氏　　名	Zhao Yue		
学位の種類	博士(マテリアルサイエンス)		
学位記番号	博材第 439 号		
学位授与年月日	平成 29 年 12 月 22 日		
論文題目	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		
論文審査委員	主査　水谷　五郎	北陸先端科学技術大学院大学	教授
	金子　達雄	同	教授
	小矢野　幹夫	同	教授
	筒井　秀和	同	准教授
	三俣　哲	新潟大学	准教授

論文の内容の要旨

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 □-sheets structures. Hence, it can be concluded that SHG is induced by □-sheets. The changes of SHG intensity with the different polarization of the incident light was also observed and it indicates that the structure of □-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.

論文審査の結果の要旨

顕微鏡技術と画像化技術は生物学と医学の発展に大きな影響を与えている。本論文では、従来と異なる方式の新しい非走査型の光第二高調波顕微鏡（SHG顕微鏡）を開発した。本論文は、非線形光学の性質を利用した顕微鏡技術の開発と、生体ポリマーにおける二次の非線形光学物性の探求の２つの観点を論じている。

二次の光高調波発生（Second Harmonic Generation, SHG）は非線形光学現象の一つである。物質に光をあてると、反転対称性が破れた構造で、もとの光の周波数が2倍に変わる。反転対称性の物質(ランダムな配向)に対してSHGは禁制である。一方、反転対称性を持たない物質（配向または分極したキラルな分子）に対してSHGは許容となる。この性質を利用した二次の光高調波顕微鏡（SHG顕微鏡）で、そのような部分が選択的に観察できる。

本論文では、再生増幅したフェムト秒パルスのビーム径を比較的緩いフォーカスで、集光系の光軸方向と角度を持つ方向から試料に照射して、試料により発生したSHGの光を光学フィルターにより選別し、イメージ増強したカメラで撮像するSHG顕微鏡を独自に開発した。

新しく開発したSHG顕微鏡を利用して、サクラン（植物の起源とされる藍藻の一種類であるスイゼンジノリにおける細胞外マトリックスから抽出した多糖類高分子）、米種子、クモの糸といった生体高分子や生体組織などのポリマーを観察した。この三つの試料はいずれも新材料あるいはそのモデルとなる材料として高い利用価値がある。

結果として、サクランにおいてSHG応答を初めて観察し、綿状の高純度サクランにおいて、同心球殻状の３層構造を持ち、各層のサクラン分子が異なる方向を向いている特殊な構造を選択的に検出した。またサクランのキャスト膜の周辺領域において、原料の水溶液が乾く際に形成すると思われる分子の配向領域があることを見出した。また、サクラン水溶液に電界をかけると、正の電荷をもつ分子が集まり、そのまわりにサクラン分子が配向することを見出した。次に同じ多糖類であるデンプンに着目し、SHG顕微鏡を用いて米の種子断面を観察し、胚と胚乳の境界に近い胚乳の一部である破砕領域の構造が、コメの種類により異なることを見出した。また、糖鎖と並んで代表的な生体ポリマーであるタンパク質であり、最近高機能な繊維材料として期待されているクモの糸において、初めてSHG応答およびSHG像を観察し、クモが作る異なる糸は異なるSHG応答を示すことを見出し、糸の中の場所により偏光特性が異なることから、構造についての情報を得た。以上本論文により、SHG顕微鏡の生体ポリマーに対する応用の可能性を大いに開拓した。

以上より、本論文は独自で開発したSHG顕微鏡を利用して、生体ポリマーにおける二次の非線形光学物性について、いままで知られていなかった新たな構造や性質を発見した。それらの発見から、反転対称性の破れを観察する二次の非線形光学顕微鏡に広い応用可能性があることを示したものであり、学術的に貢献するところが大きい。よって博士（マテリアルサイエンス）の学位論文として十分価値を有するものと認める。