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博士学位论文

面向细胞的金属纳米薄膜介导荧光技术

Metallic Nanofilm Mediated Fluorescence
Techniques for Cell Research

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摘要

金属纳米薄膜具有调控荧光团发光性质的特点，吸引了越来越多研究者的关注。它主要通过两种方式对荧光团的发光性质进行调控。1) 金属纳米薄膜表面的等离子体与荧光团发生耦合，使之发射高度定向的、偏振的荧光信号，产生表面等离子体耦合发射荧光 (Surface Plasmon Coupled Emission, SPCE)。由于表面等离子体共振 (SPR) 模式和等离子体波导共振 (PWR) 模式的存在，SPCE技术的耦合距离可从纳米级拓展到微米量级，在微米级尺度样品的研究中展现出应用潜力。2) 金属纳米薄膜反射激发光和发射光，反射光束与原光束发生干涉，产生镜面效应，从而改变荧光团的发光强度、荧光寿命和发射空间分布。镜面效应具有远场特性，可在微米级尺度范围内增强荧光信号，有望提高细胞荧光成像的灵敏度和对比度。本论文致力于发展面向细胞的金属纳米薄膜介导的荧光技术，共六章：

第一章 绪论。该章首先阐述了平滑金属纳米薄膜镜面效应增强荧光的原理、研究进展及其在细胞荧光成像中的应用；而后重点介绍了SPCE的原理和光学特性，概述了SPCE在机理研究、基底和样品尺度拓展、生化传感及显微成像等方面的研究进展。

第二章 探索了平滑金纳米薄膜对细胞荧光成像增强作用的距离效应。从荧光强度和荧光寿命两个方面系统地考察了平滑金纳米薄膜对细胞荧光成像增强作用的距离效应。基底侧、界面处和样品侧均能观察到荧光增强细胞成像。不同物镜条件下观察到不同的距离效应：当物镜数值孔径较小时，荧光增强因子不随距离发生明显变化；当物镜数值孔径较大时，基底侧荧光增强因子随距离增大而增大，而细胞侧荧光增强因子则随距离增大而减小。平滑金纳米薄膜对荧光寿命的影响也具有距离效应，与细胞深处相比，界面处细胞荧光寿命明显缩短。此外，还考察了染色层厚度和发射光波长对荧光增强作用的影响。本章为制备简单、使用方便、具有均一增强效果的平滑金属纳米薄膜在细胞荧光成像的应用推广提供了便利。

第三章 研究了从纳米到微米范围内样品尺度与SPCE性质的相关性。考察了空气体系Au膜和Al膜两种基底上SPCE性质与样品厚度的相关性，当样品厚度为几十纳米时，荧光团与表面等离子体以表面等离子体共振（SPR）模式发生耦合；当样品厚度为100 nm到1 μm 时，荧光团与表面等离子体以等离子体波导共振（PWR）模式发生耦合。SPR模式和PWR模式SPCE均具有偏振性、角度分辨性和波长分辨性。首次研究了水溶液体系样品厚度对SPCE性质的影响，观察到与空气体系类似的变化规律。当样品厚度大于1 μm 时，水溶液体系SPCE荧光信号仍具有偏振性和角度分辨性。SPCE技术在样品厚度测定、细胞研究等领域展现出应用前景。

第四章 探究了染色细胞的SPCE性质。利用反Kretschmann模式SPCE光谱系统地研究了Au膜基底上不同亚区荧光标记细胞的SPCE现象。细胞膜、细胞质和细胞核染色细胞的SPCE信号均具有明显的偏振性和角度分辨性。通过细胞密度实验和PMMA模拟实验探究了微米级尺度下样品厚度与SPCE性质之间的关系，发现了两者间新的变化规律：SPCE信号始终具有部分p-偏振性，不随样品厚度增大而发生偏振逆转；最大发射角度几乎不随样品厚度、染料层厚度、发射光波长的变化而变化。探讨了金属基底种类对细胞SPCE性质的影响，Al膜基底表现出最好的SPCE特性。此外，利用SPCE实现了细胞染色过程的实时在线检测。本章为SPCE技术在细胞膜流动性、细胞内生物分子间相互作用、细胞分区检测中的应用提供了基础。

第五章 初步探索了具有偏振调控功能的SPCE显微系统的构建。在全内反射荧光显微系统基础上，对激发光偏振性调制，并调节入射光在后焦平面的位置使之满足SPR条件，Au膜基底上可观察到细胞的SPCE荧光显微成像。与全内反射荧光成像相比，荧光强度增大且具有SPCE特征的角度分辨性。初步探索了不同金属基底的细胞SPCE显微成像，30 nm Au膜适合于细胞SPCE成像研究。实验结果表明，激发光合理的偏振取向和波长以及合适的金属基底是实现细胞SPCE成像的关键。此外，若对发射光进行检偏，纯化SPCE信号，有望提高细胞成像的SPCE特性。本章为SPCE显微系统的构建和性能提高指引了方向。

第六章 结语与展望。总结了本论文研究工作的创新性，并对研究工作的进一步开展进行了展望。

关键词：表面等离子体耦合发射荧光；镜面效应；平滑金属纳米薄膜荧光增强作用；细胞；表面等离子体耦合发射荧光显微系统

厦门大学博硕士论文摘要库

Abstract

Metallic nanofilms have attracted more and more attention owing to their characteristics in engineering the emission properties of fluorophores. Generally, there are two manners of engineering: the first is surface plasmon coupled emission (SPCE). The excited fluorophores couple with surface plasmons on metal surface and result in highly directional and polarized emission. The coupling distance of SPCE is extended from nanoscale to micrometer-scale because of the coaction of surface plasmon resonance (SPR) mode and plasmon waveguide resonance (PWR) mode. Thus, SPCE holds great potential to study micrometer-scale samples such as cells. The second is mirror effect of metallic nanofilms. The excitation light and emission light are reflected by the metallic nanofilms, leading to standing waves, which can change the excitation efficiency, radiation rate and emission pattern of fluorophores. Mirror effect has advantages in improving the sensitivity and contrast of cell imaging because of its far-field fluorescence enhancement in micrometer-scale. This dissertation focuses on metallic nanofilm mediated fluorescence techniques for cells. It consists of six chapters.

In the first chapter, a literature survey was presented. First, the principle and research progress of fluorescence enhancement based on mirror effect of smooth metallic nanofilm, and its application in cell imaging were reviewed. Second, the principle and characteristics of SPCE were introduced. Then, the development of its mechanism, the exploration of metallic substrates and sample size, and the applications in biosensor and microscopy were reviewed.

In the second chapter, the distance dependence of fluorescence enhancement based on mirror effect of smooth metallic nanofilms in cell imaging was explored. We studied the distance dependence of fluorescence intensity and lifetime in cell imaging on Au substrates. Enhancement was observed inside cells, near metal

surface, even inside the metallic substrate because of the enhanced reflection images based on the mirror effect. Different types of objectives showed different distance dependence of enhancement factors. For a low numerical aperture (NA) objective, enhancement factors almost stayed at the same level with the increase of imaging depth into either cell or substrate. While for a high NA objective, the enhancement factor increased with the increase of imaging depth in the substrate, but decreased in cell. Fluorescence lifetime also exhibited distance-dependent behavior. The lifetime of cell images near metal surface was greatly reduced, in contrast to those inside the cell. Moreover, we also investigated the impact of dye distribution and emission wavelength on the enhancement of fluorescence. Our study provided a basis to wide application of easy-accessed and convenient smooth metallic nanofilms with uniform fluorescence enhancement effect for cell imaging.

In the third chapter, the impact of sample thickness on SPCE properties was investigated. First, we studied the relationship between sample thickness and SPCE properties of Au substrate and Al substrate in air. Fluorophores coupled with surface plasmons via surface plasmon resonance (SPR) mode when sample thickness was in dozens of nanometers. While in hundreds of nanometers, they were coupled via plasmon waveguide resonance (PWR) mode. Both coupling modes of SPCE exhibited unique polarization, angle distribution and wavelength distribution. Second, their relationship in water was studied for the first time. It turned out to be similar to that in air. Interestingly, in water, SPCE signal still had polarization and angle distribution while sample thickness was larger than 1 μm . Our studies demonstrated that this technique can be used in sample thickness detection and cell research.

In the fourth chapter, SPCE properties of cell were investigated. SPCE properties of cells labeled in different subregions were studied using a reverse Kretschmann (RK) configuration SPCE spectroscopy system. Angular and p-polarized emission

was observed for cells labeled in membrane, cytoplasm and nucleus. For micrometer-scale samples, new SPCE behaviors were expected. The SPCE signals were always partially p-polarized and the maximum emission angle did not shift, regardless of the variation in emission wavelength, fluorophore distribution and stained layers thickness. We also investigated the impact of metallic substrates on SPCE properties of cell. Compared with Au and Ni substrates, Al substrate presented better performances in polarization and angle distribution. Moreover, the real-time detection of the cell labeling process was achieved by monitoring SPCE intensity. These findings provide a basis to apply SPCE technique to study cell membrane fluidity and biomolecule interactions inside the cell and to distinguish between cellular subregions.

In the fifth chapter, the establishment of SPCE microscope with the control of the polarization of excitation light was investigated preliminarily. A total internal reflection fluorescence (TIRF) microscope was derived to SPCE microscope with the modulation of the polarization and illumination position of excitation light. Compared with TIRF images, the intensity of SPCE images was greatly enhanced. The SPCE imaging of cell with different metallic substrates was also investigated. It was found that 30 nm of Au film was the suitable substrate for cell imaging. Experiment results showed that appropriate metallic substrate and excitation light with appropriate polarization and wavelength were the key to achieve SPCE imaging in cells. Moreover, the SPCE properties of cell images can be improved via setting polarizer before use detector to purify signal. Our findings provide a basis to establish a SPCE microscope and improve its performance.

In the sixth chapter, the innovative aspects were discussed and the prospect of this research was pointed out.

Keywords: Surface plasmon coupled emission; Mirror effect; Fluorescence enhancement on smooth metallic nanofilms; Cell; Surface plasmon coupled emission microscope

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