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荧光共振能量转移效率的实时定量测量

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荧光共振能量转移(FRET)广泛用于研究分子间的距离及其相互作用, 与荧光显微镜结合,可定量获取有关生物活体内蛋白质、脂类、DNA 和RNA的时空信息。随着绿色荧光蛋白(GFP)的发展,FRET荧光显微镜有可能实时测量活体细胞内分子的动态性质。提出了一种定量测量FRET效率以及供体与受体间距离的简单方法,仅需使用一组滤光片和测量一个比值,利用供体和受体的发射谱消除光谱间的串扰。该方法简单快速,可实时定量测量FRET的效率和供体与受体间的距离,尤其适用于基于GFP的供体-受体对。

REAL-TIME QUANTITATIVE FLUORESCENCE RESONANCE ENERGY TRANSFER MEASUREMENTS USING FLUORESCENCE MICROSCOPY

Fluorescence resonance energy transfer (FRET) is widely used in studies of biomolecular structure and dynamics. By combining fluorescence microscopy with FRET it is possible to obtain quantitative temporal and spatial information about the binding and interaction of protein, lipids, enzymes, DNA, and RNA in vivo. With the recent development of a variety of mutant green fluorescent proteins (GFPs), FRET microscopy provides the potential to measure the dynamic interaction of intracellular molecular species in intact living cells where the donor and acceptor fluorophores are actually part of the molecules themselves. However, present intensity-based FRET quantitative measurements suffer from cross talk of the donor and acceptor emission spectra, which cannot be corrected in a real time. We present a simple method to correct this cross talk in a real time. The data were obtained with only one standard filter set in a fluorescence microscopy. Four coefficients were introduced to eliminate the cross talk, which are constants over various FRET strengths and can be calculated ahead of the experiments based on the emission spectra of the donor and acceptor, and the spectra features of the detection channels. Our quantitative FRET measurements approach has the potential to track dynamic interactions in biological system.

关键词

能量共振转移(Resonance energy transfer efficiency); 定量FRET效率测量(Quantitative FRET efficiency measurement); 实时成像(Real time imaging); 绿色荧光蛋白(Green fluorescent proteins); 串扰(Cross talk)