Process Description:

This is the most important step, where the fully-corrected activation channel (usually FRET) is divided by the fully-corrected localization channel (usually the FRET donor). This allows changes in the activation of the protein of interest to be separated from changes in its localization, giving a measure of the protein's signaling state.

Parameter Descriptions:

Apply Masks to Ratio:

If this box is checked, any areas in the ratio image which are outside of the masks from either the numerator or denominator channels will be set to zero. This is normally desireable because areas outside the mask are background areas, and the ratio values there are very noisy and generally meaningless.

Create New Masks:

If this box is checked, new masks will be created and saved for the ratio channel, which are combinations of the masks from the two input channels: Only pixels which are included in both masks will be included in these masks. This is generally only useful if the ratio images are NOT being masked.

Numerator:

This allows you to select which channel is the numerator, or activity channel, in the ratio. This is usually the FRET channel.

Denominator:

This allows you to select which channel is the denominator, or localization channel, in the ratio. This is usually the FRET donor channel.

Numerator Mask:

This allows you to select which channel to use masks from for the numerator. Normally this is the numerator channel itself, but in the case that the numerator has poor-quality masks, a different channel can be used. As long as the channels are well-aligned, this is an acceptable alternative.

Denominator Mask:

This allows you to select which channel to use masks from for the denominator. Normally this is the denominator channel itself, but in the case that the denominator has poor-quality masks, a different channel can be used. As long as the channels are well-aligned, this is an acceptable alternative.