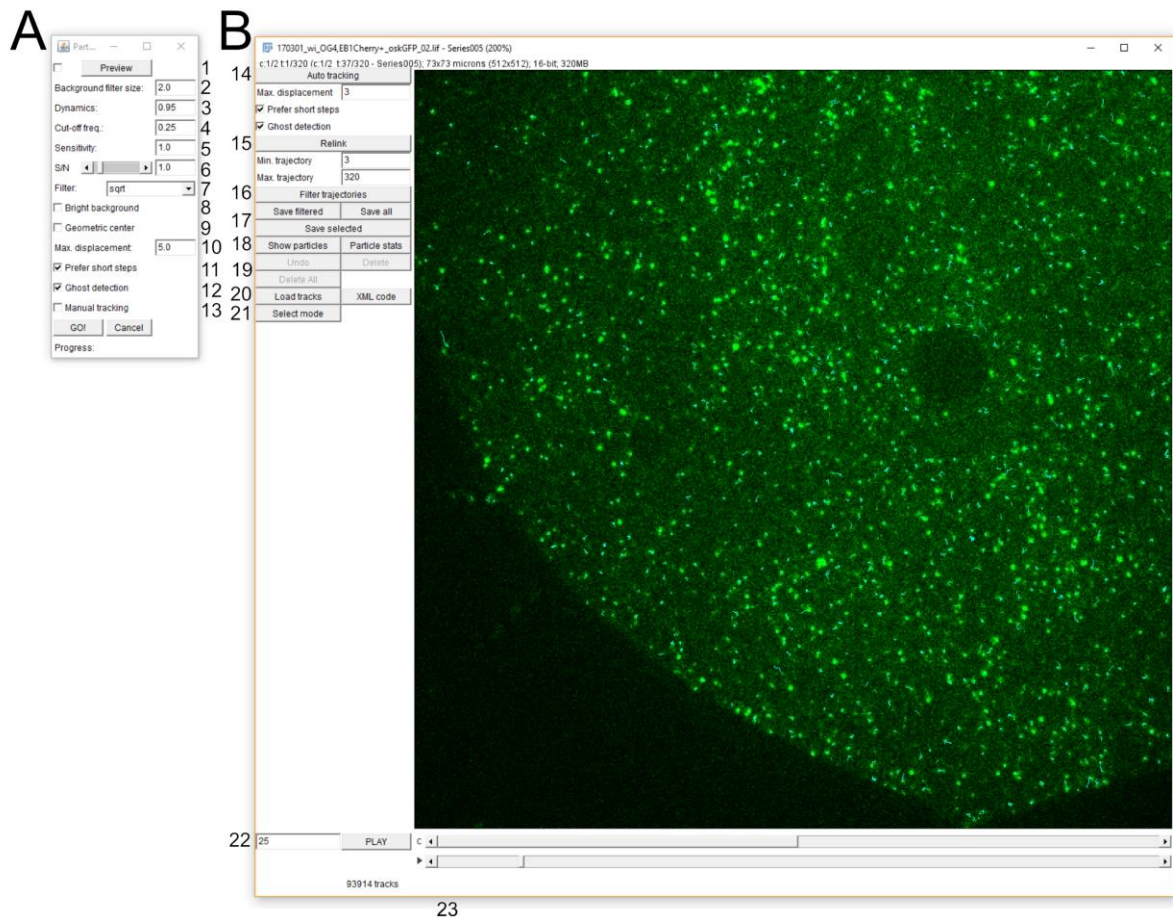


1. xsPT

Automated/assisted manual particle tracking plug-in. It works with image sequences (time or space) that contain one or multiple channels. In case of multiple channels, the channel that was selected (e.g. by the 'Channels tool') will serve as a reference and objects are recognized in this channel exclusively. The outline of these objects is transferred to the other channels and integrated signal intensity values are extracted from all channels. By starting the plug-in, a particle detector pop-up window (A) will appear.



A. Particle detector pop-up window

1. Preview the detection performance using the set parameters. The checkbox toggles the display of preview.
2. The estimated radius of the particles in pixels.
3. Threshold to discriminate between particles and noise (in percentile of pixel intensities, 0.5-1.0)
4. Threshold to trim the particles on the edges (fraction of the particle maximum).
5. Threshold of the watershed filter to merge/separate touching particles (0.5 - 1.0, merge-separate)

6. Signal to local background ratio threshold. Objects above this threshold are considered as real particles (green and yellow), object below this threshold are considered as ghosts (magenta, see at 12.)

7. Drop-down menu to select a noise reduction method (reduces the resolution).

8. If background is brighter than signal.

9. If geometric center should be used instead of centroid. Considerably faster but less accurate. However, it is recommended to use it for large objects (>10 pixels in diameters) whose signal intensity profile might be considerably different from normal distribution.

10. Maximal distance (in pixels) allowed between objects on two successive frames during tracking.

11. If long steps (shorter than the threshold at 10.) should be penalized during tracking.

12. If ghosts (see 6.) should be included in the tracking. If yes, they may be part of an existing track but cannot initiate a new track.

13. If assisted manual or automated tracking should be applied.

After hitting 'GO', particles are detected, their centroid are determined by fitting a 2D-Gaussian function and control is transferred to the main image window (B)

B. Main image window

14. Toggle between automated and assisted manual tracking.

15. In automated tracking, relink the objects using the parameters provided above the button (see description at 10-12).

16. To remove the tracks that are shorter and longer than the min. and max. trajectory (in # of frames), respectively. Also, if there is a region of interest (ROI) defined on the image using the standard ROI tools of ImageJ, only those track that have at least one object within the ROI are kept.

17. To save all, the filtered or the selected tracks. During automated tracking, tracks of interest can be selected by left-clicking on them (middle click de-selects). During manual tracking, all three buttons save the manually selected tracks.

To manually select tracks, left-click on particle to start the track. Move to another – not necessarily the next - frame where the same particle can be recognized by using either the scroll-bar, the mouse scroll or the arrow keys. The plug-in tries to link the two selected objects in time using the tracking parameters (10-12). If no route is found, most likely due to the temporal disappearance of the particle within the selected time window, the plug-in will give an alert in the status bar (22). In such cases, try to identify the frame where the particle might have disappeared. By holding down the CTRL key, left-clicking to the putative location of the particle creates an object that can be linked to the track to bridge the gap. Any non-satisfactory links can be reversed by hitting the 'Undo' button (19.). By middle clicking or hitting SPACE the track is finished and a new track can be initialized. By holding down the SHIFT key, finished tracks can be selected again by left clicking on them. These selected tracks can be continued or deleted by the user's will.

18. To show particles as an overlay on top of the image. 'Particle stats' list major parameters (position, area, maximal and integrated signal intensities, local backgrounds) of each detected objects.

19. 'Undo' and 'Delete' buttons for manual tracking (see above).

20. 'Load tracks' button to re-load saved tracking information. Note, that only the object positions but not the objects are going to be reloaded.

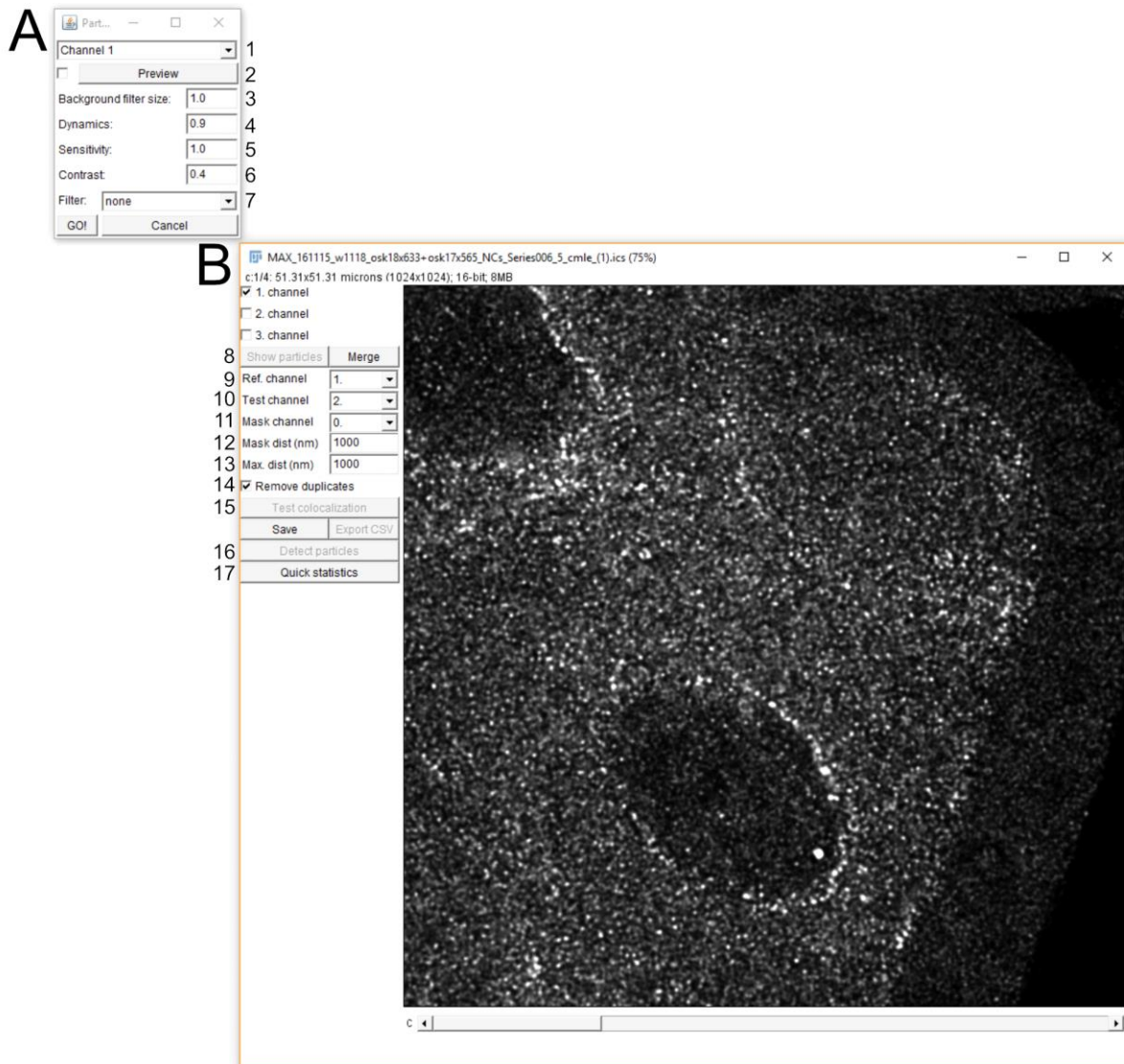
21. Toggle between 'Select mode' and 'Comment mode'. In 'Comment mode' the track can be selected by left clicking and a comment can be assigned using the 0-9 keys of the numerical keyboard. Useful for classifying the tracks.

22. Speed of playback (in FSP) and 'PLAY' button that starts the playback of the image sequence and the overlay containing the objects and the tracks.

23. Status bar

2. xsColoc

Object-based co-localization analysis on 2D images. Objects (particles) are detected in each channels separately and nearest neighbour pairs are assigned. The maximum distance between the centroids of the objects is defined by the user (max. dist. in nm). By starting the plug-in, a particle detector pop-up window (A) will appear.



A. Particle detector pop-up window

1. Drop-down menu to select the channel
2. Preview the detection performance using the set parameters. The checkbox toggles the display of preview.
3. The estimated radius of the particles in pixels.
4. Threshold to discriminate between particles and noise (in percentile of pixel intensities, 0.5-1.0)

5. Threshold of the watershed filter to merge/separate touching particles (0.5 - 1.0, merge-separate)
6. Threshold to trim the particles on the edges (fraction of the particle maximum)
7. Drop-down menu to select a noise reduction method (reduces the resolution)

After hitting 'GO', particles are detected, their centroid are determined by fitting a 2D-Gaussian function and control is transferred to the main image window (B)

B. Main image window

8. Toggle the display of the recognized particles. Use the checkboxes to select the channels to show.
9. Drop-down menu to select the reference channel
10. Drop-down menu to select the test (target) channel
11. Drop-down menu to select the mask channel (only if there are more than two channels available)
12. The distance threshold (in nm) between objects of the mask channel and the reference or the target channel. Objects in the reference and the target channel must have an object of the mask channel closer than this threshold in order to be considered in the analysis, if a mask channel is selected.
13. The maximal distance of considering nearest neighbours between objects of the reference and the target channel.
14. Checkbox to allow/forbid the recycling of objects in the target channel (i.e. whether they can co-localize with multiple or only one object from the reference channel)
15. 'Test colocalization' button to run the analysis. The analysis is either run for the entire frame or - if there is a region of interest (ROI) selected by using the standard ROI tools of ImageJ – restricted to the selected ROI. After assessing the observed co-localization between the reference and the target channel, the analysed objects of the target channel are randomly seeded back to the frame/ROI to test the likelihood of random or expected co-localization. This randomization is repeated 100 times. At the end of the analysis, a Save dialog opens. Three files are going to be saved: the actual image file (.tif), a descriptor storing the particle detection parameters and the coordinates of the ROI (.mcc) and a .csv file containing major features (e.g. position, integrated and maximal signal intensity, local background) of all analysed objects of the reference channel and features of the nearest object of the target channel (if any, otherwise -1). Furthermore, it stores hundred entries of the distances to the nearest simulated objects (if any, otherwise -1) for the hundred simulation runs.

The 'Save' button saves the image and the descriptor file, the 'Export CSV' button saves the colocalization report.

16. The 'Detect particles' button brings up the Particle detector pop-up window (A)
17. Quick summary of the number of particles detected within the frame/ROI in all the channels