Application Note

Measurement of Bioluminescence Resonance Energy Transfer (BRET) Using Luminescence Microcell Holder

KEYWORDS

Fluorescence Spectrometer, Luminescence Microcell Holder, Bioluminescence, Luciferase, Quantum Dot, Biolumenescence Resonance Energy Transfer (BRET)

INTRODUCTION

An energy transfer between molecules is mostly caused by radiation. However, in case of different fluorescent substances in close proximity (< 10nm), resonance energy transfer (RET) will occur. Förster resonance energy transfer (FRET) is a typical RET phenomenon.

In FRET, a strong light is lit directly in order to excite a donor energy, whereby unintended noise lights may be generated (Fig. 1). A residual energy that has been transferred to resonance from the donor is converted into light (bleed-through), and direct ray of lights strike unwanted fluorescent molecules such as the acceptor (cross-talk) and a natural entity in biological materials (autofluorescence). Also, many fluorescent molecules lose their fluorescence character gradually upon exposure to light (photobleaching).

For increasing the sensitivity, bioluminescence resonance energy transfer (BRET) is recently applied[1]. BRET substitutes the fluorescent donor with the bioluminescent substances. Since BRET excludes any direct ray of light, it not only blocks the cross-talk and the autofluorescence but also prevents the photobleaching (Fig. 2).

Luciferase, is widely known as the bioluminescent enzyme of a firefly, is mainly used in the BRET study[2]. In this experiment, Luc8 (*Renilla* luciferase 8) which is genetically mutated from the luciferase of sea pansy *(Renilla reinformis)* is chosen as donor (Fig. 3). As shown in Fig. 4, Luc8 has the broad bioluminescent peak near 480nm.

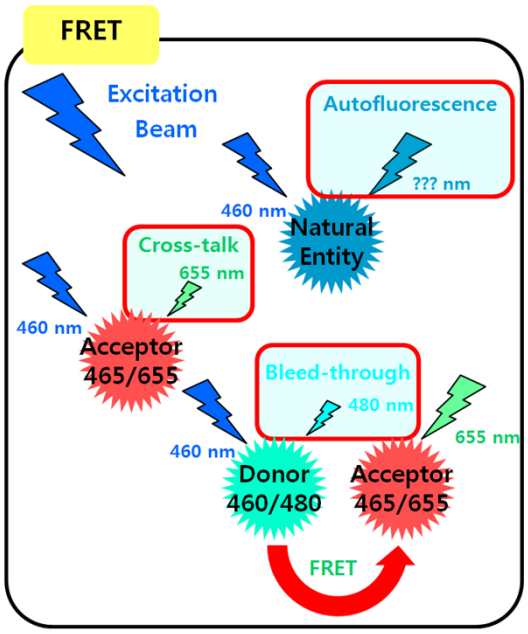

Fig. 1. Diagram of the FRET and noise generation

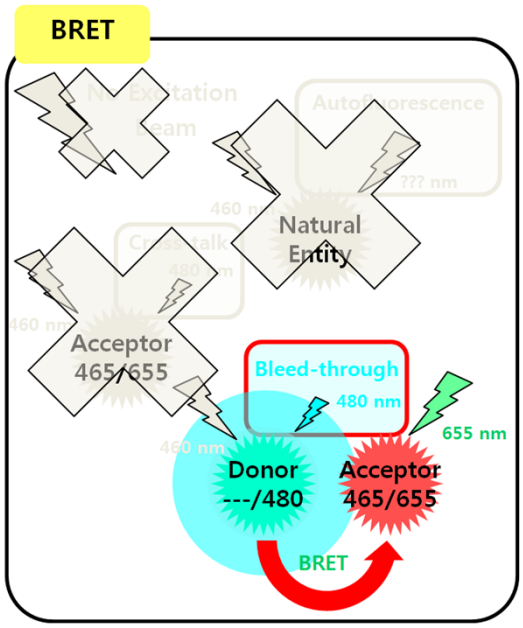

Fig. 2. Diagram of the BRET and noise elimination

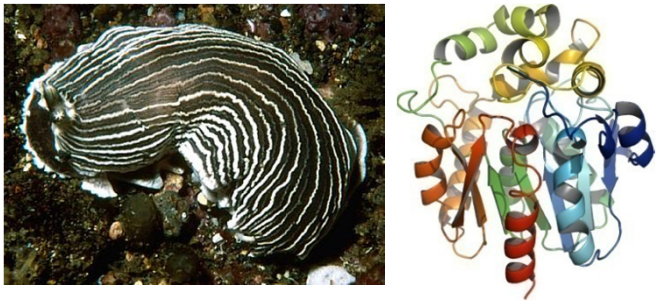

Fig. 3. Sea Pansy (Left) and the protein structure of Luc8 (Right)

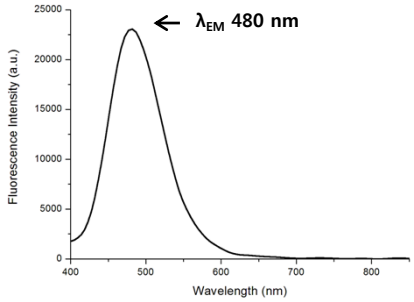

Fig. 4. The emission spectrum of Luc8

Acceptor should be determined by the emission wavelength of the donor. In this experiment 4 types of Quantum Dot (605, 655, 705 and 800) are used: they have a high quantum yield and are able to apply various fluorescence wavelength[3] (Fig. 5). These Quantum Dots absorb the light in a broad wavelength range, but emit the maximum value of the fluorescence spectrum on each 605, 655 705, and 800nm.

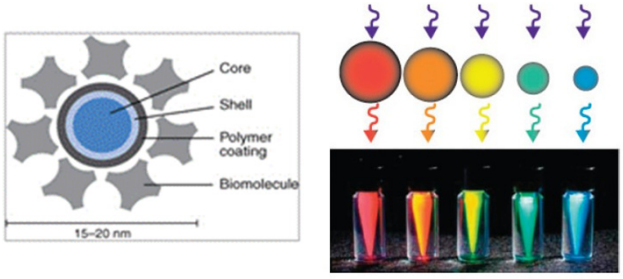

Fig. 5. Structure (Left) and fluorescence (Right) of Quantum Dots

The luminescence intensity is relatively weaker than the fluorescence intensity that is generated by a strong direct ray. Furthermore, the experiment using a micro cell obtains lesser amount of the light. In order to collect the light more efficiently while measuring BRET, a Luminescence Microcell Holder accessory, which has minimized the distance between the microcell and the emission lens, is used (Fig. 6).

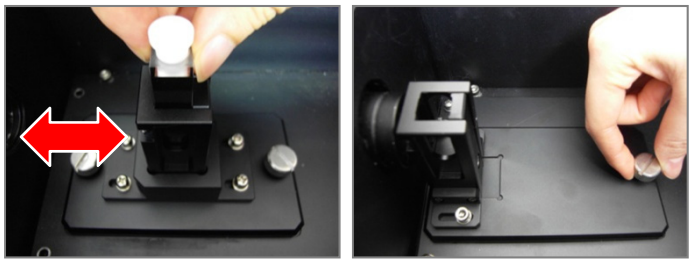

Fig. 6. Comparison between the Fluorescence Microcell Holder (Left) and the Luminescence Microcell Holder (Right)

REAGENT & APPARATUS

1. Fluorescence Spectrometer (FS-2)
2. Luminescence Microcell Holder
3. PBS Buffer Solution 0.1M (pH 7.4)
4. Luc8 150μM
   (Provided by Professor Young-Phil Kim in the life science department at Hanyang University)
5. Quantum Dot 605, 655, 705, 800 8μM
   (Invitrogen)
6. Coelenterazine H 1μg/μl in methanol (Promega)
7. $NiCl_2$ 100μM in distilled water (Sigma Aldrich)
8. 10μl Eppendorf Tubes
9. 45μl Fluorescence Microcell
10. Pipette & Pipette Tip
11. Vortex Mixer

PROCEDURE

1. Prepare four 10μl Eppendorf tubes.
2. Fill 94μl PBS buffer and 1μl Luc8 (the donor) in each tube.
3. Add 5μl Quantum Dot 605, 655, 705, 800 (the acceptor) in each tube.
4. Inject 2μl $NiCl_2$ solution (the conjugation reagent between the donor and the acceptor) in each tube and mix sufficiently on Vortex mixer.
5. Install the Luminescence Microcell Holder on FS-2, then inject the sample into the microcell and insert the microcell in the cell holder.
6. Execute the Wave Scan of the FluoroMaster Plus Software, then input the setup parameters and click the Apply button for standby status (Fig. 7).
7. Inject 2μl coelenterazine H using the pipette. After injection, close the lid of cell compartment quickly, and click the start button to measure the spectrum of each sample as fast as possible.
8. Apply Baseline Correction on the measured spectra. Click the Baseline Correction icon on the upper part of the window, then correct the baseline based on the wavelength which has the lowest bioluminescence intensity (Fig. 8).
9. Normalize the corrected spectra based on the Luc8 peak, and compare the Quantum Dot peaks. For normalizing, calculate the each conversion scalar that makes all the Luc8 peak intensities equal. Then click the Scalar Calculator icon on the upper part of the window, and normalize them by multiplying the conversion scholar (Fig. 9).

INSTRUMENT PARAMETER

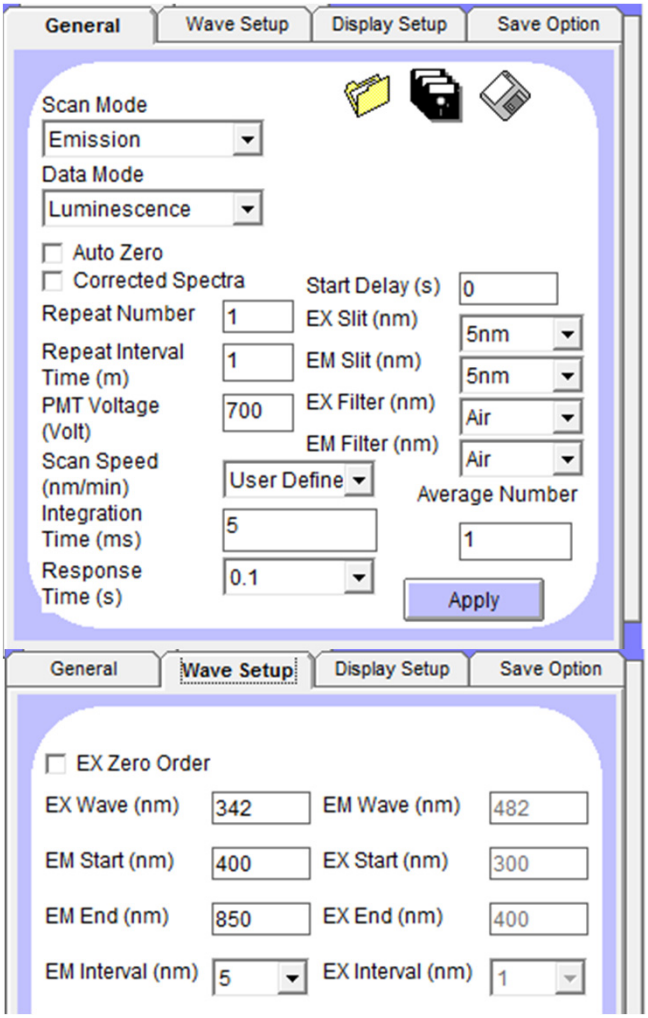

Fig. 7. Measurement condition of the Wave Scan mode

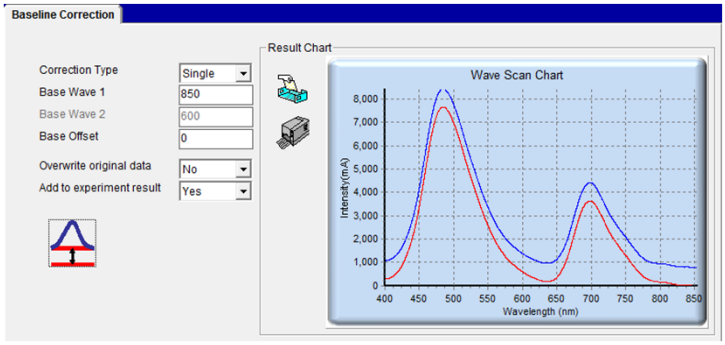

Fig. 8. Window of Baseline Correction

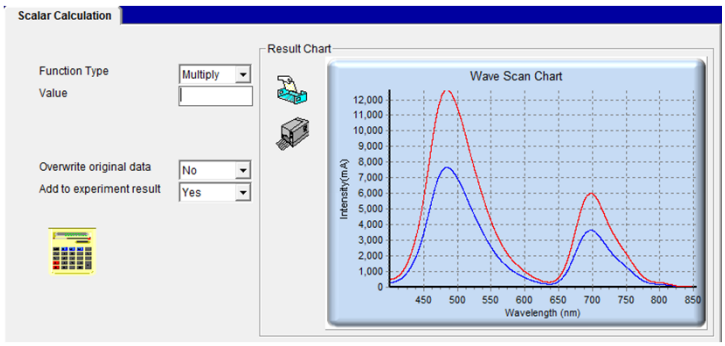

Fig. 9. Window of Scalar Calculation

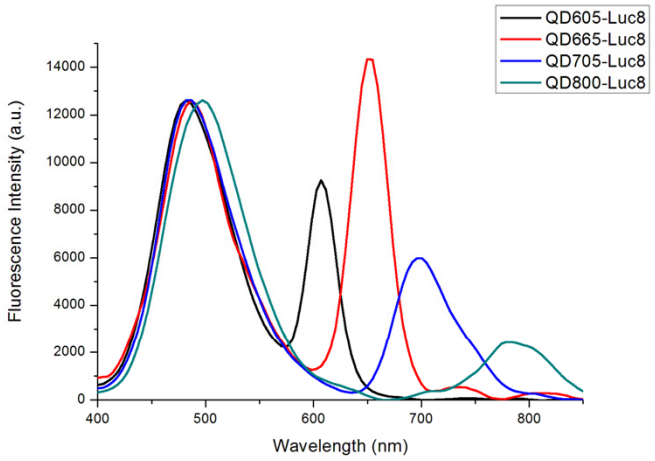

Fig. 10. BRET Spectra according to Quantum Dots

RESULT

The BRET phenomenon that transfers an energy from the bioluminescence of Luc8 to each type of Quantum Dot can be observed on all measured spectra (Fig. 10.). The BRET using Quantum Dot is quite helpful in the molecular diagnostic and imaging field since multiplexed Quantum Dot conjugation can emit many different wavelengths at one single wavelength.

Efficiency of an energy transfer by BRET is correlated with (1) the separation distance between the donor and the acceptor and (2) the overlap integral of the donor emission and acceptor excitation spectra.
By normalizing with the Luc8 peak and comparing the corrected spectra, we can verify that the Quantum Dot 665-Luc8 conjugate yields the maximum BRET efficiency among the conjugates[4] (Fig. 10).

CONCLUSION

The SCINCO's FS-2 and the Luminescence Microcell Holder accessory help you examine BRET that shows less noise than FRET. Also, we identify that the BRET phenomenon can be applied in the broad wavelength range depending on different emission wavelengths of the Quantum Dot.

REFERENCE

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