INTEGRATED DNA TECHNOLOGIES

Spectra of DNA-Conjugated Fluorescent Dyes

Fluorescent dyes have become the preferred method of detection for nucleic acids in Molecular Biology. Some common examples include automated fluorescent DNA sequencing, fluorescent genotyping, and quantitative target detection techniques (e.g., Molecular Beacons, etc.).

Fluorescent dyes absorb light at a characteristic wavelength and re-emit light at a second lower energy, longer wavelength. The wavelength (nm) where photon energy is most efficiently captured is defined as the $Absorbance_{max}$. The wavelength (nm) where light is most efficiently released is defined as the $Emission_{max}$.

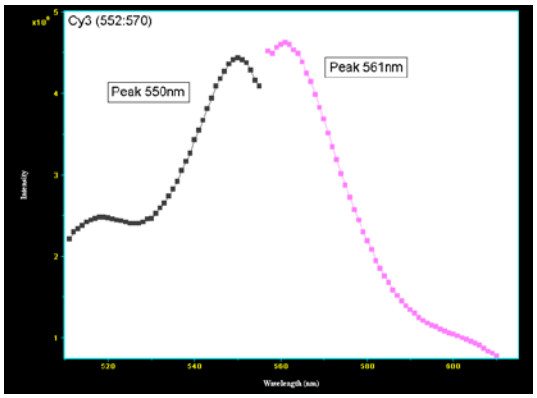
The absorbance/emission properties of fluorescent dyes are affected by their environment, including solvent, pH, and conjugation to other macromolecules (e.g., nucleic acids). For example, the fluorescent properties of fluorescein dramatically change in low pH. Free fluorescein has a pKa of 6.4 and shifts to the protonated form in low pH buffers, which is poorly fluorescent. Therefore, all studies done using fluorescein should be done at pH 7.0 or higher.

Changes in physical properties of fluorescein that occur after conjugation to DNA were studied in detail by Sjoback et al. [1]. When attached to a single-stranded oligo, the pKa of fluorescein shifts from 6.4 to 6.9 and the quantum yield decreases from 0.93 to 0.72. Further, the absorption efficiency (extinction coefficient) of fluorescein decreases by 1/3 after conjugation to DNA [2]. On the other hand, rhodamine does not undergo a similar change in absorption efficiency following conjugation. Unfortunately, this kind of comprehensive analysis is not available for most dyes.

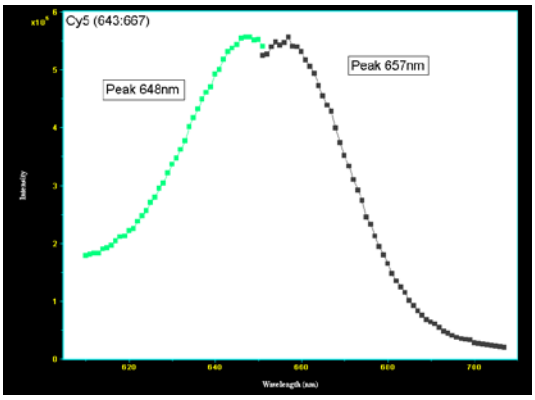
At IDT, we conducted a systematic study to assess the impact that conjugation to DNA has on the Ab_{max} and Em_{max} of 9 of the most commonly used fluorescent dyes. The results of this study are presented below. Dyes were conjugated to the 5'-end of a 12-mer oligonucleotide and HPLC purified to ensure that no free dye was present. Spectra were measured using a Photon Technology International (PTI) fluorometer at 50 nM dye concentration in a buffer similar to that used in real time PCR assays, containing 50 mM Tris pH 8.0, 50 mM KCl, and 5 mM $MgCl_2$.

For comparison, the Ab_{max} and Em_{max} provided by the dye manufacturers is shown in the upper left-hand corner. All of these measurements were collected using free dye in various solvent/pH conditions. Note that unconjugated dyes are hydrophobic and are poorly soluble in water. For this reason, many of the standard reference spectra were measured in methanol.

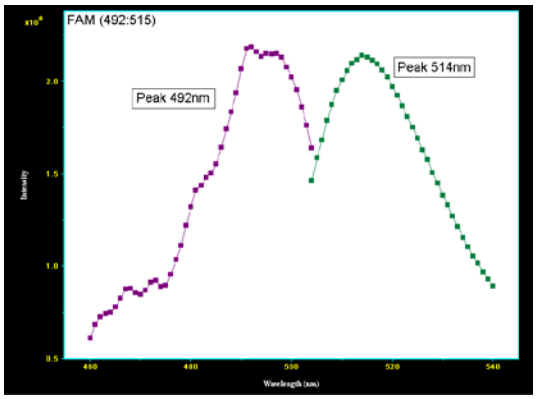
Cy3



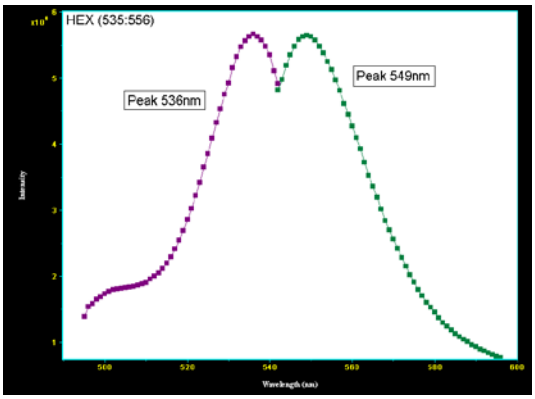
Cy5



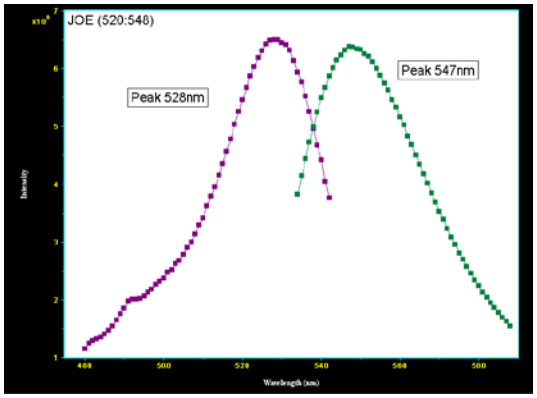
FAM



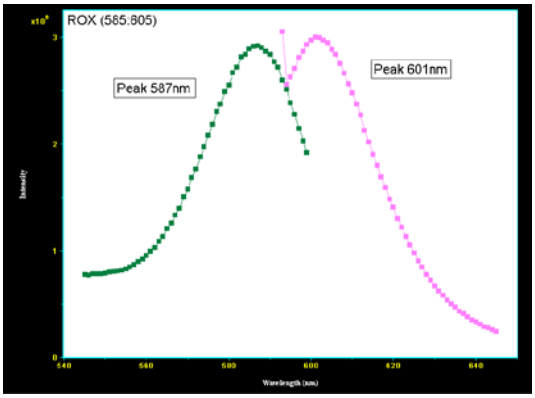
HEX



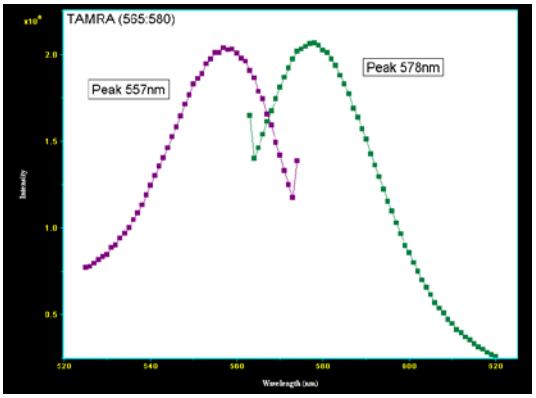
JOE



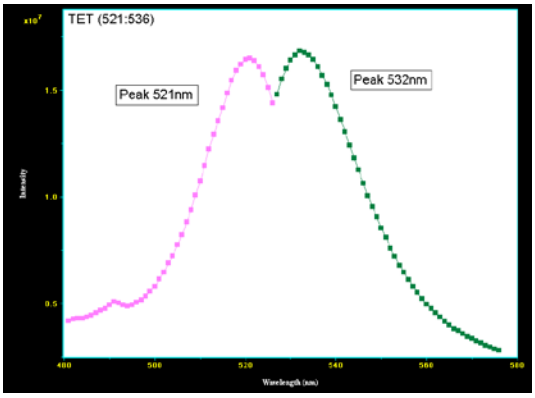
ROX



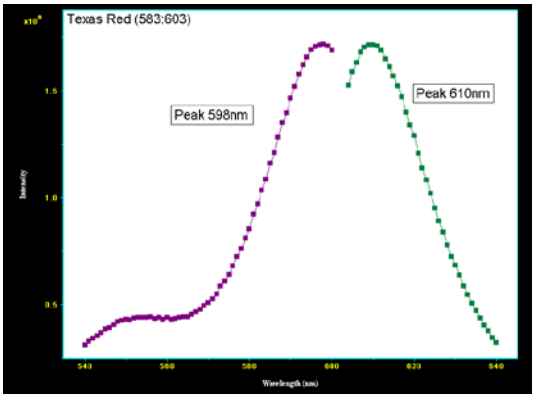
TAMRA



TET



Texas Red



As is evident from the above data, the spectral character of dyes commonly used in molecular biology change with context. Conjugation to an oligo and the pH/solvent used will affect fluorescence behavior and these factors should be considered when designing experiments or choosing dye/filter combinations, especially for multiplexed assays.

References

1. Sjoback R, Nygren J, and Kubista M. (1998) Characterization of fluorescein-oligonucleotide conjugates and measurement of local electrostatic potential. Biopolymers, 46: 445–53.

2. Clegg RM, Murchie AI, et al. (1992) Fluorescence resonance energy transfer analysis of the structure of the four-way DNA junction. Biochemistry, 31: 4846–56.

