

Ratioing 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 desirable because areas outside the mask are background areas, and the ratio values there are very noisy and generally meaningless.

Numerator Input Channel:

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

Denominator Input Channel:

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 Channel:

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 Channel:

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.