

# High-resolution, depth-resolved spheroid imaging using nonlinear fluorescence microscopy constructed with a pump-probe setup

Fumihiko Dake and Seri Hayashi

Research & Development Division, Nikon Corporation

471 Nagaodai-cho, Sakae-ku, Yokohama-city, Kanagawa 244-8533, Japan

E-mail: Fumihiko.Dake@nikon.com

**KEY WORDS:** fluorescence microscopy, nonlinear optics, stimulated emission, super-resolution imaging

We demonstrated depth-resolved spheroid imaging with a high-optical resolution using nonlinear fluorescence (NF) microscopy, which utilizes nonlinearity of stimulated transition caused by two-color laser beams [1, 2]. The resulting NF signal that undergoes such a stimulated transition process is detectable as a signal via the lock-in technique. As the NF signal is produced by the multiply combination of incident beams, the point spread function (PSF) of the signal is determined by the product of incident beams PSFs, resulting in an improvement of the three-dimensional optical resolution.

The NF microscopy was constructed with two-color continuous-wave lasers operating at wavelengths of 532 nm and 647 nm, which were used as pump and probe beams, respectively. The intensities of the pump and probe beams were temporally modulated by acousto-optic modulators at different modulation frequencies of  $f_1$  and  $f_2$ , respectively. Collinearly combined beams were focused into a sample via an objective lens. A backscattering fluorescence signal was collected by the same objective lens and filtered with an optical filter, which cut both incident laser beams. Then, fluorescence was detected with a photomultiplier and the detector photocurrent was measured using a lock-in amplifier.

Figure 1 shows imaging results of the spheroid sample at the depth of 30  $\mu\text{m}$ . The confocal image was captured with a demodulation frequency of  $f_1$ . The NF image was captured with a demodulation frequency of  $f_1 - f_2$ . Imaging results demonstrated that the NF signal has the superior optical resolution and image contrast than the conventional fluorescence signal. Several spheroid images at different depths were captured and results also demonstrated the superiority of the NF signal.

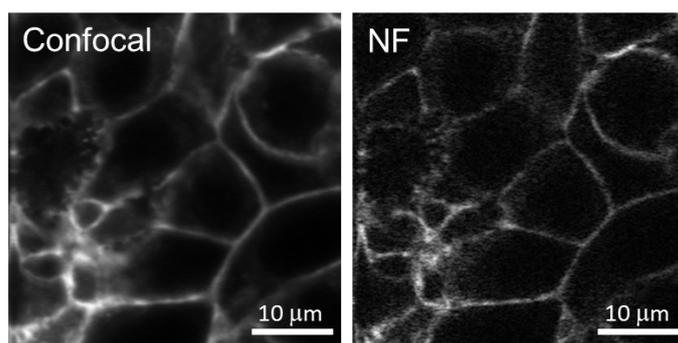


Figure 1: Imaging results of the spheroid sample (action of HT-29 cell).

## References

- [1] F. Dake *et al.*, Appl. Phys. Express **11**, 012401 (2018).
- [2] F. Dake *et al.*, Appl. Opt. **57**, 757 (2018).