APPLICATION NOTE

A1R/AX R Confocal Microscope

The Advantages of Resonant Scanning with Ultra Short Laser Exposure Times in Live Imaging

Enteroids are an excellent tool for studying intestinal epithelial functions, such as secretion of antimicrobial peptides, α-defensins by Paneth cells in innate enteric immunity. However, enteroids are very sensitive not only to such environmental factors as temperature and humidity, but also laser exposure by laser scanning microscopes, so they must be handled with care during experiments. In this application note, we will show the advantages of conducting observations with ultra-short laser exposure times using a resonant scanner by evaluating not only photobleaching of fluorescent dyes but also intestinal epithelial cell functions focusing on Paneth cell granule secretion.

Experiment Overview

Resonant scanners are capable of faster scanning than galvano scanners and can be expected to impart reduced phototoxicity to cells. In this experiment, the effects of resonant scanning and galvano scanning on secretion of Paneth cell granules rich in α-defensins were investigated using enteroids, a three-dimensional culture system of small intestinal epithelial cells.

10 μM of carbachol (CCh), a cholinergic agonist, was added 14 seconds after the first image acquisition, and images were captured using a resonant scanner and a galvano scanner. Based on the acquired images, a comparative study was conducted on photobleaching as well as effects on cell function.

Results

In the images captured by the galvano scanner, the fluorescence signals of Paneth cell granule staining with Zinpyr-1 and plasma membrane staining with CellMask Deep Red were both virtually photobleached within 6 minutes. In addition, inhibition of Paneth cell granule secretion began after about 3 minutes and completely stopped after about 6 minutes (Fig. 1a). In sharp contrast, with ultra-short laser exposure using the resonant scanner, the fluorescence signals of both stainings were not photobleached during an experiment that lasted a total of 10 minutes. Also, granule secretion was NOT inhibited after 3 minutes and continued over 10 minutes (Fig. 1b).

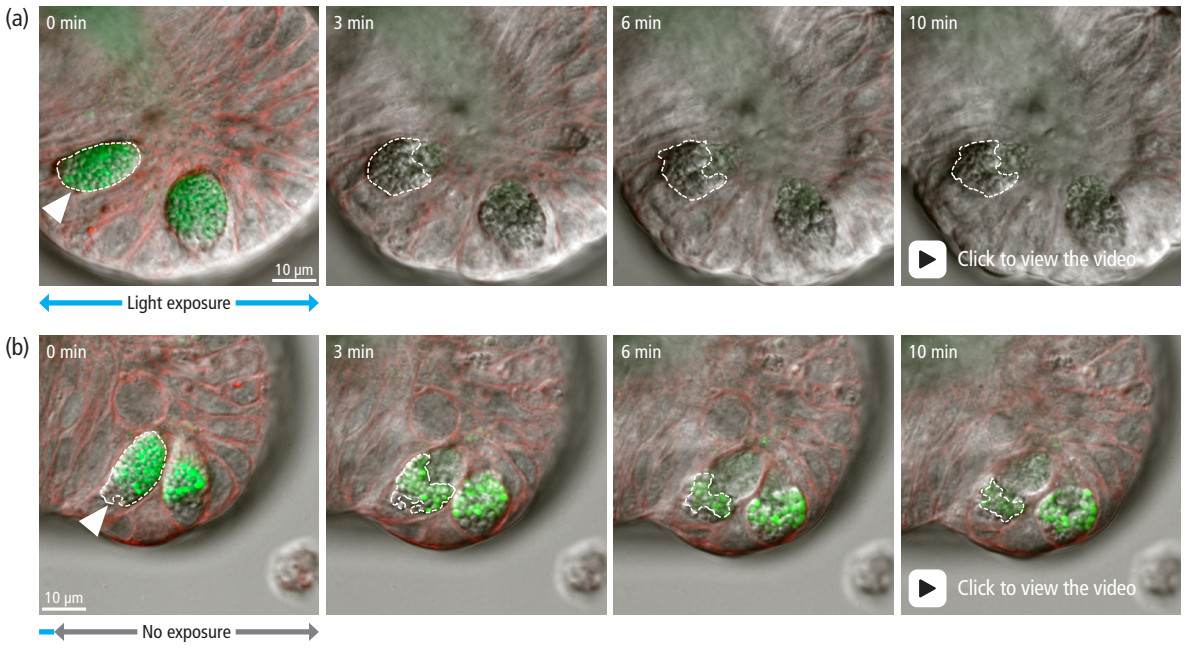


Fig. 1: Comparison of images captured with a galvano scanner and a resonant scanner

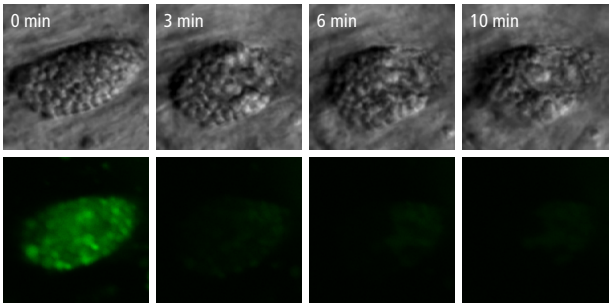
(a) Extracted images captured with a galvano scanner. Laser exposure time to one pixel: 2.18 μs. Temporal resolution: 1 sec.

(b) Extracted images captured with a resonant scanner. Laser exposure time to one pixel: 0.10 μs. Temporal resolution: 1 sec. due to intermittent image acquisition by high-speed AOTF switching.

The arrows indicate Paneth cells whose granule area was measured (refer to Fig, 3 for the measured data). The laser power (488 nm, 640 nm) and detector sensitivity settings were identical for both the galvano and resonant scanning data.

Sample video

(a) Granule secretion images captured by a galvano scanner



(b) Granule secretion images captured by a resonant scanner

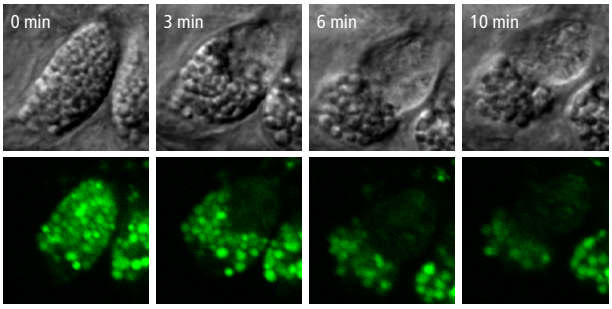


Fig. 2: Comparison of image quality between galvano scanning and resonant scanning

The image quality of galvano and resonant scanning is shown in close-up images at each point in time. In both (a) and (b), the upper row are DIC images, and the lower row are Zinpyr-1 fluorescence images. The images obtained by galvano scanning (a) have a better Zinpyr-1 S/N ratio than the images obtained by resonant scanning (b). However, each granule is unclear and appears blurry because the granules were moving.

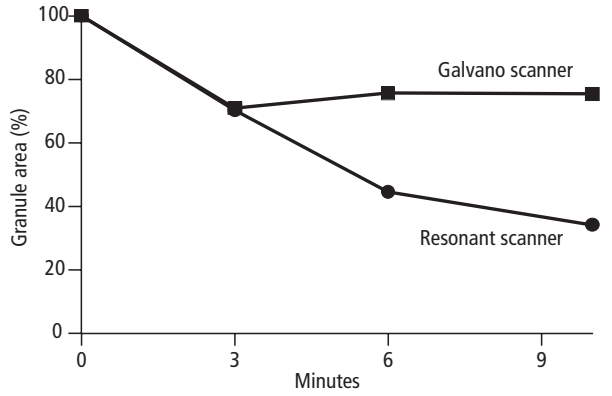


Fig. 3: Comparison of Paneth cell granule secretion visualized by galvano scanning and resonant scanning

With the resonant scanner, the Paneth cell granule area continuously decreased, which means granule secretion occurred over 10 minutes after CCh stimulation, whereas with the galvano scanner, no decrease in granule area was observed after 3 minutes. This suggests that in addition to the photobleaching shown in Figures 1 and 2, cell function may have been impaired by phototoxicity caused by the long laser pixel dwell time due to galvano scanning.

Summary

In this experiment, we evaluated the advantages of resonant scanning, which allows ultra-short laser pixel dwell time by monitoring Paneth cell granule secretion in enteroids. As a result, it was confirmed that resonant scanning can not only prevent photobleaching of the fluorescent dye, but also dramatically reduce damage to cell functions.

Resonant scanning, which imparts minimum phototoxicity on cells and provides high-resolution images, is expected to become a more useful method for elucidating the molecular dynamics of cells and their mechanisms.

Acknowledgements

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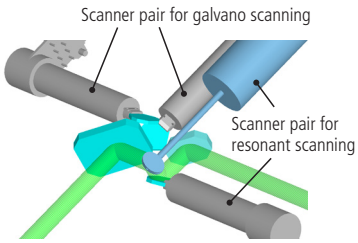
Product Information

Hybrid scanner system

Equipped with both a resonant scanner that can reduce photodamage by ultra-short laser exposure and allows high-speed (~720 fps/AXR) imaging, and a galvano scanner that can acquire high-resolution (~8K/AXR) and high-definition images, this system supports a wide range of applications with high speeds, high throughput, and high resolutions.



Silicone immersion objectives

The CFI Plan Apochromat Lambda S 25XC/40XC Sil objective uses silicone oil with a refractive index close to that of enteroids etc. to achieve higher resolution than water immersion objectives. Also, the long working distance (550 µm/25XC, 300 µm/40XC) makes it easy to find enteroids in Matrigel.