APPLICATION NOTE

AX/AX R Confocal Microscope

High Resolution Confocal Imaging that Accurately Captures Localization of Synapse Region in Cultured Neurons

A research group consisting of Professor Kohtaro TAKEI and Research associate Dr. Ryohei NISHIDA, et al., at the Molecular Medical Bioscience Lab, Yokohama City University Graduate School of Medical Life Science, is researching the involvement of the LOTUS neural circuit forming factor in the formation of synapses which are the joints of nerve cells, as well as memory function in the hippocampus, the memory-related brain region. This application note introduces a case study that accurately captured the localization of marker molecules in the synapse region, utilizing the high resolution and large field of view features of the AX R confocal microscope.

Outline of Research

The hippocampal neurons are responsible for the higher brain functions related to memory and learning, by means of the plasticity of their synapses. Synapses are constantly formed and eliminated but Nogo, known as a factor involved in synapse elimination, causes a decrease in the density of synapses by binding to Nogo receptor-1 (NgR1). Since the lateral olfactory tract usher substance (LOTUS) discovered through the research of Professor Takei et al. is an endogenous antagonist of NgR1, it inhibits binding between Nogo and NgR1 and reduces the decrease in the density of synapses. In hippocampal primary cultured neurons derived from a LOTUS deficient mouse, the location where PSD-95, the marker molecule for the postsynaptic site, and Bassoon, the marker molecule for the presynaptic site, are tightly adjacent was identified as a synapse and measured. The result showed that the density of these synapses was significantly lower than that of neurons derived from a wild-type mouse. It was also revealed that the memory formation ability of the LOTUS deficient mouse was less than that of the wild-type mouse. It was therefore revealed that LOTUS is an important protein involved in synapse formation and memory function. Here, we introduce an observational case study of mouse hippocampal primary cultured neurons.

High-Resolution Imaging with the AX R

The AX R confocal microscope features high pixel resolution and provides a maximum pixel resolution of 8K x 8K in Galvano scan mode and 2K x 2K in resonant scan mode. Figure 1 shows confocal images of hippocampal primary cultured neurons. Dendrites extending from the cell body were visualized by labeling of dendrite marker MAP2 with Alexa 647. Synapses were visualized by immunochemical labeling of both postsynaptic marker PSD-95 with Alexa 488 and presynaptic marker Bassoon with Alexa 594. Figure 1a shows the whole image of the target area obtained at 8K pixel resolution in Galvano mode. It was able to comprehensively capture a wide observation area due to the large field of view of the AX R, with a diameter of 25 mm that is twice of conventional. Figures 1b and 1c display enlarged images of a specific area, and the high resolution of the AX R allows accurate observation of the location where the post-synaptic and pre-synaptic sites are adjacent (location of synapse). In this case, it was shown that the AX R's large field of view can capture a whole picture of the sample and efficiently detect the analysis area after image acquisition, and its high pixel resolution can accurately identify the localization of the target.

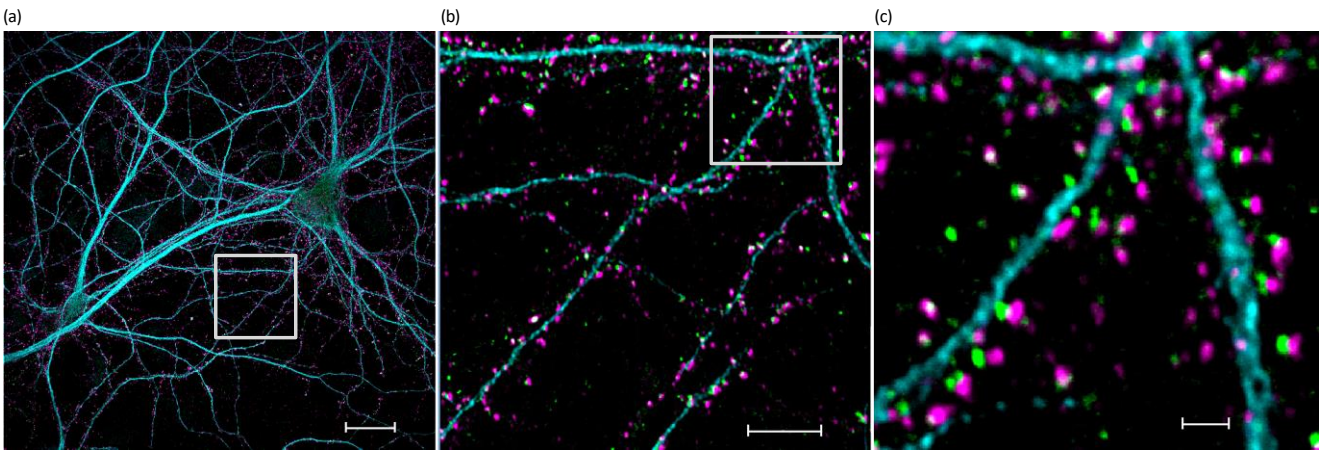

Fig. 1: Confocal images of mouse hippocampal primary cultured neurons

Mouse hippocampal primary cultured neurons on culture day 14. Green: PSD-95; post-synaptic marker (Alexa 488), magenta: Bassoon; pre-synaptic marker (Alexa 594), light blue: MAP2; dendrite marker (Alexa 647). Scale bar: Fig. 1a; 20 µm, Fig. 1b; 5 µm, Fig. 1c; 1 µm. Objective: CFI Apochromat TIRF 100XC Oil

Samples courtesy of Professor Kohtaro TAKEI and Research Associate Dr. Ryohei NISHIDA, Molecular Medical Bioscience Lab, Yokohama City Univ.

Analyzing Target Localization with NIS-Elements

Next, we introduce an example showing locations where the post-synaptic and pre-synaptic sites are adjacent, identified as synapses, using the NIS- Elements imaging software of an AX R confocal microscope, and measured the densities of these synapses. The measurement procedure is shown in Fig. 2. First, the area of interest (Fig. 2a) was obtained from the entire image and binarized for each channel. The locations of post-synaptic and pre-synaptic sites (Fig. 2b and 2c, respectively) were identified. Next, the locations of synapses where post-synaptic and pre-synaptic sites are adjacent (Fig. 2d) were identified. Consecutively, only synapses that were localized in the vicinity of dendrites were identified as synapses on dendrites as shown in yellow in Fig. 2e. In this way, it was possible to identify locations of synapses in the area of interest and measure synaptic density on dendrites using NIS-Element's image analysis tool on the image acquired by the AX R confocal microscope. Table 1 shows numbers of detected point of target in regarding to Fig2. (a)(b)(c)(d).

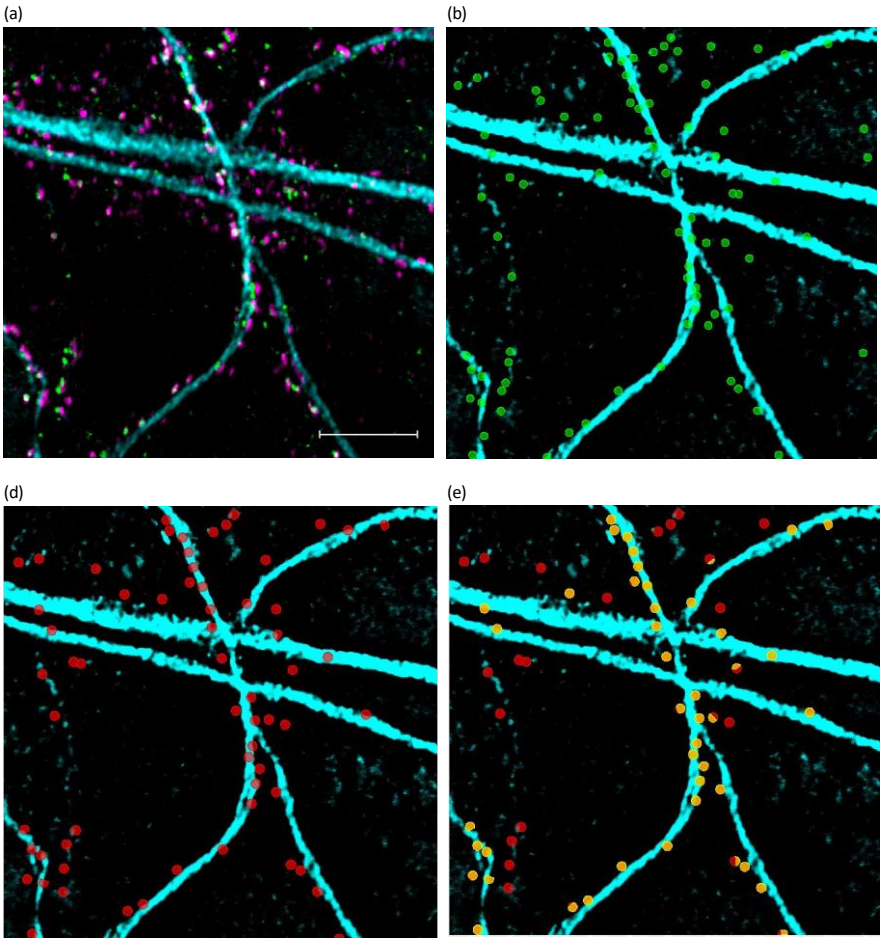

Fig. 2: Identification of locations of synapses on dendrites
(a) An image of a part of the overall image shown in Fig. 1a Scale bar: 5 µm
(b) Identified locations of post-synaptic sites (green circles)
(c) Identified locations of pre-synaptic sites (pink circles)
(d) Identified locations of synapses where post-synaptic and pre-synaptic sites are adjacent (red circles)
(e) Identified locations of synapses on dendrites (yellow circles)

Tabel1. Number of detected point of target in regarding to Fig2. (a)(b)(c)(d).

target	Number
post-synaptic sites	82
pre-synaptic sites	185
synapses where post-synaptic and pre-synaptic sites are adjacent	63
Identified locations of synapses on dendrites	45

Summary

The AX R confocal microscope features a large field of view and can capture a wide area in one image, enabling comprehensive visualization of the observation target and efficient navigation in a sample. Since the obtained image has a high pixel resolution of 8K, it is possible to accurately capture localization of the target even after magnifying the area of interest. In post-acquisition analysis, NIS-Elements software allows quick quantitative analysis and improvement of the experiment workflow.

Product Information

AX R Confocal Microscope

Provides industry-leading high-speed, high-resolution, large field of view confocal imaging. NIS-Element software supports quantitative analysis in a wide range of research aspects.

- High speed: Up to 720 fps (resonant at 2048 x 16 pixels)
- High resolution: Up to 8K (Galvano) / 2K (resonant)
- High throughput: Ultra-wide field of view of 25 mm