INTEGRATED DNA TECHNOLOGIES

Fluorescence and Fluorescence Applications
Quick Look

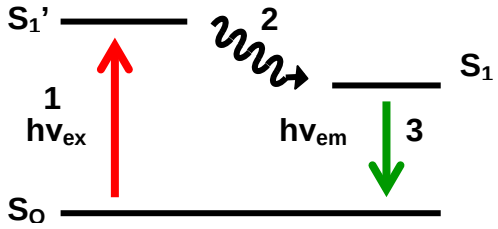
This is a modified, quick look version of the full Technical Report *Fluorescence and Fluorescence Applications.* Please see the full version for a more comprehensive explanation.

Fluorescence is a short-lived type of luminescence created by electromagnetic excitation. It is generated when a substance absorbs light energy at a short (higher energy) wavelength and then emits light energy at a longer (lower energy) wavelength (Figure 1).

Luminescence is the production of light through excitation by means other than increasing temperature. These include chemical means (chemiluminescence), electrical discharges (electroluminescence), or crushing (triboluminescence).

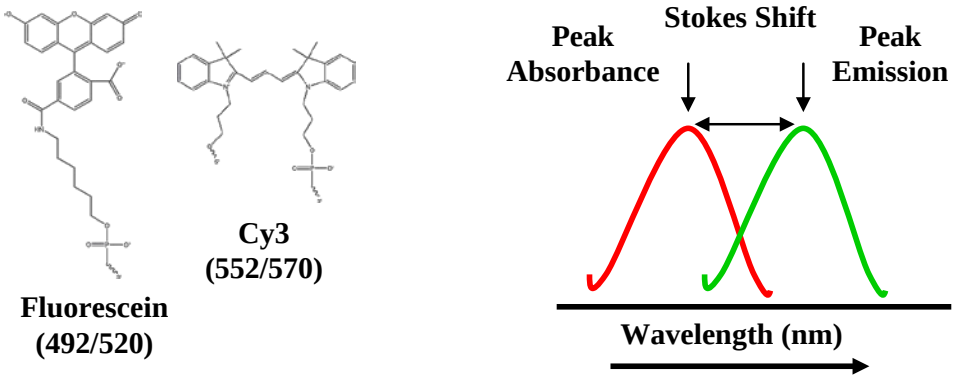
Fluorescence-labeled oligonucleotides have a wide range of applications in PCR, DNA sequencing, microarrays, and in situ hybridization. This is achieved through the Fluorescence Resonanace Energy Transfer (FRET) phenomenon where energy released upon excitation of a donor fluorophore is captured by an acceptor fluorophore or quencher.

Figure 1. Jablonski Diagram of a fluorescence event. The fluorescent molecule begins in its ground energy state, S_0, and is converted to an excited singlet state, S_1, by absorbing energy in a specific wavelength. The molecule will transition to the relaxed singlet state, S_1, by releasing some of the absorbed energy. Finally, the molecule will return to its ground energy state by releasing the remaining energy. The duration of a single fluorescence event is a few nanoseconds.



Fluorophores or **fluorochromes** are molecules that display fluorescence. Each of these molecules has a characteristic absorbance spectrum and a characteristic emission spectrum (Figure 2). **Peak absorbance** is the specific wavelength at which one of these molecules will most efficiently absorb energy and **peak emission** is the wavelength at which it will most efficiently emit energy.

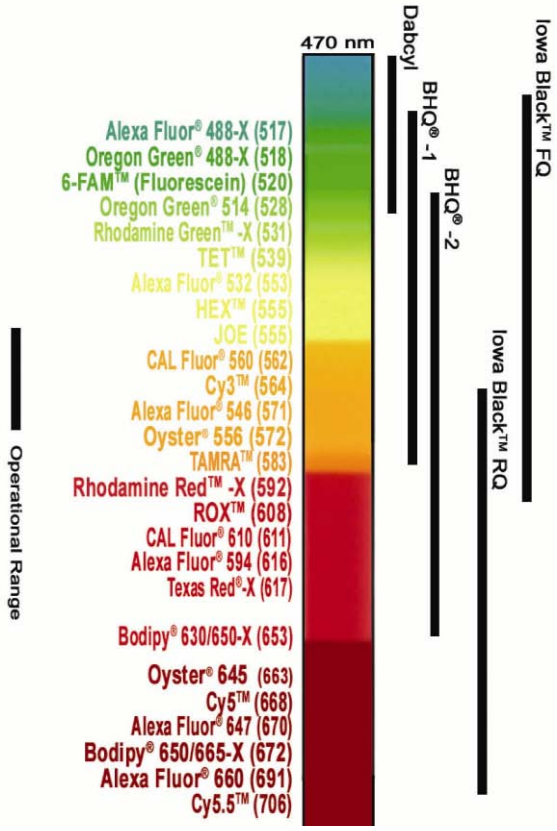
Figure 2. On the left are examples of the ring structures characteristic of fluorescent molecules. The peak absorbance and peak emission (in nanometers) of each fluorophore is shown. On the right, a generalized representation of the absorbance and emission spectra of a fluorophore is shown.



Quenchers are chemically related to fluorophores but instead of emitting absorbed fluorescence reasonance energy as light they have the useful property of transforming the light energy to heat. Heat dissipation of fluorescence energy means that replacing a fluorescent acceptor, like TAMRA, with a quencher, such as Iowa Black™ FQ, will result in an oligonucleotide construct that has no measurable fluorescence as long as the oligonucleotide tether remains intact (Figure 3). Such constructs can greatly simplify many fluorescence assays since there will be no background fluorescence. For this reason, fluorophore-quencher dual-labeled probes have become a standard in real-time PCR.

Fluorescence Resonance Energy Transfer (FRET) takes place when electromagnetic radiation excites a donor in the proper wavelength. This donor fluorophore can then directly excite an acceptor fluorophore which will emit energy at a new wavelength. Activation of this acceptor fluorophore can be detected by looking for the energy emitted at the new wavelength. Acceptance of donor energy by a FRET acceptor requires that two criteria must simultaneously be satisfied: compatibility and proximity.

Figure 3. Dynamic ranges of a number of fluorescence quenchers. The number of wavelengths over which one of these quenchers will absorb fluorescence energy provides flexibility in choosing fluorophores for multiplex assays.



Compatibility: A compatible acceptor is a molecule whose absorbance spectrum overlaps the emission spectrum of the donor molecule. If the absorbance spectrum of a molecule does not overlap the emission spectrum of the donor, the emitted energy will not be able to excite the potential acceptor. If the absorbance spectrum of the acceptor does overlap the emission spectrum of the donor, energy from the donor will excite the acceptor molecule provided that the proximity criterion is met.

Proximity is less precisely defined in operational terms. Proximity means that a compatible acceptor molecule is "close enough" to the donor for the energy to excite it. Beyond a maximum length of an oligonucleotide, with one member of a FRET pair tethered at each end, FRET will not be sufficiently efficient for reliable assays (Figure 4). In practice, this maximum length is greater than 60-70 nucleotides for many FRET pairs.

Figure 4. Representation of compatibility and proximity in a FRET donor and acceptor fluorophore pair. On the left, the relationship between the absorbance and emission spectra of the FRET pair is shown. On the right is a representation of acceptable proximity for a FRET pair in terms of their Förster radii. The tether between the two fluorescent molecules is an oligonucleotide.