MSA (Multi-Scale Automatic) Segmentation Process Description:

This process segments a single-cell image by combining multiple different segmentation results. It first computes 42 different cell masks by applying all combinations of segmentation parameters: 3 thresholding algorithms (Minmax, Rosin, Otsu), 7 different sigma values of Gaussian filters for smoothing ranging from 0 to 3 pixels, and 2 different radius parameters for morphological closing. Since it requires only one tuning parameter (either “tightness” or “number of votes”) and “tightness” = 0.5 or “number of votes” = 22 works well for many cases, it achieves almost automatic segmentation.

For more information, please see [Noh et al., Cell Systems. 13.6 (2022).](https://doi.org/10.1016/j.cels.2022.05.003)

Parameter Descriptions:

Input Channels:

This allows you to select which channels you want to segment. By default, all channels will be segmented. 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.

Use Output from Summation Channel:

Please note, this checkbox is not applicable for the Biosensors package.
If checked, the segmentation will be applied to the output of the “Generate Summation Channel process”.

Generate Summation Channel Process:

Please note, this dropdown menu is not applicable for the Biosensors package.
If more than one “Generate Summation Channel process” has been run, this allows you to select one from the drop-down menu to use for the MSA segmentation. If choosing “Select later” and more than one process exists, you will be asked at runtime which process to use.

Manually Set Tightness:

If checked, a slider bar will be available for the user to manually adjust the “tightness” parameter. The “tightness” parameter and the “number of votes” parameter are exclusive options. If “Manually Set Tightness” is checked, the “Manually Set Number of Votes” will be automatically unchecked and the “number of votes” will become “inactive”, and vice versa.

Tightness:

This slider bar allows the user to manually adjust the “tightness” parameter. The default “tightness” = 0.5 works well for most cases. If set to 1, the output is the tightest or smallest masks. If set to 0,

the output is the largest masks.

Manually Set Number of Votes:

If checked, a slider bar will be available for the user to manually adjust the “number of votes” parameter. The “tightness” parameter and the “number of votes” parameter are exclusive options. If “Manually Set Number of Votes” is checked, the “Manually Set Tightness” will be automatically unchecked and the “tightness” will become “inactive”, and vice versa.

Number of Votes:

This slider bar allows the user to manually adjust the “number of votes” parameter. The default “number of votes” = 22 works well for most cases. If set to 42, the output is the smallest mask. If set close to 0, the output mask would be larger.

Object Number:

This specifies the number of objects to keep in each mask. That is, if Object Number is set to 2, only the two largest objects in the mask will be kept. This is useful for removing larger background objects or cells which are only partially in the image, while retaining the cell or object of interest.

Final Refinement Radius:

This parameter specifies the radius of morphological closing for the final refinement.