Software Index

Software Packages:

Metamorph - Version – 7.8.2.0

Special Modules – Colocalization – Version – 7.8.2.0

Macros – none

Taskbars – none

Techniques - Corrected Fluorescence Image - ILY = IF − βICFP where (IF: excitation 430/424; emission 535/530) and (ICFP: excitation 430/424; emission 470/424) and β is defined as:

To quantify molecular interactions by sensitized emission we must separate the three spectral components from the fluorescence signals. These spectral components are the (1) direct donor fluorescence – fluorescence from donor molecules due to excitation from the light source, (2) direct acceptor fluorescence – fluorescence due to acceptor molecules that are excited by the light source and (3) the sensitized emission – fluorescence from the acceptor due to energy transfer from the donor. Measurement of the apparent FRET efficiencies requires that these three signals be isolated and scaled. Foremost to a sensitized emission FRET calculation is the isolation of the sensitized emission signal. In principle, sensitized emission can be directly measured by simply exciting the donor and measuring the acceptor emission. In practice, however, fluorophores used for FRET have significant spectral overlaps. This implies that unless the acceptor and donor both have large Stoke’s shifts there will be some excitation of the acceptor by the donor illumination and that the donor emission will spill into the acceptor emission channel. In many cases (e.g., CFP and YFP), it is possible to choose donor and acceptor excitation and emission filter combinations such that part of the donor emission and directly excited acceptor emission can be obtained independent of sensitized emission. In this case, two calibration constants can be defined that allow subtractive isolation of the sensitized emission (Youvan 1997; Gordon et al. 1998),

SE = IF βID  IA,

where SE is the sensitized emission and the three images are *I*D, the fluorescence from the donor (i.e., the CFP image, using filters for donor excitation and donor emission), *I*A, the FRET-independent fluorescence from the acceptor (i.e., the YFP image, using filters for acceptor excitation and acceptor emission), and *I*F, the mixture of signals from directly excited donors and acceptors, plus acceptors excited by FRET (i.e., the FRET image, using filters for donor excitation and acceptor emission). and are coefficients that reflect the fraction of fluorescence crossing over between channels. These parameters are simply defined for samples containing donor or acceptor only:

 IF ICFP(for acceptor only)

β IF IYFP(for donor only)

Other Software - None