様式３

論　文　内　容　の　要　旨

氏　　名　（　　天満　健太　　）	
論文題名	High resolution fluorescence microscopy using structured light for volumetric imaging (生体内超解像観察のための構造化照明蛍光顕微鏡の開発)

Fluorescence microscopy is an indispensable technique for observing biological samples owing to its noninvasiveness, target specificity, and sensitivity at the single-molecule level. In addition, the development of super-resolution techniques has overcome the long-standing limitation in spatial resolution, expanding the capability of imaging sub-organelle structures. However, most of the super-resolution techniques exhibit the resolving capability fully only in observations of sample positions near the surface because of the requirement of complex illumination patterns or single molecule detection.

In this study, I investigated the use of structured light and fluorescence signal confinement for super-resolution microscopy to image volumetric samples. To visualize submicroscopic structures at deep positions in a biological sample, the combinations of sub-wavelength patterned structured light and signal confinement techniques at sample and detection planes were examined, which efficiently carries weak high-spatial frequency signals compared to single molecule detection. For the fast and large field of view imaging, techniques based on widefield-based structured illumination microscopy (SIM), which utilizes sinusoidal illumination to detect and retrieve the high spatial frequency information, were examined. I also investigated confocal-based structured illumination microscopy (image scanning microscopy (ISM)), which achieves super-resolution by reassignment of fluorescence distribution excited by a focused light to improve the observable depth further. In both super-resolution techniques, I introduced nonlinear fluorescence responses to localize the fluorescence emission within the focus. I also explored the aberration correction techniques and high-quantum-yield fluorescent proteins to further enhance the imaging capability in volumetric samples.

I utilized the selective plane activation of reversible photo-switching fluorescent proteins to localize and detect fluorescence emission only in the focal plane of the objective lens for detection in SIM, allowing high-resolution observation of the interior of thick samples. I confirmed image contrast was improved in fluorescence images of biological samples due to the localization of fluorescent emission. I also confirmed that the developed technique achieved a temporal resolution of 1 frame/sec with 3D spatial resolution in the observation of a living cell. Furthermore, I applied 780-nm femtosecond pulsed light to induce the nonlinear response in activation, providing a higher axial resolution and background rejection capability. I succeeded in the observation of cellular nuclei at 25μm deep in a spheroid with a diameter of about 100μm with high spatial resolution.

I improved the spatial resolution of ISM in three dimensions by inducing a nonlinear response in fluorescence emission using saturated excitation (SAX). The localization of fluorescent emission in a sample by SAX is useful for both improving the spatial resolution and suppressing the background signals. I theoretically investigated the imaging property of image scanning microscopy when nonlinear fluorescence responses are introduced. I experimentally confirmed the resolution improvement in ISM by extracting up to 3rd-order nonlinear fluorescence responses and demonstrated the capability of imaging biological samples. The developed technique provided 1.47-fold SNR improvement in 3rd-order nonlinear fluorescence signals, which confirmed the improvement of spatial resolution in practical conditions.

I proposed and demonstrated the use of deflectometry to expand the aberration correction range in the observation of thick samples using adaptive optics. Deflectometry obtains the phase gradient of the wavefront from the distortion of the fringe pattern reflected by the rough surface. The dynamic range of deflectometry measurement is controllable with different frequencies of fringe patterns, which is useful for wide-range calibration of adaptive optics devices and aberration correction. I developed a technique that uses a deformable mirror to calibrate deflectometry with a high dynamic range of phase control. I also applied the technique for adaptive aperture correlation microscopy and demonstrated aberration correction in fluorescence imaging of pollen grains, confirming the signal increase and practical resolution improvement. I also performed remote-focusing fluorescence imaging by taking advantage of the high-dynamic range phase control. It was confirmed that the imaging plane can be tuned within the range of 100 μm and 20 μm without mechanical scanning in the fluorescent beads and pollen grain, respectively.

I developed a fluorescence measurement system for screening high-quantum-yield fluorescent proteins from randomly mutated variants expressed in E-coli. I proposed a technique that utilizes the saturation effect of fluorescence excitation to evaluate the quantum yield and the excitation cross-section of a fluorescent protein, which can be performed by fluorescence measurement under intensity-modulated excitation. I built an optical system that automatically detects the locations of E-coli colonies in a dish and measures the fluorescence response. Multiple CW lasers with different wavelengths and a femtosecond pulsed laser with tunable wavelengths at 350- 2500 nm are employed to evaluate varieties of fluorescent protein mutants with single-photon and two-photon excitation.

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論文審査の結果の要旨及び担当者

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	（職）	氏　名
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論文審査の結果の要旨

本学位論文は、構造化照明、非線形蛍光応答、補償光学および高量子収率蛍光プローブを利用した蛍光顕微観察法を提案し、厚みのある生体試料内部観察における超解像イメージングの実現に関する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。

シート照明と光スイッチング蛍光タンパク質を用いて蛍光発光領域を局在化することで、広視野型構造化照明顕微鏡(SIM)による試料内部の超解像観察を実現している。異なるシート照明を用いた場合の顕微鏡の点像分布関数を理論的に求め、厚みのある試料観察に適した顕微鏡を開発している。ライトシート１光子励起による局在化では1 frame/secでの経時観察および、３次元での生体試料観察を達成している。また、走査ベッセル照明を用いた２光子励起により蛍光発光を局在化させ、従来のSIMでは観察が難しい直径100μmのスフェロイドの深さ25μmの部位の超解像観察に成功している。

蛍光飽和による蛍光信号の局在化を利用し、走査型構造化照明顕微鏡（ISM)の３次元空間分解能向上を達成している。提案する顕微鏡の点像分布関数を計算し、高空間分解能かつ高信号対雑音比での超解像観察を実現する光学系と画像再構成のパラメータの最適化を行っている。直径100nmの蛍光ビーズおよび生体細胞内のアクチンフィラメントの観察により、提案する手法が空間分解能と信号対雑音比を向上できることを実験的に確認している。

高量子収率蛍光タンパク質のスクリーニングにおいて蛍光飽和を利用する手法を提案している。蛍光色素の励起―発光応答を計算し、飽和励起強度が蛍光量子収率に依存することを理論的に確かめている。実験的にも、特性既知の蛍光タンパク質の応答を測定し、提案する手法により量子収率の高い蛍光タンパク質をスクリーニングできることを確認している。

生体深部の超解像観察に有用な補償光学における収差補正のダイナミックレンジを拡大するため、ディフレクトメトリ法を用いた補償光学素子のキャリブレーションを提案している。 補償光学素子を組み込んだ蛍光顕微鏡を開発し、蛍光イメージングにおいて、従来の波面センサでは計測できない大きな変位の収差補正を達成している。

以上のように、本学位論文は非線形な蛍光応答を活用した新たな構造化照明顕微鏡法を提案し、生体試料内部での超解像観察に成功している。また、空間分解能や像コントラストの向上に貢献する蛍光プローブや収差補償を実現する新たな技術の開発にも成功している。これらの成果は応用物理学、特にナノフォトニクス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して認める。