Cost Matrix Description:

This function takes in the track segments obtained in Step 1 (frame-to-frame linking) and calculates the costs of 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. Default value of 3 generally works well.

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

How to expand the Brownian search radius with gap length:

Since a particle moves further away with longer gaps, its search radius is internally 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 length: 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. The default value of 1.5 generally works well.

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 necessary, specify a **Search Radius Lower Bound for Merging and Splitting**, usually larger than the general lower bound defined above. Setting a different lower bound for merging and splitting helps in cases where object movement is smaller than object size, in which case the apparent distance moved upon merging/splitting is dominated by the object size instead of real movement.

Linear Motion Parameters

These parameters are relevant only if the non-Brownian motion models are employed. Note that motion model choices are not allowed in Step 2, as they should be the same as in Step 1.

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 get treated accordingly.

Multiplication Factor For Linear Search Radius Calculation: Factor by which linear/directed displacement is multiplied to estimate linear search radius. Factor value depends on motion type. If motion type is directed movement WITHOUT reversal, then a value of 1 works well. If motion type is linear movement WITH reversal, then this factor is similar to the factor used to calculate the "random motion" search radius above, in which case a value of 3 works well.

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

Same concepts elements as the "random motion" search radius above, but with values depending on motion model.

<u>Examples:</u>
If motion type is directed movement WITHOUT reversal, e.g. motor-driven motion, then both scaling powers = 1.
If motion type is linear movement WITH reversal, e.g. 1D diffusion, then both scaling power = 0.5.
If motion type is linear movement WITH reversal but within a confined space, then fast expansion scaling power = 0,5, slow expansion scaling power = 0.01, and transition gap length = 2 or 3.

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