

Fluorescence Resonance Energy Transfer Microscopy

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Fluorescence is a property of certain fluorophores, which allows them to absorb light at one wavelength and emit the light at another, longer wavelength. This fluorescence can be used as a tool to study the interactions of numerous components within a cell. With the use of fluorescent probes, proteins or other cellular matter can be specifically labeled in a fixed or live cell. This allows scientists to closely monitor microscopic cellular functions in order to gain a greater understanding of living biological processes^{1,2}.

Fluorescence Resonance Energy Transfer (FRET) imaging microscopy is an important and reliable tool for studying minute molecular processes in space and time within a single cell³⁻⁹. FRET spectroscopy is a better method for studying the structure and localization of proteins in physiological conditions than are X-ray diffraction, nuclear magnetic resonance, and electron microscopy¹⁰. FRET is defined as the radiationless transmission of energy from an excited donor molecule to a near-by acceptor molecule¹¹⁻¹³. The donor is a fluorophore (fluorescent probe) with a relatively long lifetime that initially absorbs the energy, and the acceptor is a fluorophore to which the donor transfers its energy. When this transfer takes place the excitation of the donor molecule intensifies the fluorescence emission of the longer-wavelength acceptor molecule. At the same time, the donor fluorescence emission is diminishing, which makes the energy transfer easy to see. Thus, the appearance of FRET has become a well-known tool for measuring the distance between two molecules, and has been dubbed as a unique and accurate "spectroscopic ruler"¹³.

In order for FRET to occur, the process must fulfill at least three conditions: 1) The distance between the donor (D) and acceptor (A) molecules must be in the range of 1-10 nm. The amount and effectiveness of the energy transfer depends on the close proximity of the molecules. The efficiency of FRET is related to the inverse of the sixth power of the distance between the donor and acceptor molecules. 2) The absorption spectrum of the acceptor must sufficiently overlap the emission spectrum of the donor (Figure 1C). This allows the transmission to take place without the conversion to thermal energy, and without the emission or transfer of a photon. 3) The transition dipoles of the donor and acceptor must be oriented approximately parallel to each other, in order for the dipole-dipole

interaction to occur.

The rate of energy transfer between the donor and acceptor molecules is shown as¹²:

$$K_T = (1/\tau_D) (R_0/r)^6$$

where K_T is the rate of energy transfer, τ_D is the lifetime of the donor without the acceptor, R_0 is the Förster distance, which shows the distance when energy transfer is 50% efficient, and r is the distance between the donor and acceptor. The energy transfer efficiency (E_T) can be calculated by using the following equations:

$$E_T = 1 - (\tau_{DA}/\tau_D) = 1 - (I_{DA}/I_D)$$

where τ_{DA} is the lifetime of the donor in the presence and absence (τ_D) of the acceptor, or the relative fluorescence intensities under these respective conditions (I_{DA}/I_D). Using these equations, the distance between the donor and acceptor molecules can be determined by measuring the lifetime of the donor fluorophore with and without the presence of the acceptor⁶.

The discovery of new fluorescent probes has enhanced the usefulness of FRET for making real-time measurements of spatial changes of fluorescent molecules within a cell. These fluorescent dyes allow scientists to label one or more specific protein or component within a live cell. Specifically, the jellyfish green fluorescent protein (GFP) has shown to be an advantageous probe for protein labeling¹⁴⁻¹⁵. GFP creates a vibrant green color without the use of the cofactors that other fluorophores require. In addition to the non-GFP fluorophore (FITC-Rhod, GFP-Rhod, or GFP-Cy3) used for FRET imaging, the GFP fluorophore pairs are BFP-GFP, BFP-YFP, CFP-YFP, GFP-dsRED, and YFP-dsRED. For FRET imaging any fluorescent microscope can be used with the appropriate filter combination, depending on the fluorophore selected for labeling the protein. The donor (D) and acceptor (A) images are acquired by exciting the donor molecule of a double-labeled specimen. For example, for a BFP-GFP pair, in which BFP is the donor and GFP is the acceptor, excitation = 365/15, emission(D) = 460/50, and emission(A) = 520/40⁸. The detector coupled to the microscope should be a high-speed, high-sensitivity CCD camera. The FRET image is obtained by rationing the A/D images.

The fluorescence resonance energy transfer technique is a very important and sensitive tool used for visualizing the dynamics of proteins and molecules in living cells. Unlike conventional optical techniques, FRET is able to measure extremely small distances between molecules, and is therefore widely used in studying many important cellular events. For example, the alteration in

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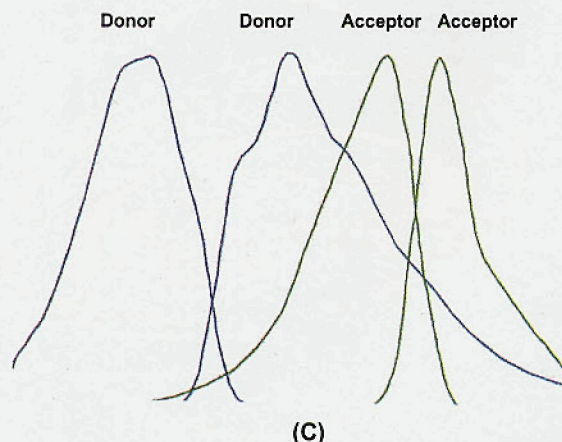
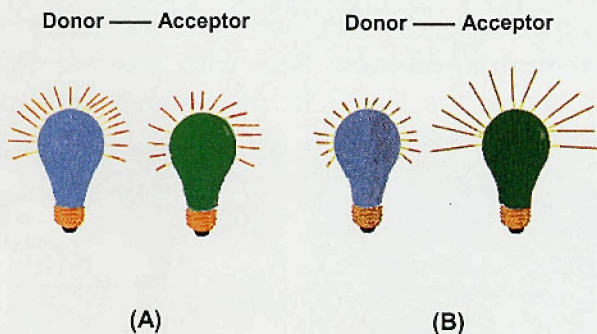
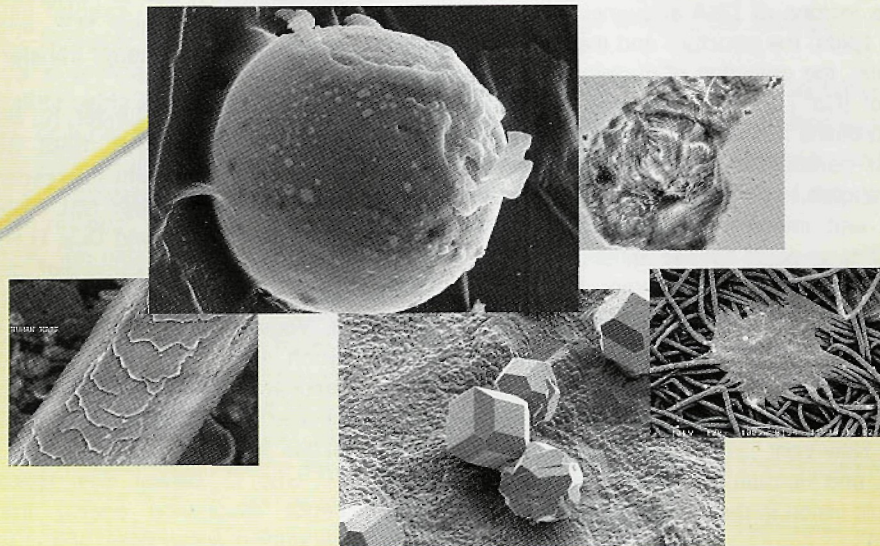


Figure 1: Representative illustration of energy transfer between two molecules, donor (D) to acceptor (A). If the conditions for FRET to occur are satisfied, then the radiationless energy transfer occurs between D and A causing the acceptor signal to increase (Figure 1B) compared to the normal condition ((Figure 1A)

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plasma membrane structure in the event of cell injury is being looked at with the FRET method¹⁶. Also, FRET is being applied to the study of the interaction between the HPV E6 protein and the tumor suppressor protein p53, in hopes of detecting human papillomaviruses more easily^{2,16}. Other applications of FRET include looking at DNA sequencing, the distribution and transport of lipids, the structure and distribution of nucleic acids and proteins, the detection of nucleic acid hybridization, the alterations of $[Ca^{2+}]$ levels^{2,17}, as well as visualizing the dimerization of proteins in two- and three-dimensions^{8,18}. With FRET and other pertinent microscopic techniques, such as confocal, multi-photon, and lifetime imaging, we have the chance to see and interpret biological processes in four-dimension (4D) that we could not see in the past.

The scientific world is growing every day with new techniques and amazing discoveries. Fluorescence Resonance Energy Transfer is one approach that is helping to further our knowledge and understanding of cellular functions. ■

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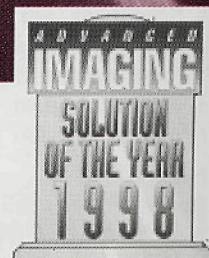
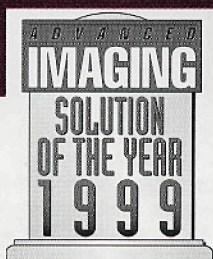




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