic imaging technique. This application note introduces an image acquisition example in which quick movement of cells rolling in the blood vessel is captured *in vivo* using Nikon's AX R confocal microscope, and by utilizing the high-speed resonant scanning that is one of the advantages of this microscope.

Background

Neutrophils are one of the immune cells that play an important role in acute inflammation. When acute inflammation occurs, neutrophils infiltrate the tissue from peripheral blood vessels and accumulate in the inflamed area to remove bacteria. However, it is known that excessive accumulation of neutrophils can impair the host organs. Understanding the process by which neutrophils that interact with the vascular endothelium are mobilized into tissues provide better understanding of the mechanism of drug action targeting this mobilization process. This understanding is expected to make a significant contribution to fully comprehending acute inflammation including sepsis and the development of treatments. The Research Group of Professor Ishii is conducting research focusing on the motility of neutrophils inside and outside the blood vessels using *in vivo* imaging. This application note introduces an example of a spatiotemporal visualization of neutrophils rolling inside the blood vessels of the pancreas using Nikon's AX R confocal microscope.

High-Speed Acquisition by Resonant Scanning

The AX R confocal microscope allows high-speed imaging with resonant scanning, and acquiring high-S/N images with shot noise removed is possible in combination with the Denoise.ai function. It is effective when high temporal resolution is required, such as when imaging cells flowing in blood vessels in mouse organs, etc. Fig. 1 shows an *in vivo* imaging of the pancreas of a LysM-EGFP mouse in which neutrophils were labeled with GFP. Blood flow is labeled with red dye. Not only in veins in which the flow is relatively slow, but also in arteries in which the flow is fast, the state of neutrophils moving at high speed in the bloodstream is clearly captured. Especially in veins, the neutrophils rolling along the vessel wall while adhering to a specific area can be observed in detail. This is an example showing that the combination of resonant scanning with this microscope and the Denoise.ai function allows clear capturing of fast dynamics.

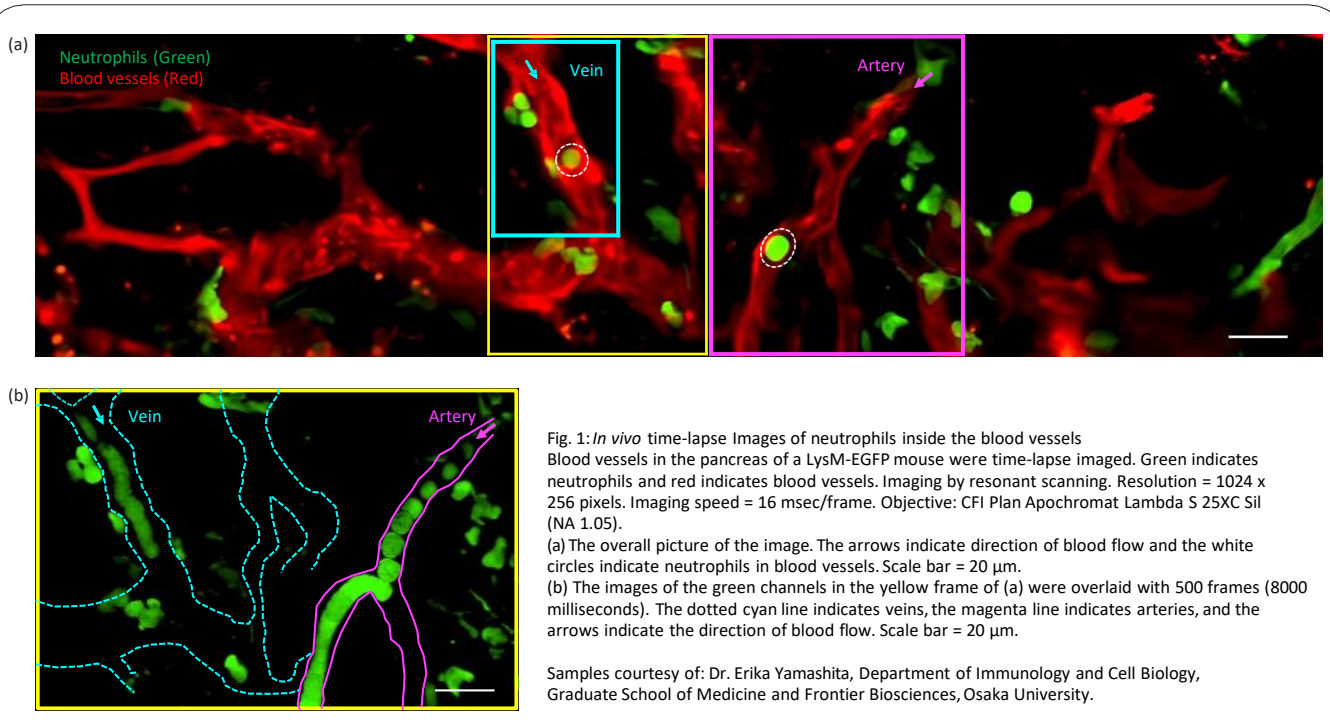

Fig. 1: *In vivo* time-lapse Images of neutrophils inside the blood vessels
Blood vessels in the pancreas of a LysM-EGFP mouse were time-lapse imaged. Green indicates neutrophils and red indicates blood vessels. Imaging by resonant scanning. Resolution = 1024 x 256 pixels. Imaging speed = 16 msec/frame. Objective: CFI Plan Apochromat Lambda S 25XC Sil (NA 1.05).
(a) The overall picture of the image. The arrows indicate direction of blood flow and the white circles indicate neutrophils in blood vessels. Scale bar = 20 µm.
(b) The images of the green channels in the yellow frame of (a) were overlaid with 500 frames (8000 milliseconds). The dotted cyan line indicates veins, the magenta line indicates arteries, and the arrows indicate the direction of blood flow. Scale bar = 20 µm.

Samples courtesy of: Dr. Erika Yamashita, Department of Immunology and Cell Biology, Graduate School of Medicine and Frontier Biosciences, Osaka University.

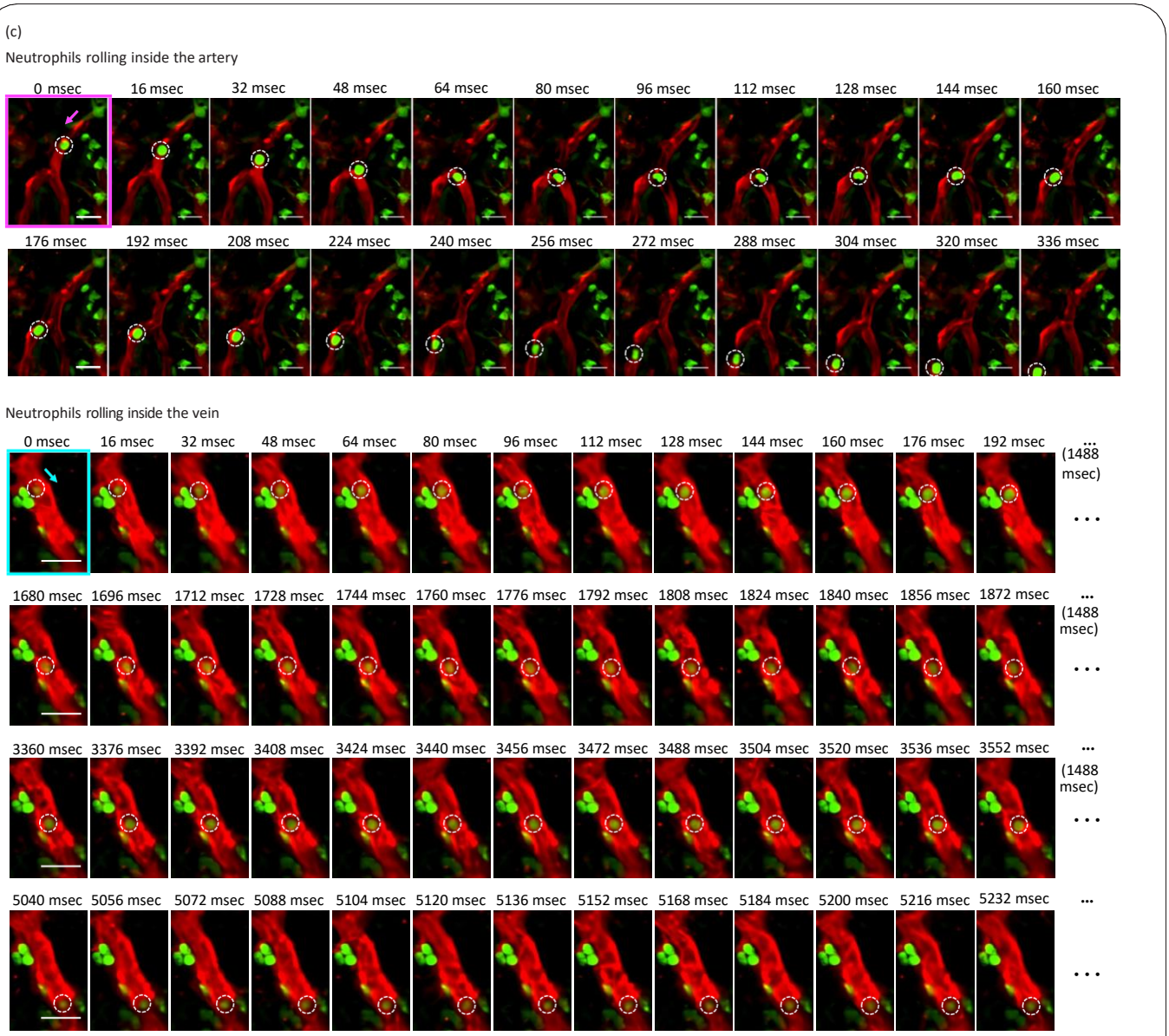

Fig. 1 (continued) : *In vivo* time-lapse Images of neutrophils inside the blood vessels
Blood vessels in the pancreas of a LysM-EGFP mouse were time-lapse imaged. Green indicates neutrophils and red indicates blood vessels. Imaging by resonant scanning. Resolution = 1024 x 256 pixels. Imaging speed = 16 msec/frame. Objective: CFI Plan Apochromat Lambda S 25XC Sil (NA 1.05).
(c) The enlarged time-lapse images of the vein (cyan frame) and artery (magenta frame) in (a). The timing when neutrophils appeared is indicated as t = 0 seconds. The arrows indicate direction of blood flow and the white circles indicate neutrophils in blood vessels. Scale bar = 20 µm.

Summary

The AX R confocal microscope allows high-speed acquisition utilizing resonant scanning to accurately capture the quick movement of cells, making it effective for analysis of dynamics, etc. In addition, it can remove shot noise to acquire images with a high S/N by applying processing with the Denoise.ai function during acquisition by resonant scanning.

Product Information

AX R Confocal Microscope

Supports fast and high resolution large FOV confocal imaging with low phototoxicity to live cells and low photobleaching.

- High speed:
  Up to 720 fps (with resonant scanning, at 2048 x 16 pixels)
- High resolution:
  Up to 8K pixels (with galvano scanning)
  Up to 2K pixels (with resonant scanning)
- High throughput:
  Ultra-large field of view of 25 mm