Process Description:

Photobleaching causes the fluorophores in each channel to lose intensity over time as they are imaged. The rate of photobleaching is almost always different in the different channels, and therefore can introduce artifacts in the ratio image if not corrected: The apparent average ratio will vary as the two fluorophores bleach at different rates. This process allows the photobleaching to be corrected by fitting a double-exponential to the intensities in each channel. After the fitting, a figure will be displayed showing the average ratio over time, and the fitted value along with confidence intervals. It is important to inspect this fit to ensure that the correction is valid, and that the confidence intervals are not excessively large. If photobleach correction is not applied, it is generally not valid to measure changes in the ratio images over time.

Parameter Descriptions:

Ratio Channels:

This channel should be set to be the same as the numerator channel used in ratioing (usually FRET).

Ratio of Averages:

This method fits a double-exponential to the ratio of the average intensity in each of the channels used for ratioing, and then uses this fit to correct the ratio images. This is standard method.

Average of Ratios:

This method fits a double-exponential to the average of the ratio image, and then uses this fit to correct the ratio images. This will suppress any long-term changes in the ratio, and should only be used if the other methods fail.

Ratio of Totals:

This method fits a double-exponential to the ratio of the total intensity in each of the channels used for ratioing, and then uses this fit to correct the ratio images. This is generally only used when there is a drastic change in area of the object being imaged during the movie. (e.g. a cell rounds up, spreads etc.). This sort of change will have a smaller effect on the total intensity than the average intensity, making the total a better measure of bleaching in these cases.