X-Cite®
Fluorescence Illumination • In Control

Successful FRET Microscopy Measurements Using White Light Solid-State Technology

Challenge
Conducting FRET with the instability of traditional fluorescence arc lamps

Solution
X-Cite® 120LED to replace existing microscope arc lamps

Benefits
Alignment-free uniform and stable illumination, fine intensity control, and long life LEDs for robust, successful FRET measurements

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INTRODUCTION

Förster (Fluorescence) resonance energy transfer (FRET) is non-radiative energy transfer from an excited molecule (the donor) to another nearby molecule (the acceptor), via a long-range dipole-dipole coupling mechanism. Since FRET is usually limited to distances less than about 10nm, FRET microscopy provides a sensitive tool for investigating a variety of phenomena that produce dynamic changes in molecular proximity in living specimens. In the branches of life sciences, FRET applications have grown exponentially as shown by the number of publications in many diverse fields since the 1990s (see details at www.kcci.virginia.edu/Literature)[1].

Widefield microscopy is the most commonly used fluorescence microscopy technique. The colorful range of available fluorescence probes can be imaged with a widefield microscope equipped with a mercury or xenon arc lamp using a correct combination of excitation, emission and dichroic filters. For quantitative fluorescence measurements, such as FRET, critical alignments of a traditional arc lamp are required to ensure an even illumination field; optical stability is unreliable due to the nature of these lamps, and a series of neutral density filters are typically used to tune the light intensity to a desired level, especially critical for live cell imaging. All these traditional requirements can now be phased out with new LED-based systems. Here, we present FRET measurement results using the X-Cite® 120LED system.

DEMONSTRATION OF THE X-CITE 120LED CAPABILITY FOR FRET MEASUREMENTS USING FRET STANDARDS

To test the capability of the X-Cite 120LED for FRET measurements, we used the FRET-standard approach developed by the Vogel laboratory (NIH)1,2. Both C5V and CTV FRET-standard constructs were expressed in live GHFT1 cells. C5V, where Cerulean and Venus fluorescent proteins are tethered by a 5 amino acid linker, gives a FRET efficiency of 40-50%. In comparison, CTV where Cerulean and Venus fluorescent proteins are separated by a 229 amino acid linker encoding a TRAF domain, should yield a FRET efficiency less than 10%. For spectral bleedthrough corrections, cells transfected with Cerulean-alone (donor-only) or Venus-alone (acceptor-only) were also used.

Data Acquisition: Images were acquired in three imaging channels: Donor (Ex. 436/20nm, Em. 470/30nm), FRET (Ex. 436/20nm, Em. 535/30nm), Acceptor (Ex. 500/20nm, Em. 535/30nm). The X-Cite 120LED light source was coupled to an Olympus IX70 widefield microscope equipped with a Hamamatsu ORCA2 camera and an Olympus 60X/1.4NA oil-immersion lens.

Data Analysis: Images were processed by the PFRET algorithm to remove spectral bleedthrough contaminations and calculate FRET signals and efficiencies3-5. The ratios between the donor and the FRET channels of C5V or CTV were calculated – a larger ratio indicates a higher FRET efficiency.

Results: The raw images acquired in the donor, acceptor, FRET channels and the processed FRET (pFRET) images of C5V and CTV are shown in Figures 1 and 2, respectively. Each image is individually contrasted for the best visualization. Each pair of the uncorrected FRET (uFRET) image acquired in the FRET channel and the processed FRET (pFRET) image are contrasted in the same range for a direct comparison. The C5V (47.7±4.8%) and CTV (5.0±2.9%) FRET efficiencies are compared in Figure 3, while the C5V (2.23±0.09) and CTV (0.87±0.06) FRET ratios are compared in Figure 4. These results clearly demonstrate the capability of the X-Cite 120LED light source for successful FRET measurements.

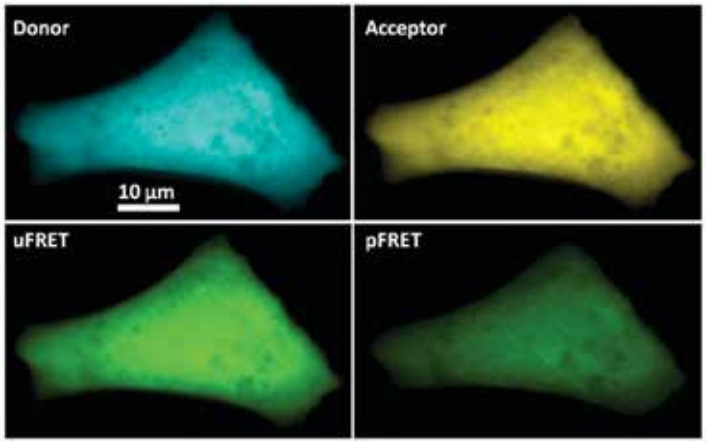

FIGURE 1: C5V donor, acceptor and uncorrected FRET (uFRET) and processed FRET (pFRET) images.

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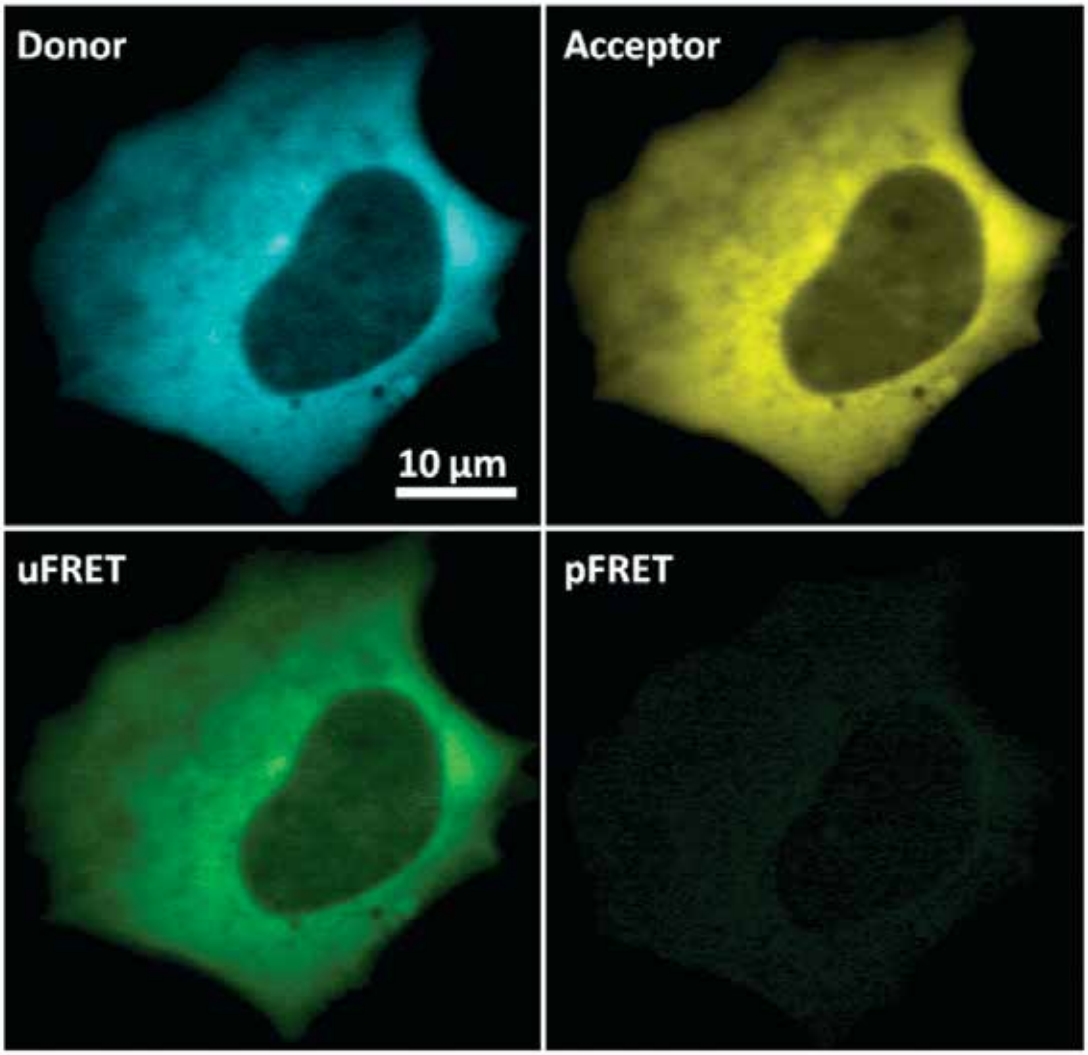

FIGURE 2: CTV donor, acceptor and uncorrected FRET (uFRET) and processed FRET (pFRET) images.

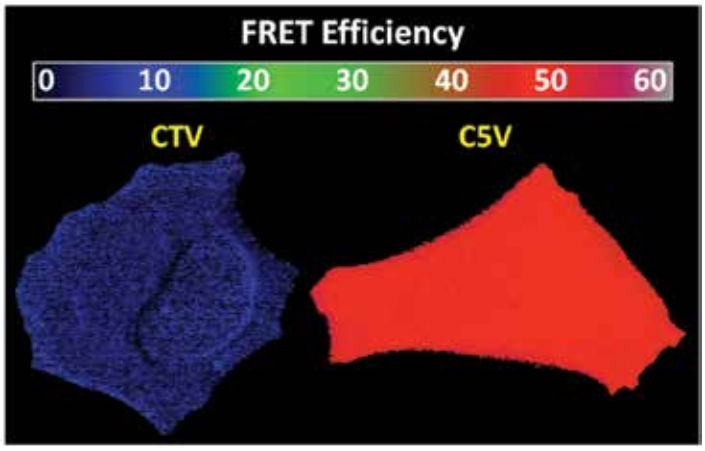

FIGURE 3: Comparison of CTV and C5V FRET efficiencies.

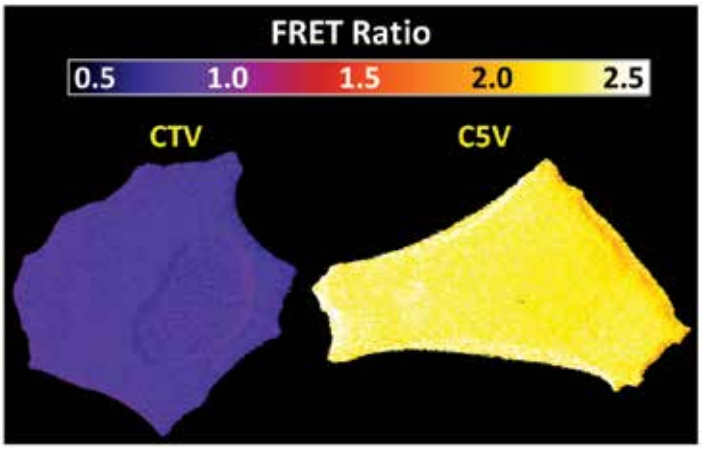

FIGURE 4: Comparison of CTV and C5V FRET ratios.

CONCLUSION

We were able to successfully calculate the predicted FRET efficiencies and ratios using the X-Cite® 120LED fluorescence light source. Along with showing that the system provided sufficient intensity to be able to excite our fluorescent probes, the X-Cite 120LED also provides the following benefits:

- Alignment Free – As there are no bulbs involved, there is no need for any alignment
- Field Uniformity – The LED based system provided good sample field uniformity
- Stability – LED technology is inherently more stable than lamp technology
- Fine Illumination Intensity Tuning – 1% intensity tuning eliminates the need for neutral density filters
- Long Lifetime – The LEDs are guaranteed for 25,000 hours

> *"The X-Cite® 120LED provides successful FRET measurements and works well with all of our fluorophores. We find it bright, uniform and easy to use."*
>
> **Dr. Ammasi Periasamy**
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