Flow Post-its
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Tandem Dyes

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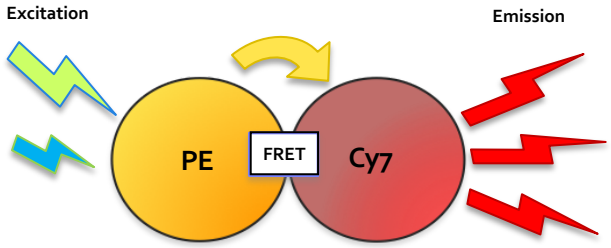
When conjugating two dyes where one fluorochrome's emission spectra (donor) overlaps the excitation spectra of a second fluorochrome (acceptor) a phenomenon called **Förster Resonance Energy Transfer (FRET)** occurs, creating a **new dye** with the excitation maximum of the donor and the emission maxima of the acceptor.
The resulting dye is called a **Tandem dye.**

Naturally occurring stokes shifts are generally small, meaning that the emission maximum wavelength is typically close to the excitation maximum. The use of tandem dyes allows for an increase in the stokes shift, and the simultaneous use of several of these dyes excited by the same laser but with increasingly larger stokes shifts can expand the capabilities of multicolor flow cytometry,.

The donor fluorochrome is excited by laser light and gives off light at a longer wavelength. This emitted light from the donor then excites the acceptor fluorochrome, which then emits the light at an even longer wavelength.



PE-Cy7 (Ex_{max}566 nm/Em_{max}785 nm)

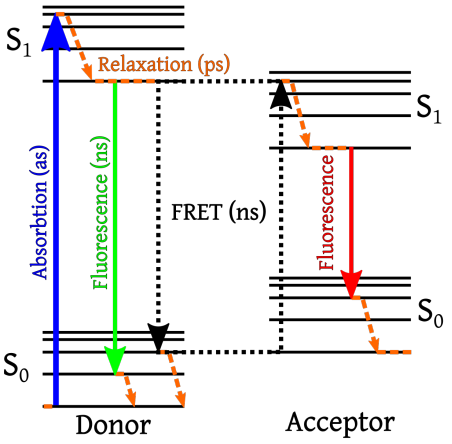


Fig. Jablonski Diagram
https://commons.wikimedia.org/w/index.php?curid=23197114

Helpful Hints

- Antibodies conjugated to tandems and any subsequent samples stained with these antibodies should be **protected from light and kept at 4C** to prevent degradation.
- Due to lot-to-lot variation, the **same antibody/tandem dye conjugate should be used** for your **controls** and **experimental samples**. In fact, considering the sensitivity of tandem dyes to light and temperature, the **same vial** should be used.
- For fix/perm, **confirm stability of the tandem dye** under experimental conditions
- **Note:** many of the brilliant & super bright fluorochromes are tandems and should be treated as such (eg. Brilliant Violet 605, Super Bright 702, etc.).