## Process Description:

This process calculates local cell edge protrusion and retraction velocities – that is, for each pixel at the cell edge, a velocity vector is calculated which connects the cell edge in that image to the cell edge in the subsequent image. These are calculated based on a minimization which balances the implied strain in the membrane and the calculated total displacement.

*Important Note: The protrusion velocities are calculated based on the cell outlines determined in the segmentation. It is therefore important that the cells be accurately segmented prior to protrusion calculation, as any errors in the segmentation WILL produce errors in the protrusion/retraction velocities.*

## Parameter Descriptions:

### Input Channels:

This allows you to select which mask channels you want to use to calculate protrusion vectors. If multiple channels are selected, the intersection of the masks will be used (only pixels which are in all mask channels). 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

### Mask process

If more than one segmentation process has been run, this allows you to select a mask process from the drop-down menu to use for calculating protrusion. If choosing "Select later" and more than one process exists, you will be asked at runtime which process to use.

*Note:* It is generally advisable to run the Mask Refinement process, and to use the masks from this process for the protrusion calculation. The protrusion calculation requires that there be only one cell or cell region in each image of the movie. If this cell touches the image border, it must touch the image border in every frame.

### Downsampling parameter:

Prior to protrusion calculation the cell-edge is sub-divided into segments in-between areas of zero velocity (i.e. where the cell outlines in two subsequent frames overlap). For each of these segments, a

protrusion vector is calculated at each pixel, UNLESS the number of pixels in that segment exceeds the number specified by this down-sampling parameter. If an edge segment has more pixels than this parameter, it will be downsampled to this number and then up-sampled after the minimization has been completed. This can speed the protrusion vector calculation on large cells in large images, with a small tradeoff in protrusion vector accuracy.

Generally the default value is suitable, but decreasing it can speed the calculations on cells whose boundary covers a large number of pixels, with a slight decrease in accuracy.

**Spline tolerance value:**

The protrusion vectors are calculated using a spline-smoothed version of the cell edge. This specifies the tolerance of that spline. Larger numbers will increase the smoothing of the mask edge. Enter 0 to use an interpolating spline.

**Note: It is generally not recommended to change this parameter from the default, as it has been chosen so as to accurately reproduce the cell outline with minimal but sufficient smoothing.**


# References