Real time polarization resolved imaging for living mice tissues by two-photon excitation spinning disk microscopy

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Second harmonic generation (SHG) is a nonlinear optical process whereby the input wavelength generates a new wavelength that is half to input. Individual noncentrosymmetric molecules generate SHG signals by high-peak-power pump pulses. Molecules arranged in a crystalline array give stronger SHG signals, so that their intensities largely depend on the polarization orientations of the molecules. In addition, SHG microscopy enables to visualize biological macromolecules, such as collagens, tubulin arrays, or sarcomeric myosins without exogenous or genetic fluorophore labeling.

Two-photon excitation laser scanning microscopy (TPLSM) system enables to visualize fluorescent signals from labeled fluorophores and SHG signals from endogeneous macromolecules, simultaneously. Recently, we developed TPLSM system equipped with a spinning disk scanner (TPLSM-SD) to achieve a high temporal resolution intravital imaging. Since the insufficient energy of conventional mode locked Titanium-Sapphire laser light sources restricted the field of view (FOV) to a narrow region [1], we introduced a high-peak-power Ytterbium laser and Neodymium laser light source to enlarge the FOV [2], and realized that bright SHG signals were also detected as well as fluorescent signals. In this study, we introduced a polarization splitting detection system for our TPLSM-SD system and developed high-speed polarization resolved SHG microscopy for living mouse tissues. By adjusting the polarization of irradiation laser lights, structural orientation of the collagen fibers of living mice skeletal muscles were successfully visualized (Figure). Since the SHG signal is located exactly at the half of the wavelength of the input laser light, SHG images were easily isolated from two-photon excitation fluorescence signals by selecting the input wavelength and/or fluorophores. We expect that real time visualizations to reveal the relationship between conformational changes of super-molecular structures and their triggers in living animals can be achieved by combining fluorescent functional indicator detections with our developed SHG imaging methodology.

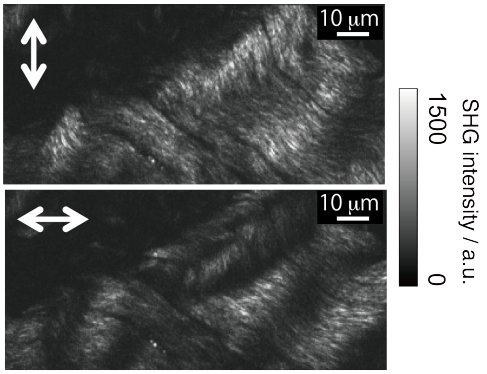

Fig. In vivo polarization resolved SHG images of collagen molecules of a Slc:ICR mouse skeletal muscle. Excitaion wavelength: 1042 nm (circular polarized), Exposure time: 300 ms.

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References
[1] Shimozawa, T., *et al., Proc. Natl. Acad. Sci. U.S.A.*, **110,** 3399-3404 (2013).
[2] Otomo, K., *et al., Anal. Sci.,* **31**, 307-313 (2015); Otomo, K., *et al.*, FOM2017.