APPLICATION NOTE

Crosstalk in Densely Multiplexed Imaging

When using multiple, densely spaced fluorophores, rapid and accurate results rely on the ability to readily distinguish the fluorescence labels from one another. This dense multiplexing of images is particularly important when doing Fluoresence in Situ Hybridization (FISH) measurements. Thus it is critical to minimize crosstalk, or the signal from an undesired fluorophore relative to that of a desired fluorophore. The table below quantifies crosstalk values for neighboring fluorophores when using a given BrightLine FISH filter set. Values are determined from the overlap of typical, normalized fluorophore spectra, the filter design spectra, and an intense metal halide lamp.

Relative Fluorophore Contributions for Each Filter Set

Filter Set \ Fluorophore	DAPI	SpAqua	SpGreen	SpGold	SpOrange	SpRed	Cy5 / FRed	Cy5.5	Cy7
DAPI	100%	30%	0%						
SpAqua	0%	100%	1%	0%					
SpGreen	0%	0%	100%	3%	0%				
SpGold		0%	2%	100%	49%	1%			
SpOrange			0%	36%	100%	11%	0%		
SpRed				0%	15%	100%	1%	0%	
Cy5 / FRed					0%	12%	100%	53%	1%
Cy5.5						0%	53%	100%	6%
Cy7			Grey combinations are not recommended					12%	100%

As an example, when imaging a sample labeled with the SpectrumGreen™, SpectrumGold™, and SpectrumRed™ fluorophores using the SpectrumGold filter set, the undesired SpectrumGreen signal will be less than 2% of the desired SpectrumGold signal, and the SpectrumRed signal will be less than 1%.

Amazing Spectra for Minimizing Crosstalk

These BrightLine filter sets are meticulously optimized to maximize brightness for popular fluorophores, while simultaneously minimizing unnecessary background as well as crosstalk with adjacent fluorophores. The graph below shows an example of the filter spectra for the SpectrumRed filter set (blue, green, and red solid lines), as well as the absorption and emission curves for SpectrumGold, SpectrumRed, and Cy5™ (left to right). Crosstalk is kept to only a few percent or less, as quantified in the table above.