

Optimizing Optical Filters for Quantum Dot Applications

Fluorophores & Filter Set Design

Typical organic fluorophores, including Fluorescent Proteins, have excitation and emission spectra that are often overlapping. The peaks of these asymmetric, bell-shaped curves can be quite close together, defining the Stokes shift of that fluorophore. For example, for FITC the distance between excitation and emission peaks, or Stokes shift, is 24nm. For TRITC the Stokes shift is 25nm.

In order to maximize signal of fluorophores with small Stokes shifts, excitation and emission filter transmission bands need to be placed close together. To minimize background, this filter pair must have discrete bands of transmission that do not overlap, requiring that the passbands edges are as steep as possible. For fluorophores with small Stokes shifts, filter designs with the characteristics described above are a necessity for optimal performance.

Quantum Dots & Filter Set Design

The basic fluorescent characteristics of semi-conductor nanocrystals, or quantum dots, allows for an alternative filter design strategy. Qdots™ (Invitrogen/Molecular Probes) are all excited in the UV/deep blue with emission at much longer wavelengths dependant on the physical size of the dot. For the shortest wavelength Qdot, with emission centered at 525nm, the Stokes shift is >100nm, with the Stokes shift increasing as the emission wavelength increases. Contrast this with a Stokes shift for FITC of 24nm.

While the large Stokes shifts of Qdots allows for the use of less steep-edged filters, their extremely bright signal and relatively narrow Gaussian distribution allows the use of emission filters with relatively narrow emission bandwidths. If this design strategy is followed, emission filters function equally well for single label or multiplexing applications, and they are less expensive without compromising performance.

Optimized Quantum Dot Filter Performance

It is understandable to try and use existing filter sets optimized for comparable fluorophores when first trying Qdots. A substitution in these cases, however, leads to poor performance because of the inherent differences in the fluorescent properties of Qdots and most organic fluorophores. Use of optimized filters and filter sets is critical in Qdot applications for all instrument platforms and applications in order to obtain best experimental results.

Figures – Comparison of Qdot 525 & FITC Filter Sets

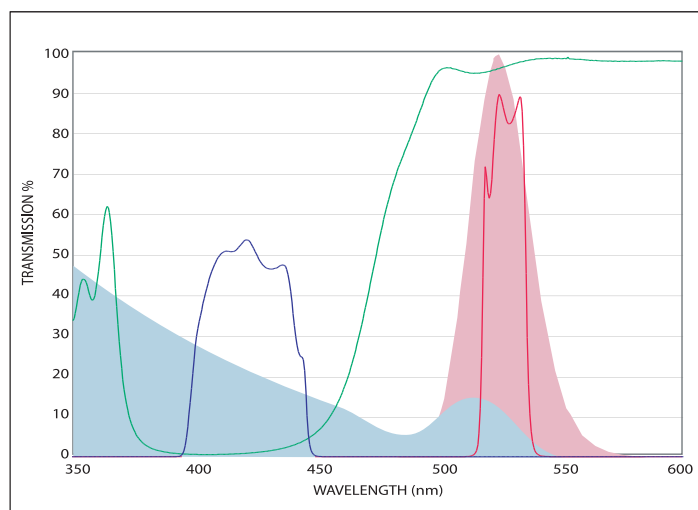


Figure 1: Qdot 525 spectra with overlay of Qdot 525 Filter Set (XF301-1)

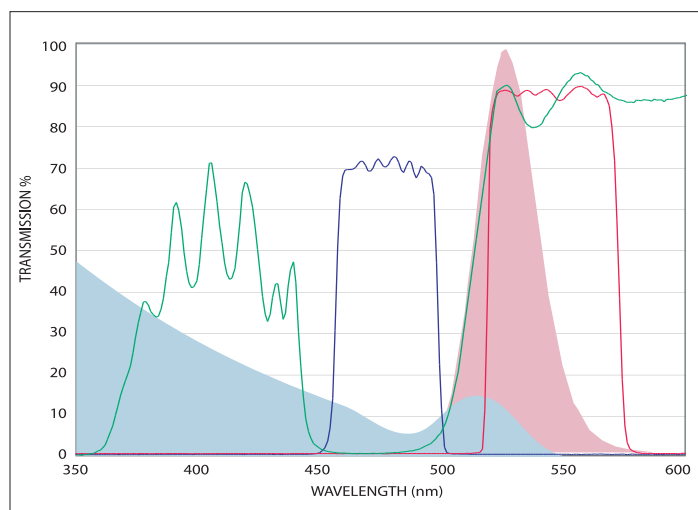


Figure 2: Qdot 525 spectra with overlay of FITC Filter Set (XF100-2)