Cost Matrix Description:

This function takes in the track segments obtained in Step 1 (frame-to-frame linking) and calculates the costs of potentially linking them in order to close gaps and capture merging and splitting events.

Parameter Descriptions:

Brownian Search Radius: Define **Lower Bound** and **Upper Bound**. NOTE: These are the search radius lower and upper bound for frame-to-frame linking, and are thus usually given the same values as defined in the cost function for frame-to-frame linking. The search radius will be internally expanded for gap closing, where a particle disappears for a number of frames and then reappears.

Multiplication Factor for Brownian Search Radius Calculation: Factor by which displacement standard deviation is multiplied to estimate search radius. Usually same as value used in frame-to-frame linking cost function.

Check "**Use nearest neighbor …**" to use particle density, in addition to motion, to estimate search radius. If unchecked, only motion is used.

How to expand the Brownian search radius with gap length:

Since a particle moves further away with longer gaps, its search radius is expanded with gap length.

This expansion can have two phases, a fast one and a slow one. Thus define the "**Scaling Power in Fast Expansion Phase**", the "**Scaling Power in Slow Expansion Phase**", and the "**Gap Length to Transition from Fast to Slow Expansion**".

To have only one expansion phase, make "Scaling Power in Fast Expansion Phase" and "Scaling Power in Slow Expansion Phase" equal, and use "Gap Length to Transition from Fast to Slow Expansion" as the Maximum Gap to Close.

<u>Examples:</u>
For tracking particles exhibiting free diffusion, use 0.5 for both scaling powers.
For tracking particles exhibiting confined diffusion, use 0.5 for the fast phase, 0.01 for the slow phase, and 2 or 3 frames for the transition gap length.

Penalty for increasing gap: Define the penalty for longer gaps. 1 means no penalty. A value $x > 1$ means that a gap of length n will be penalized by a factor x^n.

Merging and splitting:

Check "**In merging and splitting …**" to use particle intensities as a cue for merging and splitting. Specifically, the code calculates the ratio of the intensity after merging/before splitting to the sum of particle intensities before merging/after splitting, and expects this ratio to be close to 1. If checked, specify the **Minimum Allowed** and **Maximum Allowed** intensity ratios. If unchecked, only distance information is used.

If desired, specify a **Search Radius Lower Bound for Merging and Splitting**, usually larger than the

general lower bound defined above.

Linear Motion Parameters

These parameters are relevant only if “Allow direction motion position propagation” is checked.

Allow directed motion position propagation: This is not optional in this step. To check/uncheck, go back to the cost function for frame-to-frame linking.

Allow instantaneous direction reversal: This is not optional in this step. To check/uncheck, go back to the cost function for frame-to-frame linking.

Minimum Track Segment Lifetime for Classification as Linear or Random: To classify a track segment as random or linear, it has to last for at least the specified number of frames. If shorter, it will remain unclassified and treated accordingly.

Multiplication Factor For Linear Search Radius Calculation: Factor by which linear displacement standard deviation is multiplied to estimate linear search radius. Similar to the factor used to calculate the “random motion” search radius above.

How to expand the linear motion search radius with gap length:

Same elements as the “random motion” search radius above.

Examples:
For tracking motor-driven motion, use 1 for both scaling powers.
For tracking motion that is similar to one dimensional diffusion, use 0.5 for both scaling powers.

Maximum Angle between Linear Track Segments: Maximum angle between the directions of two linear segments that can be potentially linked.