Description:

This function calculates average bleedthgough coefficients based on the input movie. This movie should have one channel (A) with a fluorophore and illumination and filters specific to this fluorophore, and another channel (B) imaged with it's own illumination and filters, but with no fluorophore. The bleedthrough of the fluorophore in channel A to the image in channel B will then be calculated. This allows, in a later experiment, the image in channel B to be corrected for this bleedthrough. This calculation requires that the movie have been segmented already, as only areas within the cell(s) will be used for coefficient calculation.

Since time information is not needed in bleedthrough calculations, this function assumes that each image in the movie is of different cell(s), and therefore calculates a separate coefficient for each frame. The average of all the coefficients should then be used in later corrections.

NOTE It is required that you use fully-corrected, images for both channels used in this calculation!! This means that all channels used must AT LEAST have been background subtracted. It is strongly recommended that a transformation also be applied if dual-camera acquisition is used. Also, be sure that none of your images have saturated areas, as this will cause error in the calculated coefficient.

Parameter Descriptions:

Fluorophore channel:

This allows you to select which channel has the fluorophore (channel A above) in the input movie.

Bleedthrough channel:

This allows you to select which channel has No fluorophore (channel B above) in the input movie.

Fluorophore Mask:

This allows you to select which channel to use masks from for the fluorophore channel. Default is to use masks from the fluorophore channel itself.

Bleedthrough Mask:

This allows you to select which channel to use masks from for the bleedthrough channel. Default is to use masks from the bleedchannel itself.