APPLICATION NOTE

AX/AX R Confocal Microscope

Efficient Confocal Imaging of Dynamics and Changes of Cells utilizing a Large Field of View

The Research Group led by Professor Masaru Ishii (immunology and cell biology) at the Graduate School of Medicine and Frontier Biosciences, Osaka University, is working on visualizing tumor cell movement *in vivo* using the microscopic imaging technique. This application note introduces an image acquisition example in which the dynamics and state changes of tumor cells were accurately captured using Nikon's AX R confocal microscope, and by making the maximum use of the large field of view that is one of the advantages of this microscope.

Background

Cell "motility" supports fundamental life phenomena such as immunity and biogenesis, and it is an important property in determining cell function. On the other hand, for tumor cells, motility allows infiltration and metastasis, and it will lead to cancer recurrence. Various studies have been conducted on molecules related to tumor cell motility, but the actual movement of tumor cells *in vivo* remains unclear. The research group of Professor Ishii has actively conducted intravital imaging research focused on tumor cell motility. The group has clarified that the metastasis of colon cancer cells depend on the cell cycle, and that the movement of leukemia cells is deeply involved in chemotherapy resistance. These findings are expected to lead to the development of next generation cancer treatment which targets the movement of tumor cells. This application note introduces an example of a spatiotemporal visualization of tumor cell dynamics using Nikon's AX R confocal microscope.

Large Field of View Imaging with AX R

The AX R confocal microscope can capture images with a large field of view with a diameter of 25mm. As shown in Fig. 1, the field of view size is approximately 1.25 times larger than that of the average of other companies. Even with cells that move around in a wide area like immune cells, and tumor cells (leukemia cells) like those used in this example, the change of cell state can be captured efficiently. In this experiment, induction of apoptosis in leukemia cells was observed. Specifically, leukemia cells expressing the apoptosis imaging probe were seeded on a 35 mm dish, an apoptosis-inducing reagent (staurosporine) was added, and time-lapse imaging was performed. The fluorescent color of leukemia cells changes from YFP to CFP due to apoptosis. As shown in Fig. 1, the number of cells expressing YFP decreased as time passed and, conversely, the number of cells expressing CFP increased. Many cells are observed in one field of view, and this is an example showing that the large field of view, which is an advantage of this microscope, allows efficient time-lapse imaging.

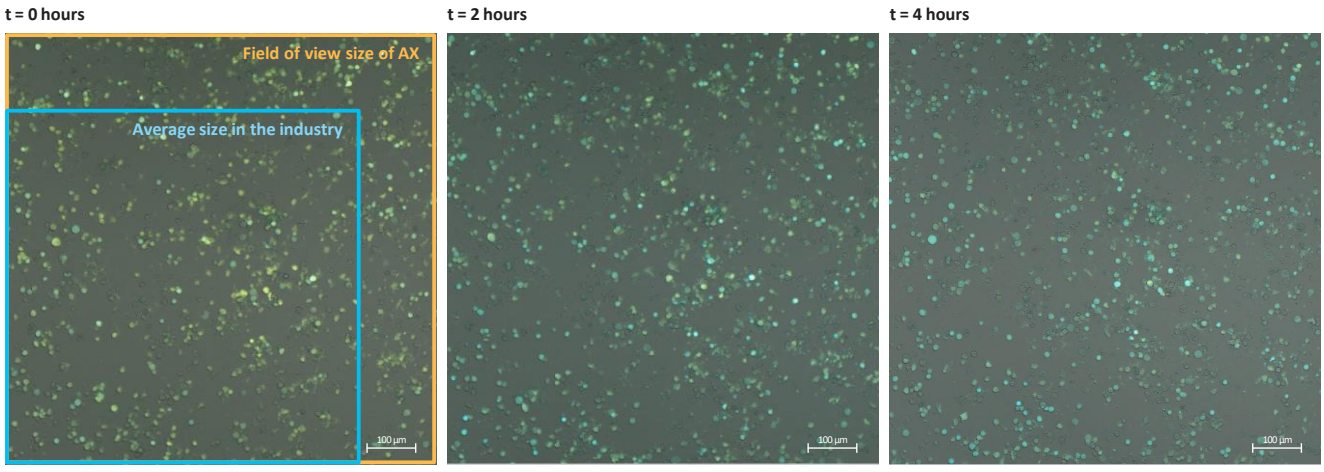

Fig. 1: Time-lapse images of tumor cells expressing apoptosis imaging probe
Staurosporine was added to the leukemia cell line expressing apoptosis imaging probe, and images were acquired every 20 seconds for 4 hours. Yellow indicates live cells and cyan indicates apoptotic cells. Scale bar = 100 µm. Imaging by resonant scanning. Resolution = 2K x 2K pixels. Objective: CFI Plan Apochromat VC 20x DIC N2.
Samples courtesy of: Dr. Erika Yamashita, Department of Immunology and Cell Biology, Graduate School of Medicine and Frontier Biosciences, Osaka University

The intensity ratio (YFP/CFP) in the captured images was displayed using the rainbow spectrum to clarify the color change from YFP to CFP shown in Fig. 1. The result of t = 0 hours is shown in Fig. 2(a). In addition, using the “GA3” image analysis function of NIS-Elements imaging software, the number of cells in which CFP was dominant was measured from the image with the intensity ratio shown in Fig. 2(a). As shown in Fig. 2(b), the number of cells in which CFP is predominant increases over time. In this way, with this microscope the analysis results can be confirmed easily and quickly by using NIS-Elements consistently, not only during image acquisition but also in the subsequent quantitative analysis.

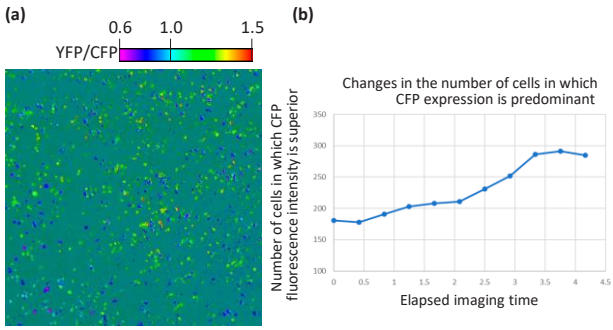

Fig. 2: Changes in the number of cells in which CFP is predominant over time
(a) Image of the intensity ratio of fluorescent protein (YFP/CFP) in Fig. 1 at t = 0h, displayed using the rainbow spectrum.
(b) A graph showing the changes over time of counting cells having a fluorescence intensity ratio (YFP/CFP) of 0.9 or less from the intensity ratio image shown in Fig. 2(a).

Denoise.ai supports image quality improvement

The excitation light should be as weak as possible for long-term confocal imaging without damaging cells. However, the detected fluorescence intensity becomes weaker with weak excitation light and the image will thus have a poor S/N ratio. By using the Denoise.ai function, unnecessary noise elements are removed from the acquired image, and an image with a high signal-to-noise ratio can be observed. The images with a high S/N ratio which captured the dynamics of tumor cells using the Denoise.ai function is shown in Fig. 3. Noise-free images can be obtained even with a long-term time-lapse imaging of 18 hours, and changes in cell morphology and color can be tracked in detail.

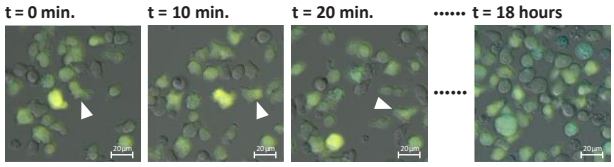

Fig. 3: Time-lapse images of tumor cells
Time-lapse images of leukemia cells. The fluorescence images were overlaid on the diascopic images. Scale bar = 20 µm. Imaging by resonant scanning. Resolution = 2K x 2K pixels. Objective: CFI Plan Apochromat VC 20x DIC N2

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

Summary

The advantage of the AX R confocal microscope is having a largefield of view, which increases the number of cells that can be observed in one field of view and improves experimental efficiency.In addition, post-acquisition analysis can be supported by NIS-Elements imaging software, enabling quick quantitative analysis.
Moreover, combining AX R with the Denoise.ai function allows long-term time-lapse imaging with a high S/N ratio while reducingdamage to cells.

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