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Multimodal nonlinear spectral imaging of tissue samples with CARS molecular fingerprint

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1. Introduction

Among recent progress of optical bioimaging techniques, nonlinear optical imaging is one of the promising new-generation bioimaging methods. Nonlinear optical imaging is capable of rapid image acquisition, three-dimensional sectioning, label-free analysis, and simultaneous multimodal imaging. The multimodality of nonlinear optical processes enables us to obtain various signals simultaneously such as second harmonic generation (SHG), third harmonic generation (THG), third-order sum frequency generation (TSFG), and coherent anti-Stokes Raman scattering (CARS) [1]. Since images obtained by each process are based on different contrast mechanism, the combinational analysis of various nonlinear optical processes provides more detailed information on the sample. In the present study, we report recent our development of multimodal nonlinear spectral microscope and its applications to imaging of tissue samples. In particular, multimodal imaging with spectral analysis of the CARS molecular fingerprint provided molecular structural information of the tissue sample.

2. Experimental

We used our lab-made multimodal nonlinear spectral imaging system [1]. Briefly, the light source is a cw Q-switched Nd:YAG laser. The fundamental 1064 nm radiations are firstly divided into two. One is used as an excitation radiation (ω_1), and the other is introduced into a photonic crystal fiber (PCF). PCF converts the 1064-nm laser pulse to white-light supercontinuum (SC). The SC is used as the ω_2 excitation light. Two radiations are superimposed and introduced into a microscope. The sample is placed upon a piezo electric stage for raster scanning. The forward propagating signal radiation is introduced into spectrographs, and detected by CCD cameras. Sample was a frozen section of rat eye tissue, the thickness of which was 20 μm. The sample was treated with formalin before the measurement.

3. Results and discussion

Multimodal spectral images of cornea and retina are shown in Fig. 1. In particular, at the positions of retina, SHG signals were detected around visual pigment (Fig. 1(f)). This area corresponds to the position of new visual pigment. It suggests that outer segment where visual pigment exists have non-centrosymmetric structure.

References

[1] H. Segawa, M. Okuno, H. Kano, P. Leproux, V. Couderc, and H. Hamaguchi, Opt.Express 20, 9551(2012).

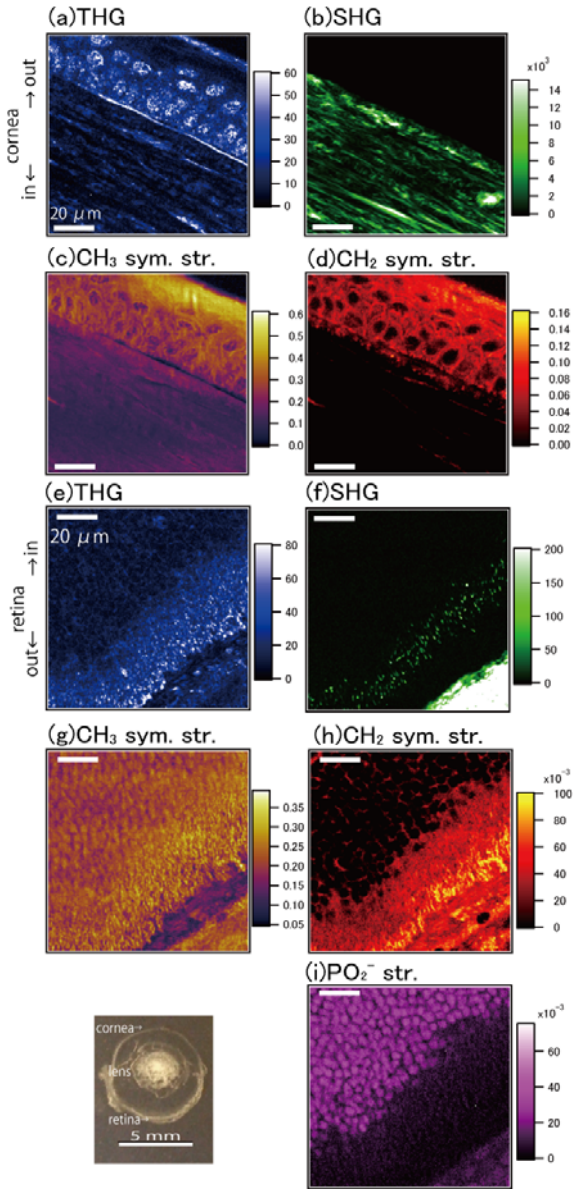

Fig. 1. Multimodal and multiphoton (THG, SHG and multiplex CARS) images at cornea (a-d) and retina (e-i), and optical image of the frozen section of rat eye tissue