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以超連續譜進行全頻域全光纖時間解析生物螢光感測(I)

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摘要

本計劃旨在研發一新穎的全頻域即時遠距螢光檢測技術。為瞭解細胞中不同分子在複雜的生物反應中的作用,能夠同時量測多種不同的生物標記是至關重要的技術。然而,受限於激發光源的波長,傳統的螢光檢測技術只能同時量測有限頻寬內的螢光分子,而無法同時量測整個可見光至紅外光波段的所有螢光分子。為了量測到更廣頻帶的螢光分子,現行的作法是增加不同波長的激發雷射並加入許多濾光鏡以增加量測的(頻域)通道數。然而,這麼一來整個系統將變得十分複雜且有很大的螢光訊號損失。這麼一複雜又龐大的系統很難運用在實驗室外的實地量測或是生物體內的即時量測。然而,為真正了解細胞中生物反應的進行,能夠即時在其生長的生物體內進行直接測量是很重要的一環。為根本解決上述問題,本計劃將發展一全新的螢光量測技術,方法簡述如下:我們將用單一雷射產生的超連續譜(supercontinuum)脈衝光源激發所有在此頻域內的螢光分子,並將其產生的各個不同波長的螢光訊號同時收集回來,此方法將不使用帶通濾

波片(filter)或分色鏡(dichroic mirror)，因此可完全使用超連續譜的所有頻帶，且所激發之螢光亦包含所有頻帶，而在頻率域重疊的激發光與螢光信號將用時間解析的方法在時間域分開來。為實現一個能夠即時遠距進行量測的單分子頻譜儀，我們將利用光纖元件製造一精巧、可攜的原型，我們將用一根高數值孔徑(numerical aperture) 雙包覆光纖(double-clad fiber)作為探頭(probe)，激發的光脈衝將經由光纖之蕊心 (core)傳遞至待測物，而螢光信號將經由高數值孔徑之內包覆(inner cladding)傳回。這個柔韌的光纖探頭不僅解決了在混濁環境（如生物組織）中光學量測的最大問題：光的散射與吸收，同時加長了被散射的激發光脈衝與螢光信號之間的時間延遲，因此我們可以更有效率地濾除雜散的激發光，進而得到更好的信號雜訊比。除此之外，此雙包覆光纖的內包覆之高數值孔徑(~0.5)提供了極佳的螢光偵測效率，非常適合用來做生物體內單分子的量測。配合適當的光纖探頭設計，此種量測技術將可應用在多種常用的生物醫學光學量測技術上，從而產生出精簡可攜式的螢光顯微鏡、流式細胞儀等。研究成果將可能對立基於分子診斷學的生物醫學及保健工業造成深遠的影響。

關鍵字：螢光檢測；光纖光學；非線性光學；
超連續譜；單分子頻譜學

Time-Resolved Full-Spectrum All-Fiber Fluorescence Biosensing with Ultrafast Supercontinuum(I)

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Abstract

The primary goal of the proposed research is to develop a novel methodology for remote multicolor fluorescence detection. There is a great demand to concurrently distinguish a variety of different biomarkers in order to study different cellular functions and biological processes. However, in conventional fluorescence detection, a single excitation wavelength can excite only a limited number of fluorophores. A full coverage of simultaneous excitation over the visible to near infrared region has not been realized with conventional systems due to the limited number of discrete excitation wavelengths available. Conventional systems seek to meet the increasing demand for multicolor fluorescence detection by increasing the number of excitation sources and detection channels by using a number of filters. Consequently, these systems become very complicated and lossy. In addition, the bulky instrumentation limits its application in situ or in vivo. However, it is critical to be able to quantify fluorescence biomarkers in the native environment of the cells to understand the underlying mechanism in the biological processes. The proposed approach employs a fundamentally different mechanism that overcomes the conventional detection limits. We propose to use a single supercontinuum source to simultaneously excite all conceivable dye molecules and collect the entire individual spectra of the fluorescence emissions. The entire spectrum of the supercontinuum generated from a femtosecond laser will be used without filtering. The fluorescence signal will be separated from the scattered excitation light in the time domain. This novel detection scheme significantly simplifies the optical configurations through elimination of multiple lasers, a number of band pass filters and dichroic mirrors. To further realize a compact, portable versatile single-molecule spectrometer for in vivo applications, a monolithic fiber-optic probe prototype will be constructed. A single fiber probe will be used to simultaneously delivery the excitation pulses and collect the fluorescence signal. The fiber will introduce extended time delay between the scattered excitation light and the fluorescence signal, making the gating of signal and excitation pulses

more efficient, hence increasing the signal to noise ratio. In addition, the flexible fiber probe makes in vivo or remote detection in turbid environments possible by circumventing the scattering and absorption tissues. High numerical aperture double-clad fibers will be used as the probe to enhance the detection sensitivity. The proposed methods address the fundamental issues for remote multicolor detection. We believe the successful development of the proposed detection scheme in conjunction with proper design of the flexible fiber probe will lead to remarkable improvements of various kinds of optical instruments, such as fluorescence microscopes, microplate readers, and flow cytometers, enabling a wide range of biomedical applications.

Key words: Fluorescence detection;Fiber optics;Nonlinear optics;
Supercontinuum;Single-molecule spectroscopy