Process Description:

This process creates masks for the movie which seperate objects (e.g. fluorescent cells, single fluorophores etc.) from the background. Masks are binary images which contain 1 where there is an object of interest (cell), and zero where there is background. Thresholding creates these masks by finding areas of the image which are brighter than a specific value, called the threshold. These will be created for any channels the user selects, or for all channels by default. The masks can be checked by clicking the "Result" button, which will show the outline of the mask on top of the fluorescence image.

Parameter Descriptions:

Input Channels:

This allows you to select which channels you want to create masks for. Generally, this is only done for fluorescence channels, and DIC/Phase images are not thresholded. Select the channels by clicking on them in the "Available Input Channels" box and then clicking "Select->" to move them to the "Selected Channels" box. You can un-select a channel by clicking the "Delete" button

Use Automatic Thresholding:

Select this box to allow the software to attempt to automatically select a threshold level for each image in each selected channel. Depending on the data, this automatic thresholding may fail in some cases. In this case, manual thresholding can be used.

Fixed Threshold:

If you un-check "Use automatic thresholding", then this option allows you to manually specify an an intensity value to use for thresholding for each channel. This value should be just barely above the average backround intensity in the image. You can use the matlab function imtool, or ImageJ to determine a threshold to use.

Maximum Threshold Jump:

This option should be used if the automatic thresholding works on most frames of the movie but fails on a few. If selected, this sets an upper limit on the change of the threshold between frames. For example, if this was set to .5 and the automatically-selected threshold changed by a factor of .51 between two consecutive frames, this new threshold would be ignored and the last good threshold value used.