Title	フェムト秒レーザー光第二高調波顕微鏡を用いた生体分子ポリマーの構造分析
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Femto-second laser optical second harmonic microscope for biomolecular polymers structural analysis

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Second harmonic generation (SHG) is one of nonlinear optical phenomena. SHG occurs in asymmetric media and the SHG frequency will be double of the incident one. We can use this property to construct an SHG microscopic system. One of the characteristics of this SHG microscopy lies in detecting separately the asymmetric part of a substance such as polarized or oriented chiral molecules. Application of the SHG microscopy is expected to various fields such as the development of new materials, elucidation of physical properties, medical diagnosis and so on. In this study, the main research content is the development of the second-order nonlinear optical microscopy by using a femtosecond pulse laser. A technology of SHG microscopic images observation in a short time and low excitation power by using a femtosecond pulse laser has been established. This second-order nonlinear optical microscopy can be applied to observe and analyze the biomolecular polymers. In this study, the sacran, rice and spider silk were selected as the observation samples. The operation of this new SHG microscopy system was demonstrated on the three biomolecular polymers.

First, sacran with the largest molecular weight was focused in this study. In order to understand the complex structures of sacran, observation of its molecular chains by SHG microcopy was concentrated. The clarification of the origin of SHG in sacran can contribute to elucidation of macroscopic structure in the sacran molecule. As the results of experiment, the SHG images of the pure sacran cotton lump, the sacran fibers, and the sacran films were observed. SHG images of the pure sacran cotton lump showed some bright spots with size of several tens of micrometers. Further, I kept the same of the incident optical power, the observation position and integration time, and changed the observation angle only. As a result, even if the same position was measured, the intensity of the SHG has changed. The dependence of the SHG images on the polarization of the incident light was observed. This indicates that sacran molecules in aggregates have anisotropic structure. The polarization dependent SHG microscopic images also showed multilayer structure of liquid crystal domains, and it means that each domain structure has its own orientation. On the other hand, in the film made from sacran aqueous solution, more continuous SHG signals were observed near the edges of the films. One of the candidate origins of this more continuous SHG is a non-uniform concentration distribution of sacran in the films caused by different evaporation velocity of water from the solution droplet on the substrate during the sample preparation process. Sacran films in concentric circular electrodes were fabricated, and the sacran molecules were found to generate SHG only near the negative electrode.

The second observed sample is an important food, the rice. Rice is an angiosperms that form embryos and endosperm by double fertilization. Starch as the main component of the endosperm is an energy source for animals and plants on the earth. So far the components and their spatial distribution in the seeds of rice have been examined morphologically by chemical and physical methods such as iodine starch reaction and scanning electron microscope (SEM). Endosperm starch of rice is composed mainly of amylose and amylopectin, but it is difficult to distinguish them from the coloring by iodine starch reaction because the color spectrum is complicated. In this study, we used a combined second-harmonic generation (SHG) microscopy and iodine starch reaction to distinguish between amylose and amylopectin in rice seeds with the aid of the SHG activity difference between them. The result suggested that the distribution of starch types in embryo facing endosperm region (EFR) depends on the type of rice. On the other hand, the glucose and maltose are a trigger as the energy source for seed germination. They are known to be present in embryos of rice seeds, but a more accurate distribution has not been elucidated. An observation result by our SHG microscopy method suggested that glucose and maltose are localized on the testa side of the embryo. These distribution map of glucose in the seed is indispensable information for the interpretation of the mechanism of energy consumption at the beginning of germination. These morphological results are expected to provide important information to plant breeding and agriculture.

Finally, the sample is spider silk. Spider silk has always been a coveted material. Its tremendous mechanical strength in spite of its thin width has been already paid attention and it has been applied in some fields. Spider silk has properties of high toughness and high strength and is being studied as attractive materials. Many marvellous molecular structures and mechanisms of spider silk have been discovered so far. However, the reason for the high strength of spider silk is not well understood yet. Therefore, it is important to analyze the configuration structure of the spider silk. Traditional analytical methods are nuclear magnetic resonance, micro-Raman spectroscopy and micro X-ray diffraction, etc. In this study, the SHG images observed from the radial line are considered to generate from the orientation arrangement of the macroscopic non-centrosymmetric structures. However, no SHG signal was observed from the spiral line. The difference of the compositions between the radial and spiral lines is that the former contains β-sheets structures. Hence, it can be concluded that SHG is induced by β-sheets. The changes of SHG intensity with the different polarization of the incident light was also observed and it indicates that the structure of β-sheets is anisotropic. A model of the microscopic structure of the spider silk was proposed. This study proposed a new and effective method to observe the macroscopic structure of spider silk. A simple, quick observation method in this study can provide an effective and direct way to monitor the properties of the natural and artificial spider silk. The SHG image of spider silk reflects also what is happening in the spider's gland. Thus in the future, the secretory glands of the spider can be directly observed to find the mechanism of the production of the silk by this microscope. This work should promote the biomimetics of spider silk.

In this study, oriented structure in various materials were observed extractively by a homemade femtosecond pulse laser secend-order nonlinear optical microscope. The biological polymers were clearly observed by using this femtosecond pulse laser secend-order nonlinear optical microscope. Therefore, the performance of this microscope was evaluated. It can contribute to elucidating new properties and structures of materials such as sacran, spider silk and rice. These findings suggest that a new science was found. It will be useful for biology and materials science.

Keyword: Nonlinear optics; SHG microscopy; Sacran; Rice; Spider silk.