様式３

論　文　内　容　の　要　旨

氏　名　（　望月健太郎　）	
論文題名	Nonlinear Fluorescence Probes: Development and Application to Super-Resolution Microscopy (非線形発光プローブの開発と超解像顕微法への応用)

論文内容の要旨

Two-photon excitation microscopy (TPEM) provides spatial resolution beyond the diffraction limit of light using the nonlinear response of fluorescent molecules. One of the strong advantages of TPEM is that it can be performed using a laser-scanning microscope without a complicated excitation process or computational post-processing. However, TPEM has not been recognized as a super-resolution microscopy due to the use of near-infrared light as excitation light source, which provides lower spatial resolution than visible light. In this thesis, I combined visible light and nonlinear fluorescence responses in order to achieve super-resolution fluorescence imaging. To realize this aim, fluorescent probes providing stepwise multi-photon excitation were developed.

As a first approach, I adopted the use of photoinduced charge separation (PCS) to achieve stepwise two-photon excitation. A fluorescent probe with two electron donors and one electron acceptor was designed and named nitro-bisBODIPY. The first incident photon incident to the probe generates a charge-separated pair between one of donors and an acceptor, and then the second photon can be used for fluorescence excitation of the other donor. The 2nd order nonlinear fluorescence response of nitro-bisBODIPY was confirmed by exciting the probe by CW laser light at 488 nm. Using the same experimental setup, fluorescence imaging of biological cells stained with nitro-bisBODIPY was conducted, and an improvement in image contrast and resolution was confirmed.

The photophysical mechanism of the nonlinear fluorescence response via PCS was investigated by constructing the model of the energy diagram of nitro-bisBODIPY. To quantify the parameters of electronic transition in the model, especially which were difficult to be provided theoretically, transient absorption spectra of nitro-bisBODIPY was measured. Referring the model and parameters, the fluorescence response of the probe was simulated. The good consistency of the simulated and experimentally-measured fluorescence responses indicated that the photophysical mechanism of the nonlinear optical response via PCS was based on the proposed energy diagram.

Secondly, I provided a concept to realize 3rd order nonlinear fluorescence response by fluorescence switching based on spirocycle opening/closing. In this concept, stepwise three-photon excitation was expected to occur through two-photon excitation for spirocycle opening and subsequent one-photon excitation for fluorescence emission by visible-wavelength pulsed light excitation. Fluorescence response of three kinds of spirocyclic compounds (HySOx, MMSiR, RSA) under the visible wavelength pulsed excitation were measured, of which absorption peaks and lifetimes of spirocycle opening were different. As results, a 3rd order nonlinear fluorescence response was obtained with MMSiR under 610 nm pulsed excitation.

To apply the concept based on the modification of probes also to Raman microscopy, I investigated the development of a probe to image intracellular molecules via surface enhanced Raman scattering (SERS). The probe was composed of gold nanoparticles and fabricated at desired positions in cells using laser-induced photoreduction. The volume of the aggregated gold nanoparticles was controlled by modifying the excitation intensity and exposure time for photoreduction. And the detection of SERS spectra using the probes photoreduced inside cells was confirmed.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本学位論文は、可視光励起により非線形蛍光応答を示す蛍光プローブの開発を試みた研究をまとめたものである。その成果は以下の通りである。

・光誘起電荷分離による蛍光スイッチングに基づき、２光子の入射により蛍光発光するプローブ nitro-bisBODIPY を開発している。nitro-bisBODIPY を可視・連続波レーザーで励起することで、２次非線形蛍光応答の取得を実現している。さらに、nitro-bisBODIPY を細胞の蛍光イメージングに利用することで、空間分解能が向上することを確認している。

・nitro-bisBODIPY のエネルギー準位図を構築し、光誘起電荷分離を利用した２次非線形蛍光応答を示す物理モデルを提案している。エネルギー準位間の遷移確率の定量化にあたり、理論的考察が困難であった電荷分離状態形成/解消の速度定数を過渡吸収分光法により測定している。構築したエネルギー準位図を基に nitro-bisBODIPY の蛍光応答をシミュレーションし、実験結果と比較することによりモデルが妥当であることを確認している。

・より高次の非線形蛍光応答を実現するために、分子内スピロ環化に基づく蛍光スイッチングを利用している。スピロ環構造をもつ蛍光分子を可視パルス光で励起することで、２光子励起によるスピロ環開環および１光子励起による蛍光発光から成る３次非線形蛍光応答の取得を達成している。

以上のように、本学位論文では、可視光励起により非線形蛍光応答を示す蛍光プローブの開発を達成している。開発したプローブを蛍光イメージングに利用にすることで空間分解能の向上を実現しており、従来のような複雑な励起方法やコンピューターによる後処理を必要としない超解像顕微法として応用できることを見出している。これらの結果は、応用物理学、特にナノフォトニクスの発展に寄与するところが大きい。よって本論文は博士論文として価値あるものと認める。