Process Description:

"**Sub-Resolution Object Detection**" identifies sub-resolution objects in the images and returns their sub-pixel positions and intensities. For a description of the algorithm, see **Jaqaman et al., Nature Methods 5: 695-702 (2008), Supplementary Note 2.**

Parameter Descriptions:

Gaussian Standard Deviation: The standard deviation of the point spread function, defined as 0.21*(emission wavelength)/(numerical aperture). To get value in pixels, divide by pixel size. This field is automatically filled using the movie information supplied when movieData is set up.

Camera Bit Depth: This field is also automatically filled using the movie information supplied when movieData is set up.

Local Maxima Detection:

Alpha-value for Comparison with Local Background: This value is used in the hypothesis test to decide whether a local maximum is significantly brighter than what is expected from background and noise fluctuations. The smaller the alpha-value, the more strict the selection.

Check "**Use Rolling Window Time-Averaging**" to average consecutive frames using a rolling window prior to searching for local maxima. This option can help in the case of low signal-to-noise ratio if objects do not move much from one frame to the next. This averaging is used ONLY for local maxima detection, and not for any of the consecutive steps.

If option is checked, define "**Window Size**": 1 means no averaging, 3 means you average 3 frames at a time (i.e. 1, 2 and 3; 2, 3 and 4; etc.), and so on.

Check "**Use Absolute Background**" to use absolute background information (in addition to local background information) for local maxima detection. This option will be ignored when analyzing multiple movies with the same parameter settings.

If option is checked, define an **alpha-value** to compare the brightness of local maxima to the absolute background. This alpha-value is usually stricter than that used for comparing to local background, as defined above.

Click "**Open**" if you already have background images that you can supply.

Click "**New**" if you want to crop background images out of the images used for detection.

Gaussian Fitting at Local Maxima

Choose "**Iterate to estimate Gaussian Standard Deviation**" to estimate the Gaussian standard deviation from the data, instead of using the theoretical one defined above.

If chosen, enter the "**Maximum Number of Iterations**" used to determine the Gaussian standard deviation.

Choose "**Do Iterative Gaussian Mixture-Model Fitting**" to attempt to iteratively fit multiple Gaussians at each local maximum, as described in the paper. If not chosen, only one Gaussian is fitted

at each local maximum.

For the fitting, define the following hypothesis test alpha-values:

Residuals: Alpha-value to decide whether n+1 Gaussians fit the image significantly better than n Gaussians. Needed only if "Do Iterative Gaussian Mixture-Model Fitting" is checked.

Amplitude: Alpha-value for testing whether the amplitude of the fitted Gaussian (reflecting object intensity) is significantly different from zero, given the uncertainty in the amplitude.

Distance: Alpha-value for testing whether the distance between two fitted Gaussian centers (reflecting the underlying object positions) is significantly different from zero, given the uncertainty in the positions.

Final: This test is disabled, and there is nothing to define.

Input and Output

Define frame range to analyze.

Define name and location of .mat file where results will be saved.

Check "**View results immediately frame by frame**" to view results as movie is being analyzed. Recommended only when analyzing a small number of frames for parameter testing. When whole movie is analyzed, it is more efficient to view results *after* the analysis is finished (by clicking on the "Result" button in the U-Track package control panel.

The important output variable stored in the .mat file defined above is "movieInfo", which contains the detected particle information:
For a movie with N frames, movieInfo is a structure array with N entries.
Every entry has the fields xCoord, yCoord and amp.
If there are M features in frame i, each one of these fields in moveiInfo(i) will be an Mx2 array, where:
the first column is the value (e.g. x-coordinate in xCoord and intensity in amp),
and the second column is the standard deviation.