Process Description:

"Bleedthrough" is when the fluorophore from one channel contributes to the intensity in another channel, due to spectral overlap or imperfect filtering. With single-chain (intramolecular) biosensors, the localization of the fluorophores in each channel is always identical, so the bleedthrough contribution is automatically canceled out during the ratioing step. With dual-chain (intermolecular) biosensors, the localization is almost always at least slightly different for the two fluorophores, meaning that bleedthrough must be corrected. This is accomplished by determining bleedthrough coefficients in a separate experiment where each half of the sensor is imaged independently. These coefficients are then used to correct the actual fluorophore images. The coefficients may be calculated from these bleedthrough experiments using the bleedthrough coefficient calculation tool in the “tools” menu (see the user's manual for more information).

Parameter Descriptions:

Input Channel:

This allows you to select the channel to bleedthrough correct. This should be the activity channel (usually FRET) which will be the numerator in the ratio.

Bleedthrough Channels:

This box allows you to select the channels which contain fluorophores which are bleeding through into the channel selected for correction (usually CFP and YFP).

Bleedthrough Coefficients:

This is where you can input the bleedthrough coefficients that you have determined previously. You must input one coefficient for each bleedthrough channel selected above. Type in the coefficient in the box and then click the "Add->" button. Coefficients can be determined from bleedthrough experiments using the function calculateMovieBleedthrough.m (see the user's manual for more information).