

# MSP-toolbox 0.3

Manual

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The MSP framework contains two elements: MSP-tracker for particle tracking task and MSP-viewer to examine the extracted trajectories.

# 1 Installation

To run the MSP framework, you first need to install the Conda package manager and use the command line to install and run the software. You can download the software from github: [github.com/MariaaVision/msp\\_tracker](https://github.com/MariaaVision/msp_tracker). Installation steps are described below.

## Mac

1. Install [conda for Mac](#)
2. Open the folder which contains the software in Terminal (in finder, right click on the folder and select New Terminal at Folder)
3. In the terminal run **conda env create -f environment\_mac.yml**. It should install all the required packages
4. To run the software, in the software folder activate the environment with the installed packages **conda activate msp** and run **python msptracker.py** (MSP-tracker) or **python mspviewer.py** (MSP-viewer).

## Windows

1. Install [Anaconda](#)
2. Launch Anaconda Prompt
3. Navigate to the directory with MSP code and create the conda environment: **conda env create -f environment\_win.yml**. It should install all of the required packages.
4. To run the software, in the software folder activate the environment with the installed packages **conda activate msp** and run **python msptracker.py** (MSP-tracker) or **python mspviewer.py** (MSP-viewer).

## Linux

1. Install [conda for Linux](#)
2. In the terminal open the directory with the software and create the environment **conda env create -f environment\_linux.yml**.
3. To run the software, in the software folder activate the environment with the installed packages **conda activate msp** and run **python msptracker.py** (MSP-tracker) or **python mspviewer.py** (MSP-viewer).

## 2 MSP-tracker

The MSP-tracker is an interactive tool for single particle tracking. The main window of the MSP-tracker has three tabs: detection, linking and run tracking. The detection and linking tabs allow the user to set tracking parameters and view the results in the provided image viewer. The "run tracking" tab provides an overview of the set parameters and runs the tracking algorithms for the entire image sequence.

### 2.1 Data requirements

The data loaded to the MSP-tracker should be a single channel 8 or 16 bit tiff image sequence.

### 2.2 Setting the detection parameters

The detection method first produces a number of candidates using the Multiscale Spot Enhancing Filter (MSSEF), then these candidates are pruned using a light CNN network. The detection section is organised according to the process.

The "detection" tab allows the user to load the image sequence ("Select image sequence" button) and provides an image viewer to inspect the original sequence ("original image" option), the pool of candidates ("candidates" option) and the final detections ("detection" option). The user can then set detection parameters with the option to save or load parameters. To view the candidates and final detections with the current parameter setup, the user needs to run the algorithm for the current frame by pressing the "Run test" button.

#### 2.2.1 Parameter descriptions

The detection parameters are divided into two groups: candidate detection and candidate pruning. Table 1 provides descriptions of the parameters and their range.

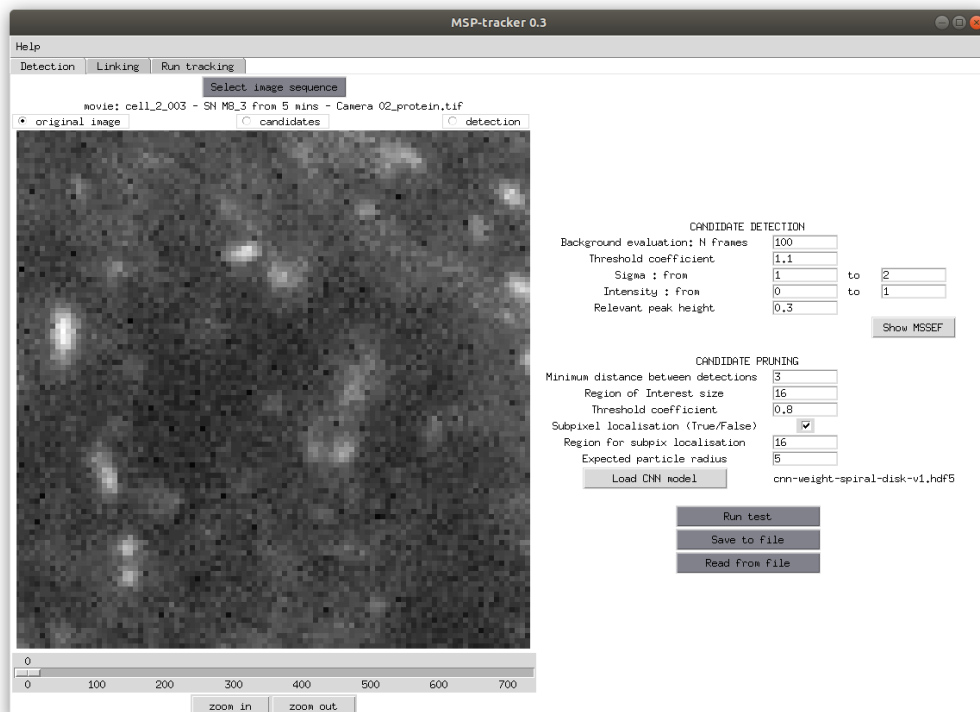
#### 2.2.2 Proposed tuning workflow

1. Set parameters for candidate detection.
  - (a) Set **Background evaluation: N frames**. This parameter is set based on the changes in the background over time. Start using default 100 frames and make changes if necessary. Set a larger number to take into account more frames, too small a value can lead to removing some of the stalled or slow moving particles. Set the parameter to 0 if you want to skip step.
  - (b) Adjust the MSSEF by choosing a **Threshold coefficient** and **Sigma range**. You can use the "Show MSSEF" button to check the enhanced spots. The ideal image shows all the particles of interest as separate spots without any holes inside and the area outside the region of interest doesn't produce any spots.

Parameters	Description	Value range
CANDIDATE DETECTION		
Background evaluation: N frames	Number of frames taken into account for the background subtraction. Start with default 100 frames and make changes if necessary. Set a larger number to take into account more frames, too small a value can lead to removing some of the stalled or slow moving particles. Set value to zero to skip the background subtraction.	integer [0, sequence length]
Threshold coefficient	Parameter of the MSSEF which is related to the intensity of the detected spots. Decrease the value to include darker areas, increase to eliminate them.	float [0, 10]
Sigma (from to)	Parameter of the MSSEF which influences size range of the spots. Decrease if targeting smaller spots, increase to include larger particles. We recommend to start with (1 - 2) and make changes based on the MSSEF image.	float [0.0001:10]
Intensity	Relative intensity range of the detected peaks, where 0 - is the minimum intensity value of the image sequence and 1 - the maximum intensity value. Set the range to eliminate too bright or too dim spots.	float [0,1]
Relevant peak height	Proportion of the detected peak in relation to the surrounding environment. Increase the value to detect only brighter/sharper particles and decrease it if darker/misty spots have to be detected as well.	float [0,1]
CANDIDATE PRUNING		
Minimum distance between detections	Minimum number of pixels expected between centers of two particles. If you have a densely populated area, decrease the value, a larger value allows you to eliminate noisy closely located detections.	float
Region of image size	Region of Interest for CNN classifier. The number depends on the loaded model (image size it was trained on).	integer (8, 16, 32)
Threshold coefficient	Threshold for the classifier. Decrease the threshold to allow more candidates though the pruning process.	float [0, 1]
Subpixel localisation	on/off subpixel localisation mode. When the mode is off the coordinates of the detected particles are integers, with mode on, the coordinates are refined to provide subpixel location.	True/False
Region for subpixel localisation	Region of interest for the task of subpixel localisation. The value relates to the expected particle size and should be adjusted so that the complete particle can fit in. The value should be larger than the expected diameter of the particle (larger than the double of the expected particle radius).	int
Expected particle radius	Expected size of the particle. Adjust the value based on your particle size. Measured in pixels.	odd integer
Load CNN model	Trained model for candidate pruning.	provide path

Table 1: Detection parameters of the MSP-tracker.

Figure 1: Detection tab layout



- (c) Adjust the intensity range of the detected spots with the **Intensity** range values. The values are presented in relation to the intensity of the image sequence: 0 is the minimum intensity value and 1 - the maximum intensity value. Set the range to eliminate too bright or too dim spots.
  - (d) Adjust the **Relevant peak height**. Use the "candidate" option above the image viewer to monitor the result. Press the "Run test" button to run the detection algorithm for the current frame for candidates to show up in the viewer. Increase the value to detect only bright sharp spots and decrease it if darker dim spots have to be detected as well. Use the "Run test" button to update the results after changing the parameter.
2. Set parameters for candidate pruning.
    - (a) Load the **CNN model** that you are planning to use for detection. First try the existing options, but if it doesn't perform well, train the model with your data (described in the next Section).
    - (b) Set the **Region of Interest size** based on the chosen CNN model.
    - (c) Adjust the **Threshold coefficient** after running a test and looking at the "detection" mode of the image viewer. Decrease the threshold to allow more candidates though the pruning process. Set coefficient to 0 to skip the candidate pruning step.

- (d) Adjust the **Minimum distance between detections**.
  - (e) Adjust settings for the subpixel localisation. The **Region for sub-pixel localisation** should be larger than double the **expected particle radius**.
3. Test the results on at least a couple of frames. Adjust parameters if required and run a test every time you want to see the updated detection result.
  4. Save parameters for future use (optional).

You can test the detection on the current frame – button “Run test”, save the parameters into a file – button “Save to file” and load previously saved parameters “Read from file”.

### 2.2.3 Training the CNN model

To train the model with a new dataset, use the bash script **run\_train\_CNN.sh**. The following variables should be adjusted:

- **NUMBER\_FILES** - number of image sequences used for the training
- **MOVIE\_PATH\_1 ... MOVIE\_PATH\_N** - paths to the image sequence (should be tiff format, single channel)
- **POSITIVE\_COORDINATES\_PATH\_1 ... POSITIVE\_COORDINATES\_PATH\_N** - paths to the txt file with coordinates of the positive samples
- **NEGATIVE\_COORDINATES\_PATH\_1 ... NEGATIVE\_COORDINATES\_PATH\_N** - paths to the txt file with coordinates of the negative samples
- **IMAGE\_PATH** - path where the positive and negative samples can be stored
- **MODEL\_PATH** - path where the new weight for the model will be stored
- **ROI\_SIZE** - size of the region of interest used for training (16 or 32), the value depends on the particle size

Provide all the paths required in the line: **python tracking\_lib...** to include all the training files. Keep the same order of tiff sequences, positive and negative samples.

Train the model by running in command line **bash run\_train\_CNN.sh**.

Copy the trained model **cnn-model-best.hdf5** to the folder with the existing weights (dl\_weight), rename it and select it as a new CNN model in MSP-tracker detection tab.

**Preparing data for training:** The training data should contain image sequences paired up with two txt files. The txt files contain coordinates of the positive samples (particles of interest) and negative samples (non-particles) in separate files. One row represents a single sample with the following order (position, x, y, frame):

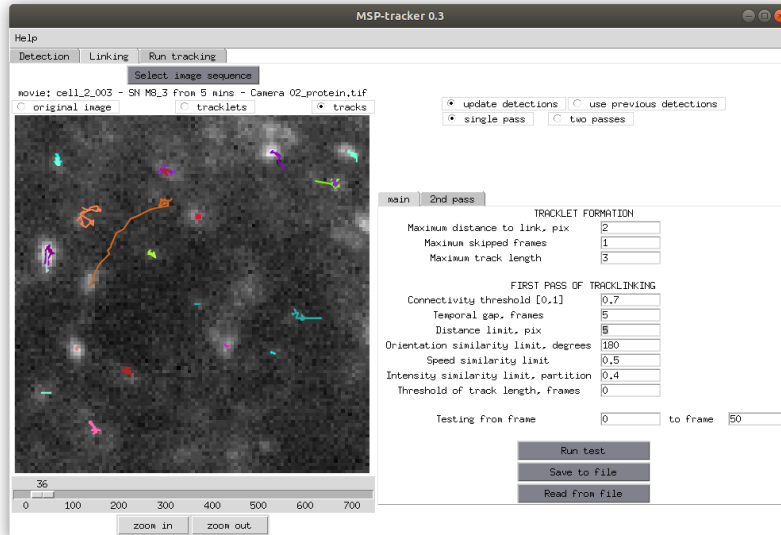
42	211.000	338.000	3
43	222.000	314.000	3
44	201.500	300.000	3
45	181.500	273.500	3
46	211.000	72.000	10
47	239.500	84.500	10
48	226.500	84.000	10
49	226.500	219.000	10

To create the txt file, you can use ImageJ multi-point tool with measure tool (ctrl+M) to extract coordinates and copy them into a new txt file. The coordinates in the txt file should be represented in pixels (not in real world measurement). It is important to include a large variety in the particle class. The non-particle class should include background, bright spots which are not particles of interest, noisy areas without vesicles. Please, select at least 200 samples for each class.

## 2.3 Setting linking parameters

The linking process is divided into a few steps. Firstly, tracklet formation is performed, where short tracks (tracklets) are formed based on the distance between detections. Secondly, these tracklets are connected between each other (tracklinking step). It is possible to use a single tracklinking pass (common solution) or choose to use two passes to extract trajectories for more complex scenarios. The output of tracklet formation and tracklinking can be viewed in image viewer using the option "tracklets" and "tracks".

Figure 2: Linking tab layout





### 2.3.1 Parameter descriptions

The linking parameters are divided into two groups - tracklet formation and the tracklinking with a choice of "single pass" vs "two passes" for the tracklinking step. When "single pass" is selected only one tab of the parameters should be filled, for the "two passes" option set parameters for the second tracklinking pass in a separate tab. The linking parameters are described in Table 2.

### 2.3.2 Proposed tuning workflow

1. Choose linking interval ("Testing from frame - to frame"). It can be about 10-40 frames or more depending on the movement you want to capture. The interval should cover the most challenging part of the sequence.
2. Choose between "update detections" and "use previous detections". For the first test run select "update detections" (detection will be run for the selected time interval), but when changing parameters for the same frame sequence switch to "use previous detections" (existing detection results will be used). It eliminates the detection process and reduces computation time.
3. Choose the number of passes. This is the number of tracklinking passes. In most cases one pass is enough, but in case of dense vesicle populations or differences in speed movement it can be beneficial to use two passes.
4. Set parameters for the tracklet formation. At this step short tracklets are formed based on the distance. You need to specify three parameters, press the button "Run test" and use the "tracklets" option above the image viewer to check the formed tracklets. Make sure that the only correct links are created, that means that the links between the points inside each tracklet are correct. Change the parameters if required, until the result is satisfying.
5. Set parameters for the first pass of the tracklinking, then if you choose two passes move to the second one. If you have two passes the idea is first to connect slowly moving vesicles:
  - higher values for speed, intensity, and orientation, as it is not important at the first pass
  - smaller value for temporal gaps - the connections should be close to each other in time
  - smaller value for the distance limit - this value depends on vesicle movement

And with the second pass faster moving vesicles will be linked:

- decrease values for speed, intensity, and orientation value
- increase the temporal gap
- increase the distance limit

Parameters	Description	Values
TRACKLET FORMATION		
Maximum distance to link	Number of pixels between detections which still can be linked. Increase the number to connect faster moving vesicles, decrease to create more reliable connections.	float
Maximum skipped frames	Number of frames which can be skipped in the same tracklet between two detections. It is preferable to use a small value (0-3 frames) to keep the connections reliable. Increase the value to compensate for persistent failed particle detection.	integer
Maximum track length	Number of frames in one tracklet. Larger value provides faster tracklinking step, but it can cause false linking. We suggest to use 5-10 frames in general, but for dense movement it should be about 3-4.	integer
FIRST/SECOND PASS OF TRACKLINKING		
Connectivity score	Final threshold which decide tracklet connection. The value is calculated based on the tracklet parameters and shows the probability of two tracklets being connected. Therefore 1 means that only perfectly matching tracklets will be linked, while 0 will allow any sequenced tracklets to be linked. We proposed to use 0.6-0.8 value, increasing it to eliminate unwanted linking.	float [0,1]
Temporal gap	Maximum temporal gap (number of frames) between two connections (when the particle is not detected for a number of frames).	integer
Distance limit	Maximum allowed distance between two detections.	float
Orientation similarity limit	Acceptable difference in orientation.	integer [0, 180]
Speed similairty limit	Acceptable proportional difference in speed.	float [0,1]
Intensity similarity limit	Acceptable difference in intensity.	float [0,1]
Threshold of track length	The parameter limits trajectory length. The final tracks shorter than the number will be removed. Should be 0 for first pass if the second pass is used.	integer

Table 2: Linking parameters of the MSP-tracker.

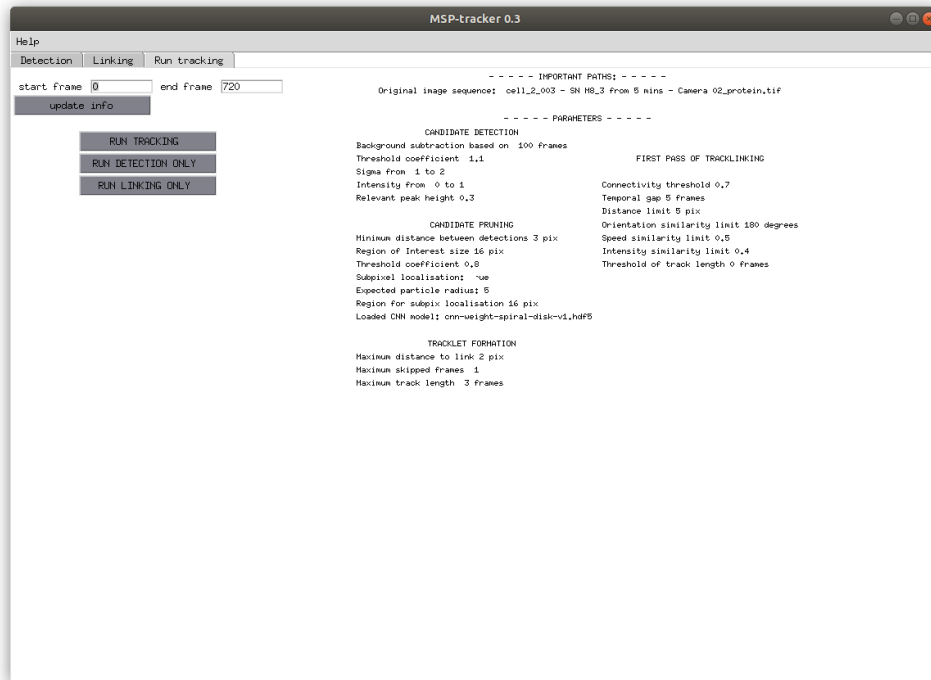
- Run a test and change parameters until satisfied with the results and save parameters (optional) for future use.

You can test the linking on the frame range – button “Run test”, save the parameters into a file – button “Save to file” and load previously saved parameters “Read from file”.

## 2.4 Running the tracker

This tab provides an overview of the set parameters, allows the user to set the final result path and run the algorithm for the entire sequence for the given frame range (set with "start frame" and "end frame", entire sequence by default). Use "update info" button to visualise latest updates in parameters and file path names.

Figure 3: Run tracking tab



You can run the MSP-tracker in the following order:

1. Check the parameters.
2. Choose start and end frames
3. Run the tracker with button "RUN TRACKING" to run both detection and linking, use "RUN DETECTION ONLY" and "RUN LINKING ONLY" to run a single step of the tracker.

You should not close the software until the tracking is complete. You can follow the progress of the algorithm in the terminal. The processing time depends on the size of the image sequence, set parameters and computational power of the machine it is running on. When finished, the final tracks will appear in the linking window. The saved tracking results can be opened in the MSP-viewer for review and analysis.

### 2.4.1 Output file format

The extracted trajectories/tracks will be saved in two formats: csv and json. In the csv file each row represents a single detection with a timestamp and the trackID it belongs to, while the json file is organised trajectory wise. Each trajectory is described by its ID, coordinates and frames. Both files can be read by the MSP-viewer.

### 2.4.2 Run the MSP-tracker without GUI

It is possible to run the tracker without the GUI when the parameters for the detection and linking are saved to a file. Use the **run\_msp\_tracker.sh** bash script to run the tracker. It is preferable to use this option when the image sequence is large and the tracking can take some time, also it can be beneficial to run the code on a server with higher computational power.

Make sure that the name of the image sequence and parameter files do not have any spaces inside the name, as it can cause a reading error in the bash script.

Adjust following variables in the bash script **run\_msp\_tracker.sh** before running the code :

- **MOVIE\_PATH** - path to the image sequence (the file should be tiff format, single channel)
- **DETECTION\_PARAMETERS\_PATH** - path to the file with detection parameters (created with the MSP-tracker GUI)
- **LINKING\_PARAMETERS\_PATH** - path to the file with linking parameters (created with the MSP-tracker GUI)
- **USE\_EXIST\_DETECTION** - "True" or "False": set it to False to run the detection part of the tracker, and True to use existing detections
- **DETECTION\_PATH** - path to the file with the detection for the current image sequence. When **USE\_EXIST\_DETECTION** is "False" - detections will be saved there, otherwise (when True) - detections will be read from the file
- **RESULT\_PATH** - path to save the trajectories

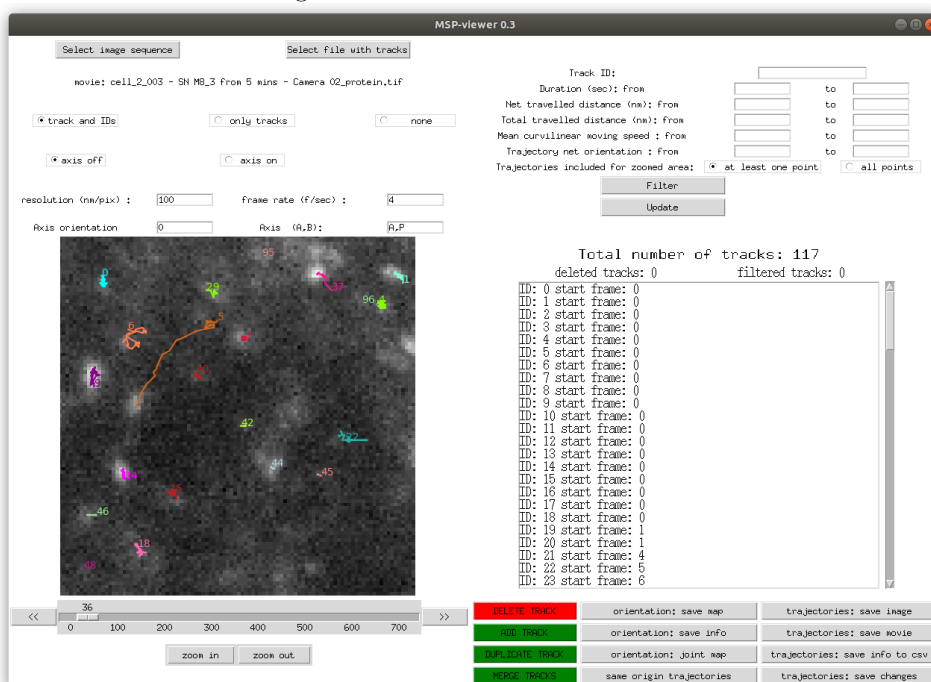
Run the script from the command line **bash run\_msp\_tracker.sh**

## 3 MSP-viewer

### 3.1 Interface overview: main window

The MSP-viewer is a tool for exploring extracted tracks (trajectories). It allows the user to view the tracks, correct them if required, plot them, and looks at trajectories' characteristics.

Figure 4: MSP-viewer interface



#### 3.1.1 Loading data

The image sequence with particles of interest should be a single channel 8-bit or 16-bit tiff stack. Trajectories can be loaded from a csv-file or txt-file with json format. Both formats are provided by the MSP-tracker, but can be imported from a file generated by any other software if the data structure is the same. Use "select image sequence" and "select file with tracks" to load the data. Lastly, provide the image sequence resolution and frame rate, otherwise default parameters will be applied. You also can provide an axis orientation and name. If the loaded trajectory file includes information about the resolution and axis, it will be updated automatically.

#### 3.1.2 Viewing trajectories

The Image viewer displays the image sequence with plotted trajectories. You can select to view trajectories with their ID, just trajectories without ID, or the

original image without anything plotted over it: "track and ID", "only tracks" and "none" respectively. The axis can also be switched on and off: "axis off", "axis on", by default the axis is not visible.

### 3.1.3 Trajectory filter

The loaded set of trajectories can be filtered based on duration(length), net and total travelled distances, mean curvilinear moving speed and trajectory orientation. The trajectories also can be filtered using the zoom option, for this you can zoom on the image and press "filter" button. The software will filter out all the tracks which are not located in the visible zone. The filter can be set to include or exclude tracks partly appearing in the zoomed area (select from the options for the trajectory included for zoom area). The duration (in seconds), travelled distance (in nm) and mean curvilinear moving speed (nm/sec) is calculated based on the provided resolution and frame rate. You also can choose a particular trajectory by providing its track ID. Press "Filter" button to apply the filter.

You can monitor the number of filtered tracks above the trajectory list. Empty the filter and press "Filter" button again to return to the original state.

### 3.1.4 Removing and adding tracks

The existing tracks can be deleted, duplicated and merged. To delete a track you should first select it, press the button "DELETE TRACK" and confirm the action. The deleted track cannot be restored, but the changes will not be saved in the original file until you press "trajectories: save updates". You can monitor the number of deleted tracks above the trajectory list.

To duplicate a track, select it and press "DUPLICATE TRACK" button, provide new track ID or use the suggested track ID value. To merge tracks use the "MERGE TRACKS" button and provide IDs of the tracks you want to merge. The merged tracks will not be deleted, the new merged track will appear with a new track ID.

A new track can be created by pressing on the "ADD TRACK" button. It can be filled with points (coordinates values and frames) in the individual trajectory window.

### 3.1.5 Trajectory details for the entire image sequence

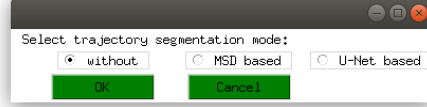
There are two options to visualise the tracking results:

- plot all the trajectories in a single frame ("**trajectories: save image**")
- create an image sequence (movie) with plotted trajectories ("**trajectories: save movie**").

Deleted and filtered out trajectories will not appear in the exported image/movie.

A number of parameters can be exported all together to a csv file ( "trajectories: save info to csv"). The list of parameters can be found in Table 3. The particle speed is evaluated for the entire trajectory and for its moving segment. Therefore, you will be asked to select the type of trajectory segmentation. The choice "without" will not provide any information about the motion, "MSD

Figure 5: Trajectory segmentation menu for "trajectories: save info to csv"

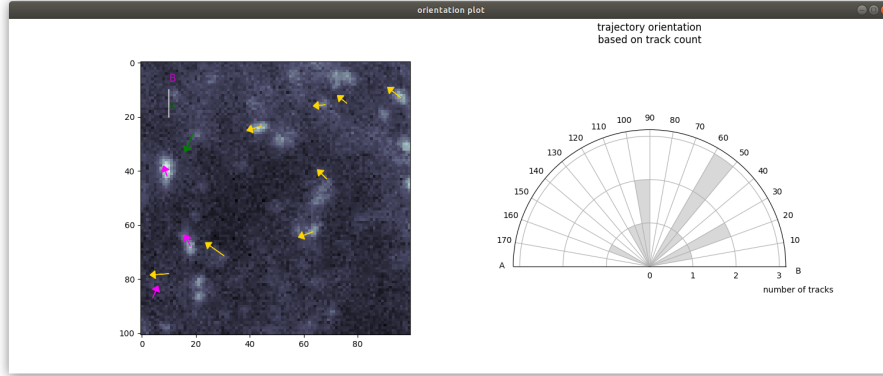


based" will use Mean Square Displacement (MSD) to segment directed motion of the trajectory path, while "U-Net based" will provide segmentation using the trained network. After selection of the trajectory segmentation mode, you will be redirected to provide the file name.

To save the changes use **"trajectory: save update"** button and provide a new filename or select the existing one to overwrite it. If you use the existing name, but doesn't provide the extension, the software will not ask you if you want to overwrite the file but will add the date and time to the provided name. It helps to avoid overwriting my a mistake.

The MSP-viewer provides an option to plot a map with trajectory orientations (taking into account the first and the last coordinates of each trajectory) and a polar diagram of the orientations for all the trajectories (Figure 6). Arrow colours on the orientation map represent the direction of movement and relate to the given axis ( $\pm 45^\circ$ ) with magenta and green, while yellow represents an orientation in-between.

Figure 6: Trajectory orientation map and polar diagram



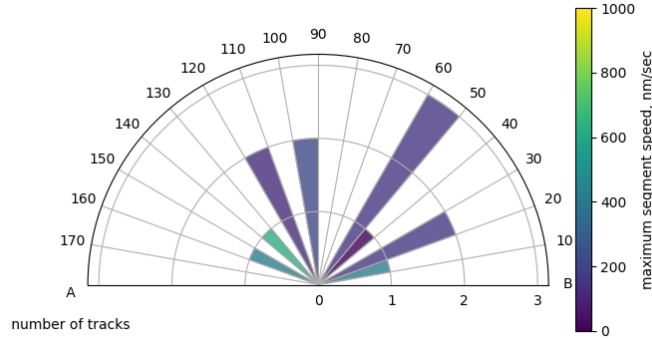
Firstly, the user is asked some additional information required to plot the diagram (Figure 7). The diagram can be based on the track count or the net distance travelled. The user can set a display range by providing a maximum value for the diagram. It can be helpful when looking at results from different experiments that require a comparable scale. Also, scale factor can be provided to normalise the values, which can be used, for example, to compare tracks extracted from movies of different length.

Default colour of the polar diagram is grey, but it can be coloured based on the particle speed per track, Figure 8. Only maximum speed from moving segments is taken into account. The user should select speed range based on the

Figure 7: Menu for trajectory orientation map

expected maximum speed, trajectory segmentation mode and a segment speed evaluation time interval for the maximum speed evaluation.

Figure 8: Polar diagram of the trajectories' orientation



Additionally, the user can select to view the axis on the image, as well as change the arrow size to adjust the visualisation parameters to the current dataset.

The angle of trajectory orientation is calculated in relation to the given axis, where  $B$  is  $0^\circ$  and  $A$  is  $180^\circ$  with anticlockwise direction of the angle count, Figure 9 (A).

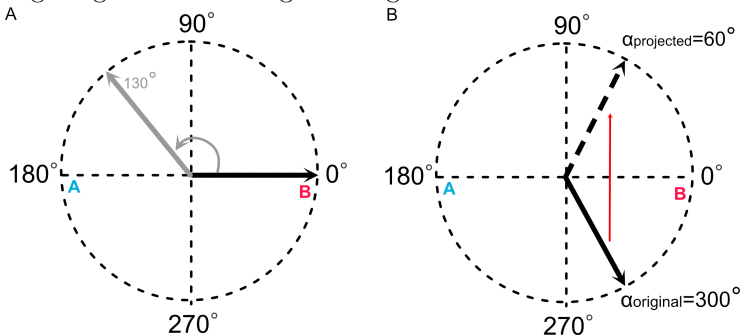
The polar diagram displays orientation in the range from  $0^\circ$  to  $180^\circ$  by mapping large angles ( $180^\circ - 360^\circ$ ) into the given range:

$$\alpha = \begin{cases} \alpha_{original}, & \text{if } \alpha_{original} \leq 180. \\ 360 - \alpha_{original}, & \text{if } \alpha_{original} > 180. \end{cases} \quad (1)$$

Example of the projection is illustrated in Figure 9 (B).



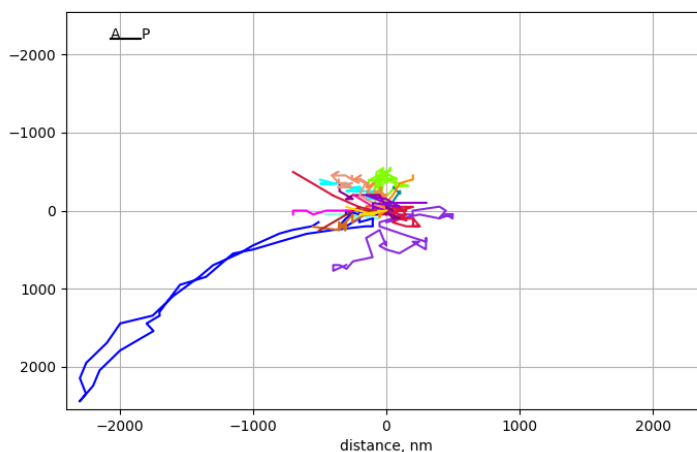
Figure 9: Trajectory orientation: (A) calculating orientation angles, (B) projecting large angles into the diagram range



Use **"orientation: save map"** to create a png file with the orientation map and diagram of the current image sequences with loaded trajectories. If you want to plot the results of multiple sequences on the same polar diagram, firstly, save the orientation data with **"orientation: save info"** for each sequence, secondly, load all the saved orientation files together with **"orientation: joint map"**. The software will ask to provide the location for the plotted polar diagram.

There is an option to plot all trajectories with the start point from the same origin - **"same origin trajectories"**, Figure10 . The user is asked to provided select txt a file with trajectories and axis orientation info. To generate the file use **"trajectories: save changes"** to save the trajectories you want to plot, the axis orientation information will be saved automatically.

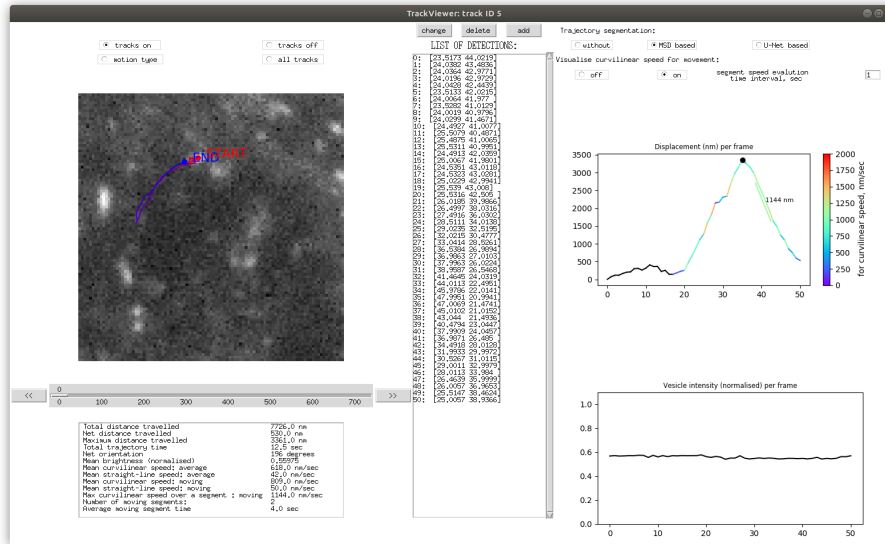
Figure 10: Plotting trajectories from the same origin



## 3.2 Individual trajectory window

The individual trajectory window can be opened by double clicking on the track ID in the trajectory list.

Figure 11: MSP-viewer: individual trajectory window



The window displays an image viewer, a list of quantitative trajectory measures, a tool to correct the trajectory, a list of coordinates, and plots representing both trajectory displacement and changes in intensity of the particle. A red colour of a position in the coordinate list highlights discrepancies in the frame order. It is not advised to have any gaps or overlaps of the detections in the trajectory as it can influence the trajectory segmentation and speed evaluation. However, as it is not always possible to void the gaps, current version of the software detects inconsistencies, and takes them into account for the speed evaluation.

The image viewer provides an option to visualise the trajectory itself or view the other tracks located nearby. There are four preferences:

- "tracks on" option to view the trajectory itself (red colour represents start of the trajectory and blue the end point),
- "tracks off" option to view the original frames,
- "motion type" to colour the trajectory based on the motion type (green - moving particle and red - stalled particle),
- "all tracks" to view other tracks in the area.

The motion type analysis can be selected on the right top side of the window. The trajectory will be segmented based on the motion type analysis selected:

- "without" will not provide any information about the motion,
- "MSD based" will use Mean Square Displacement (MSD) to segment directed motion of the trajectory path,

- "U-Net based" will provide segmentation using trained 1D U-Net.

There is an option to visualise the curvilinear speed for the moving segments of the trajectories ("Visualise curvilinear speed for movement" switch). The speed is colour-coded with a colour map provided on the side of the plot.

### 3.2.1 Quantitative trajectory measures

There are a number of quantitative measures evaluated for each trajectory. The descriptions of the measures are provided in the Table 3.

Measure	Formulation
Total distance travelled	$d_{total} = \sum_{i=1}^{N-1} d(p_i + p_{i+1})$ , where $p_i$ is a position of the $i^{th}$ particle
Net distance travelled	$d_{net} = d(p_i + p_N)$
Maximum distance travelled	$d_{max} = \max_i d(p_1 + p_i)$
Total trajectory time	$t_{traj} = (N - 1)t$
Net orientation	$\alpha$
Mean brightness (normalised)	$\frac{1}{b_{max}N} \sum_{p=1}^N b_p$ , where $b_p$ - pixel brightness, $N$ - number of pixels in the ROI, $b_{max}$ - value of the brightest pixel in the image sequence
Mean curvilinear speed	$\nu = \frac{1}{N-1} \sum_{i=1}^{N-1} \frac{d(p_i + p_{i+1})}{t}$
Mean straight-line speed	$\nu_{line} = \frac{d_{net}}{t_{traj}}$
Max curvilinear speed over a segment	$\max(\nu_1, \nu_2 \dots \nu_w)$ , where $\nu_w$ is a mean curvilinear speed of a single window (window length is provided by the user)
Number of moving segments	$N$
Average moving segment time	$t_{avg} = \frac{\sum_{j=1}^N t_j}{N}$ , where $t_j$ is a length of the $j^{th}$ moving segment

Table 3: Quantitative trajectory measures

The speed parameters are provided for the entire trajectory (average) and for the segments where particle has directed motion (moving).

### 3.2.2 Correcting trajectory points

Trajectories can be corrected by adding, removing or changing the points/detections. To delete a point select it in the list of detections and press "delete" button. You can select multiple position in the list to delete them simultaneously.

Confirm the action in the window which appears. To add a new trajectory, just use the "add" button and provide the frame number and coordinates. You can click on the viewer to grab the position and frame from the image sequence. Use "apply" to confirm the action and "apply and add" to confirm the action and add another detection.

To change the coordinate values for a particular point - select the point in the list and press "change". If multiple positions are selected only the earliest one (with the smallest frame number) will be changed. The coordinates can be specified by typing them manually or you can click on the image window.

### 3.2.3 Displacement and intensity plots

Two plots are provided to represent trajectory characteristics: a displacement plot (on the top) and intensity plot (on the bottom).

The displacement plot represents a displacement of the particle in relation to the first point of the trajectory. The color of the line highlights the motion type: green for a moving particle segment, and red for a stalled (not moving) particle. The user can select the segmentation mode: "without" (all trajectory is assigned a "not moving" class), "MSD based", and "U-Net based".

The plot can also display curvilinear speed values. To display the speed switch on the "Visualise curvilinear speed for movement". The displacement graph will change the colour-code. It will have black colour for non-moving segments and colour-coded area for moving segments. The colour map on the right side can be used to identify the speed value for each step. The software also provides a maximum curvilinear speed calculated for a given time interval ("segment speed evaluation time interval"). The speed is defined by a sliding window over the moving segments and calculating the curvilinear speed for each window. The maximum speed calculated is displayed on the figure next to a line highlighting the fastest interval.

The intensity plot shows intensity of the particle along the entire trajectory. The intensity is calculated for a region of interest (ROI) with the centre at the detection. The intensity is normalised by the maximum intensity of the entire image sequence.