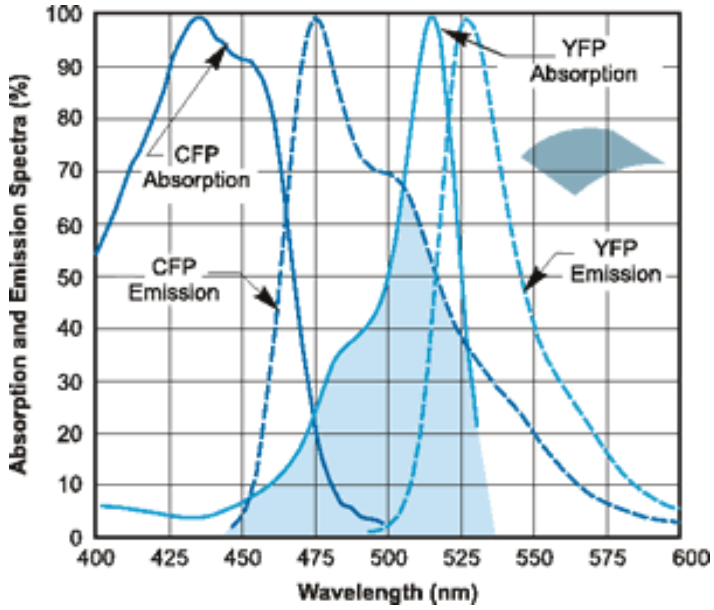
FRET

An overview of Andor's solutions for FRET

FRET (sometimes called Förster Resonance Energy Transfer), is an increasingly popular microscopy technique used to measure the proximity of two fluorophores.Resonance energy transfer occurs only over very short distances, typically within 10nm, and involves the direct transfer of excited state energy from the donor fluorophore to an acceptor fluorophore as an alternative to fluorescence emissive decay from the donor. Upon transfer of energy, the acceptor molecule enters an excited state from which it decays emissively (always of a longer wavelength than that of the acceptor emission). Thus, by exciting the donor and then monitoring the relative donor and acceptor emissions, either sequentially or simultaneously, one can determine when FRET has occurred and at what efficiency.Since fluorophores can be employed to specifically label biomolecules and the distance condition for FRET is of the order of the diameter of most biomolecules, FRET is often used to determine when and where two or more biomolecules, often proteins, interact within their physiological surroundings. Since energy transfer occurs over distances of 1-10nm, a FRET signal corresponding to a particular location within a microscope image provides an additional distance accuracy surpassing the optical resolution (~0.25 mm) of the light microscope. Aside from spatial proximity, for efficient FRET to take place the FRET dye pair must also exhibit significant overlap of the donor's excitation spectrum with the acceptor's absorption spectrum. Herein though lies one of the experimental paradoxes of FRET. The spectral profiles of the FRET pair cannot be so separated that we have poor overlap, yet one wants to avoid "cross-talk" between the two imaging channels, i.e. ideally the donor emission filter set must collect only the light from the donor and none from the acceptor, and vice versa. In practice, this can be somewhat realized by employing short bandpass filters that collect light from only the shorter wavelength side of the donor emission and the longer wavelength side of the acceptor emission. This can limit somewhat the photon flux from both donor and acceptor during a typical exposure, especially when we bear in mind that these measurements are best performed under conditions of reduced excitation power, such that we do not accelerate the rates of bleaching.

BFP-GFP; CFP-dsRED; BFP-GFP; Cy3-Cy5; CFP-YFP Alexa488-Alexa555; Alexa488-Cy3 FITC-TRITC; DiSBAC4(3)-CC2-DMPE (a voltage sensitive FRET pair)

Andor's EMCCD cameras, whether as a key component in our Revolution confocal live cell imaging system, or as an EMCCD + iQ imaging software solution, is a well-established technique for FRET imaging. EMCCD enables high resolution, high signal-to-noise (S/N) determination of FRET interactions throughout the imaged area or volume of the cell and help counter the photon throughput sacrifice involved when using narrow-band filters This combined with careful choice of filter sets ensures high integrity of FRET data.Since EMCCDs overcome the noise floor detection limit at any readout speed, molecular interactions can be followed dynamically with high accuracy. Furthermore, through reducing the excitation power, phototoxic and photo-bleach effects are minimized, enabling molecular interactions to be followed for much longer periods.



Absorption and emission spectral profiles of the CFP-YFP FRET pair.