Ratio Output Process Description:

This process allows you to export the ratio images you have created in step 10 (or step 11, if photobleach correction has been applied) as .tif images. This requires multiplying the images by a large number called a "scale factor". This is necessary because the ratio images themselves are small, non-integer values (usually between 0.5 and 5), while .tif images can only store positive integer values. If these ratios are converted to integers without multiplying by a scale factor, there would be severe rounding error in the images. Alternatively, you can use the ratio images themselves, which are stored as floating-point MATLAB .mat files in the movie's output directory.

Parameter Descriptions:

Ratio Channel:

This box allows you to select the channel which is the NUMERATOR of the ratio images you want to export (usually the FRET channel).

TIF export options:

Scale Factor:

This is the number that the ratio images will be multiplied by before being saved as .tif images. It is recommended that this number be fairly large (1000 is a good starting point) to minimize rounding error. It is also important that this number be kept constant among different experiments, if the resulting ratio images are to be compared.

Select Path:

This allows you to specify the directory to save the .tif ratio images to. They will be saved to a subdirectory of this folder called "ratio_tiffs", with one .tif file per ratio image.

Make ratio movie:

This allows you to export the ratio output as a movie (in .avi and/or .mov format).

Movie options:

Use a constant scale throughout the movie:

If checked, the color scale used for the first image will be used throughout the movie. Otherwise, the range will be selected separately for each frame.

Fraction of saturated ratio values:

This allows you to specify the fraction of the ratio values to allow appearing saturated in the color scale. That is, if it is set to 0, the color scale will cover the entire range of the ratio values, from maximum to minimum (no saturation). If it is set to 0.05, then 95% of the data will fit between the max and min of the color scale, but values outside this range will be saturated. Smaller values for this

parameter will make small variations in the ratios easier to see but will saturate very active/inactive areas.

Display color bar:

If checked, a bar showing the color scale, i.e., the ratio value associated with each color in the movie, will be displayed.

Create .avi/.mov movie:

If checked, the movie will be saved as a .avi/.mov.