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Detection of the homo- and hetero-interaction of proteins using fluorescence resonance energy transfer spectrum method

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Abstract

Fluorescence spectrometry based on fluorescence resonance energy transfer (FRET) principle is a simple but effective tool for investigating protein–protein interactions. In this paper, we report a spectrometry to quantify FRET efficiency based on our home-designed spectral probe system and spectral data-processing procedure. In our method, the fluorescence spectrum from each specimen is recorded at two wavelengths 454 and 502 nm. Least-squares linear fitting algorithm is applied directly to decompose the spectra of donor and acceptor under these two wavelengths to obtain FRET efficiency, which takes both spectral intensity and spectral profile into account compared with traditional three-step analysis. This system and the data-processing procedure enabled us to detect the homo-interaction and hetero-interaction of proteins in living cell.

Keywords: Fluorescence spectroscopy; Spectral unmixing; FRET; CFP; YFP

1. Introduction

Since the last decade of the twentieth century, fluorescence resonance energy transfer (FRET) technique has found widespread applications in various fields owing to its advantage of providing direct and noninvasive detection of protein interaction *in vivo* [1,2]. Up to now, most FRET detection is achieved in fluorescence microscope through two- or multi-channel imaging [3,4]. In those FRET imaging systems, emission filters are specially designed to collect emissions only in the short wavelength range of the donor or in the long wavelength range of the acceptor in order to avoid cross-talk among the image channels. The problem is that for FRET to work, the donor emission and acceptor excitation spectra must overlap (high overlap is good), but for good signal-to-noise ratio imaging one must avoid collecting the "wrong" photons [5].

A potential alternative collection method is fluorescence spectrometry that would collect all the photons from both the donor and acceptor simultaneously without the requirement of any filter. Therefore, fluorescence spectrometry could produce straightforward and instrument-independent FRET result for recording full-spectrum information instead of only images' intensity. Accompanied with the increasing application of spectral FRET, data-processing methods to extract FRET information from fluorescent spectrum

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have also drawn great attention. Overton and Kota have established three-step analysis procedure to quantify FRET efficiency [6,7], which determine emission induced by FRET by subtracting the cell autofluorescence, then emission of donor, and the last the acceptor emission due to direct excitation. Although the three-step analysis procedure is a robust method, it can be a tough exercise, requiring the precise control of cells number in each sample and the precise calculation of normalized factor. And the three-step analysis procedure is costly in terms of time, requiring performing all the subtractions step by step. Furthermore, the nature behind this approach is to quantify FRET only from spectral intensity without considering spectral profile. Recent publications have demonstrated the feasibility of spectrally unmixing procedures [8–10] in quantitative FRET studies. Gu and his colleagues succeeded to resolve the spectral bleed-through by combining the spectral unmixing technique with FRET by acceptor photobleaching, and retrieved the FRET efficiency by spectrally unmixing the donor emissions before and after photobleaching [11]. Raicu et al. [12] demonstrated collection of 16 images of fluorescence emissions from each sample over 5 nm-wide windows separated by 1 nm gaps to form emission fluorescence spectra using a wavelength-tunable confocal laser scanning microscope, and then separated the spectra generated by donor and acceptor through spectral unmixing. However, those unmixing methods applied for FRET microscopy, either combined with acceptor photobleaching or confocal microscopy, may all complicate the experimental operation or the data analysis.

In this paper, we report a spectrometry based on a simple spectral linear unmixing algorithm and a self-developed compact spectral probe system for studying the interaction of proteins inside living cells. In our approach, spectral unmixing is applied directly to spectral decomposition of fluorescence spectra obtained in our probe system. No spectrum construction or other supplementary technology is needed. The fluorescence spectrum from each specimen (co-transfected with donor and acceptor) is recorded at the two wavelengths, which are 454 and 502 nm (for the cyan fluorescent protein (CFP)–yellow fluorescent protein (YFP) FRET pair in our case). Least-squares linear fitting procedure gives out the best-fitting curves to experimental composite spectra based on the knowledge of member spectra at the two wavelengths, respectively, and the fraction of each component can be worked out. Through comparing the relative fraction factors of acceptor emission components under two wavelengths, the FRET efficiency is obtained. Our approach takes both spectral intensity and spectral profile into account compared with three-step analysis, and is more simple and straightforward to quantify FRET efficiency compared with other spectral unmixing microscopy. We applied our system to detect FRET between proteins of interest fused with CFP and YFP in living Cos7 cell. FRET information from specimen is analyzed by spectral unmixing and comparing with three-step analysis procedure. Two approaches gave the similar results, suggesting homo- and hetero-interaction of target proteins *in vivo*, which implies that our system is sufficient for investigating the interaction of proteins in living cell conveniently.

2. Material and method

2.1. Spectral probe system

The experimental setup is shown in Fig. 1. An Ar ion, nine lines, single-mode laser (MellesGriot, CA, USA) was used as the excitation light source. The cooling fan was mounted away from optical table to prevent fluctuation of laser power .The laser beam with a diameter of 2 mm was focused with an achromatic lens ($f = 100$ mm) into a $10 \times 10 \times 40\,\text{mm}^3$ quartz sample cell. A shutter is placed in front of the laser source to avoid excess illumination of sample while no signal collection is undertaken. The fluorescence signal that transmitted at the right angle was focused into the slit of a monochromator (Triax180, Jobin-Yvon, Horiba, France) through a focusing lens ($f = 50$ mm), and the front entrance slit of monochromator was set as 20 μm to ensure a high spectral resolution as well as a good collection efficiency of the fluorescence photons. A 16bit CCD (Jobin-Yvon, Horiba, France) was mounted at the output of the monochromator to record the fluorescence spectra.

2.2. Cell culture and transfection

Cos7 cell line were seeded in 12-well overnight with Dulbecco's modified Eagle's medium (DMEM), which contains 10% fetal bovine serum and glutamine. Cells

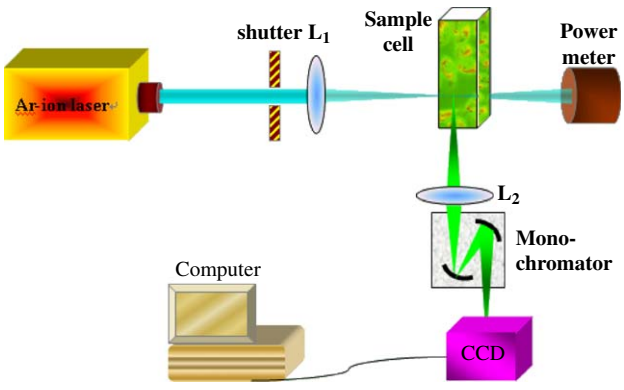

Fig. 1. Schematic drawing of FRET spectral probe system.