

Process Description:

This process applies manual or automatic thresholding to every frame of the input movie and then writes the resulting mask as a binary .tif file.

Parameter Descriptions:

Input Channels:

This allows you to select which channels you want to perform shade correction on. This should be applied to all channels that are going to be used for ratioing or bleedthrough correction. 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 unselect a channel by clicking the "Delete" button

Standard deviation of Gaussian filtering:

This allows to pre-filter each image with a low-pass Gaussian filter before thresholding. If the value is set to zero, no pre-filtering is applied. Else the value specifies the standard deviation of the Gaussian kernel (in pixels).

Use automatic thresholding:

If checked, the process automatically computes the threshold value based on the image intensity histogram using a given thresholding algorithm.

Automatic thresholding

Thresholding methods:

This dropdown menu allows the user to select a method among a list of popular thresholding algorithms. Algorithms currently available for selection include:

- **MinMax:** this algorithm first fits a spline to the intensity histogram and then determines the position of the intensity minimum immediately after the first intensity maximum.
- **Otsu:** this method uses the Matlab implementation of the Otsu algorithm.
N. Otsu. *A threshold selection method from gray-level histograms*. IEEE Transactions on Systems, Man, and Cybernetics, 1979. **9** (1): p. 62–66.
- **Rosin:** this method implements Rosin's unimodal thresholding algorithm.
P.L. Rosin. *Unimodal thresholding*. Pattern recognition, 2001. **34**(11): p. 2083-2096.
- **Gradient-based:** This function selects a threshold, which corresponds to a strong spatial gradient in intensities between background and foreground.

Maximum threshold jump:

The thresholds are calculated for each frame individually. This option allows you to suppress unrealistically large changes between frames. If the maximum threshold jump is non-zero, any changes in the automatically selected threshold value greater than the value specified will be suppressed by using the most recent good threshold. That is, if the value is set to 2.0 and the threshold changes by a

factor of 2.2 between two consecutive frames, the new threshold will be ignored and the previous threshold will continue to be used until the automatic threshold is less than 2.0 times different. This option is ignored if the user specifies a threshold.

Fixed threshold

Threshold value:

This allows you to select a manual value for the thresholding step.

Set:

This allows you to add the threshold value to the list of thresholds. If the list contains only one value, this value is used for all channels. If several values are entered, each element specifies the value to use for a specific channel.

Preview thresholded image:

If checked, the result of the thresholding (after image pre-filtering if applicable) for the selected channel and the specified frame will be displayed in a separate figure.