

Process Description:

If flow tracking has been performed, speckles positions are interpolated using the tracked flow. Then, for each frame, speckles within the search radius are linked together. Once speckles have been linked for the entire movie, single-frame gaps are closed. The gap between two tracks is closed if an insignificant speckle is found within the search radius of the propagated position in the missing frame.

Parameter Descriptions:

Input Channels:

This allows you to select which channels you want to perform speckle tracking on. This should be applied to all channels that are going to be used for calculating the noise parameters. 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

Search radius (pixels):

This value sets the search radius to be used when linking speckles from frame to frame.

Correlation length:

This value sets the correlation length of the flow. This length is used when 1) tracks are interpolated during hierarchical tracking and/or 2) the tracked flow is interpolated by the Gaussian weighting method.

Perform hierarchical tracking:

If selected, the tracker uses the results of the first linking step as an initializer. This option is strongly recommended if the flow tracking has not been performed on the movie.

Flow interpolation method:

This menu allows the user select a method to interpolate positions where no flow was computed during flow tracking. The following three interpolation methods are available:

- 1) No interpolation in which speckles with no estimated flow will be considered as speckles with zero-magnitude flow,

- 2) Nearest-neighbor flow interpolation which speckles with no estimated flow will use the nearest finite flow
- 3) Gaussian weighting interpolation in which speckles with no estimated flow will integrate the flow of all neighboring speckles using a Gaussian weighted interpolation.

References

- Ponti, A., P. Vallotton, et al. (2003). "Computational analysis of F-actin turnover in cortical actin meshworks using fluorescent speckle microscopy." *Biophys J* 84(5): 3336-3352.
- Li, J. and Danuser, G. 2005. Tracking quasi-stationary flow of weak fluorescent signals by adaptive multi-frame correlation. *Journal of microscopy*. 220:150-167.