

## Cost matrix description:

This function takes in the detected objects and calculates the cost of linking them between consecutive frames.

## Parameter Descriptions:

**Allow directed motion position propagation:** If unchecked, particles are assumed to exhibit random “Brownian” motion,” in which case there is no position propagation. If checked, particles are allowed to exhibit directed movement, in which case the velocity of each particle is estimated from its track via the Kalman filter and its position is first propagated to the next frame before searching for potential links. Note that checking “allow directed motion” does not mean that every particle has to exhibit directed movement; the motion model for each particle is determined from its track, so that some particles can have directed movement and other only Brownian movement. Using directed motion position propagation depends on the particles' motion type. For example, motor-driven movement requires this option, while simple diffusion does not.

### **Allow instantaneous direction reversal**

If checked, a particle can not only exhibit directed movement but can also reverse direction instantaneously, keeping the same speed. This option is useful if particles exhibit a 1D diffusion type motion, for example.

Checking/unchecking these options will be propagated to the cost function of Step 2.

**Brownian Search radius:** Define **Lower Bound** and **Upper Bound**. NOTE: The lower bound does NOT mean that a particle has to move at least that number of pixels, it simply sets a lower bound on the search radius. The particle can still move anywhere from zero pixels up to its search radius. The upper bound on the search radius is a critical parameter that must be optimized per movie type.

**Multiplication Factor for Brownian Search Radius Calculation:** Factor by which displacement standard deviation is multiplied to estimate search radius. The default value, 3, works in the majority of cases.

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.

**Plot histograms of linking distances:** If checked, the histogram of linking distances up to the specified frames will be plotted. This option can be used to check whether the search radius upper bound is reasonable or too small. A chopped off histogram means that the search radius upper bound is too small. HOWEVER, a histogram smoothly decaying to zero does not imply that good tracking has been achieved – one should check the tracking results by making static and dynamic plots of the tracks to make the final decision on the search radius lower and upper bounds.