

# AnimalTA user manual

*v 4.2.0*



## Index

I.	Installation.....	5
	AnimalTA on MacOS.....	5
II.	Updates .....	5
III.	Generalities and project management .....	6
	1. Language .....	6
	2. Information panel .....	6
	3. Settings.....	6
	Ergonomic options .....	6
	Tracking options.....	6
	4. Create a new project.....	7
	5. Open a project.....	7
	6. Save a project.....	7
	7. Autosave.....	7
IV.	Gestion of videos.....	8
	1. Add videos.....	8
	2. Import videos from another project .....	9
	3. Suppress video .....	9
	4. Duplicate video .....	9
	5. Concatenate videos.....	9
	6. Reorganize the videos.....	10
V.	Main project table.....	10
	1. Video identity.....	10
	2. Video orientation .....	10
	3. Frame rate.....	11
	4. Cropping.....	12
	5. Stabilization.....	13
	6. Background .....	14
	7. Definition of the arenas .....	15
	8. Definition of the scale .....	16
VI.	Video reader.....	17
VII.	Tracking preparation.....	19
	Original video .....	19
	Color mode.....	19

Apply corrections .....	19
Background.....	20
Threshold.....	21
Erosion/Dilation .....	22
Area selection.....	22
Distance threshold .....	23
Number of individuals per arena .....	24
VIII. Tracking process.....	25
1. Automatic tracking.....	25
2. Manual tracking .....	26
IX. Visualization and correction of the trackings.....	26
1. Visualization .....	26
2. Set the identities of the targets .....	27
3. Corrections .....	27
Find missing values.....	27
Punctual error .....	28
Interpolation .....	28
Redo part of the tracking .....	29
Invert coordinates .....	29
Delete existing values.....	29
Add/Remove an individual.....	29
Fusion trajectories.....	29
Validate corrections .....	30
X. Prepare analyses .....	30
1. Smoothing filter .....	31
2. Perspective correction .....	31
3. Analyses of individual trajectories .....	32
4. Spatial repartition .....	33
Elements manipulation .....	33
Types of elements .....	33
Expend elements to other arenas .....	35
5. Interindividual distance.....	35
6. Exploration .....	36
Individual centered .....	36
Meshes .....	36
7. Sequences .....	37

XI.	Run analyses.....	40
	Results per individual .....	40
	Results of interindividual distances.....	43
	Spatial.....	43
	Detailed data .....	43
XII.	Export a video .....	45
XIII.	Confidentiality.....	46

## I. Installation

AnimalTA is a program developed in Python for usage under the Windows operating system. The AnimalTA installer can be downloaded from: <http://vchiara.eu/index.php/animalta> or <https://github.com/VioletteChiara/AnimalTA/releases>.

Download the file AnimalTA.installer.exe, launch it and follow the installer instruction.

If you want to know which version of the program you are using, click the “?” button on the top left corner of the program, this will also provide information on how to cite the program.

### *AnimalTA on MacOS*

AnimalTA has been developed for Windows operating system and is not available on MacOS. For MacOS users, it is however possible to use it via virtualization softwares. We have good feedback from users using UTM (<https://mac.getutm.app/>) or Parallel Desktop (<https://www.parallels.com/products>).

## II. Updates

To keep AnimalTA alive and improve it through time, we frequently release updates of the program. If you have internet connection, AnimalTA will inform you automatically if an update is available. If you want to update the program, three options are available:

- *Manual update*

This is the most time-consuming option, but it allows you to control everything and will not allow AnimalTA to download anything by itself. To update the program manually, download the last installer at: <http://vchiara.eu/index.php/animalta> or <https://github.com/VioletteChiara/AnimalTA/releases>. Launch then the installer and follow the instructions.

- *Semi-manual update*

If AnimalTA detects that a new update is available, it will give you the possibility to download and install it by itself. To do so, click the “Do the update (only this time)” button. Note that by clicking the button, you allow AnimalTA to download and install the latest version of the program at this precise moment. For each new update the program will ask you the same question.

- *Automatic update*

This is the least time-consuming option. You can enable this option in the program settings ([see section: Ergonomic options](#)), or by clicking the “Do update and activate Auto-update (for future updates)” button which appears when a new update is detected. If you activate this option, AnimalTA will be downloading and installing the latest versions as a background process. Downloads will be done without you noticing while you use the program. The next time you open the program, the update will be performed automatically. A loading panel will also inform you that the update is in progress and toward which version the program is updating.

### III. Generalities and project management

#### 1. Language

A drop menu is available on the top right corner of the AnimalTA window to change the displayed language. Note that changing the language will automatically close AnimalTA. Just reopen it to have the new set language.

#### 2. Information panel

An information panel is always visible in the top right corner of the application. This panel aims to provide the user with information about the different components of the window simply by putting the mouse over them.

#### 3. Settings

Some defaults settings can be changed using the <...> button in the top left corner of the program. Once your settings done, click the validate button to return to the main menu.

##### *Ergonomic options*

- Set AnimalTA to low priority. This option allows to limit AnimalTA's memory usage. We advise selecting this option when your computer is running other tasks at the same time as AnimalTA and that those others tasks are slowed down.
- Choose the theme color of AnimalTA. Note that the program will be closed each time you ask for changing the theme color for this theme to be applied.
- Activate automatic update ([see section Updates](#))
- Sound/visual alert when the trackings are completed.
- Change the default size of the tool used to modify the background.
- Change the size of images (first image, background and arenas) displayed for verification.

##### *Tracking options*

- Use Kalman filter. This filter is used to predict the future position of a target based on its previous coordinates. When this option is selected AnimalTA will use the predicted coordinates to solve the cases or targets crossing (see Figure 1) instead of the last known position of the targets. Note that using the Kalman filter will increase the time needed for the tracking.

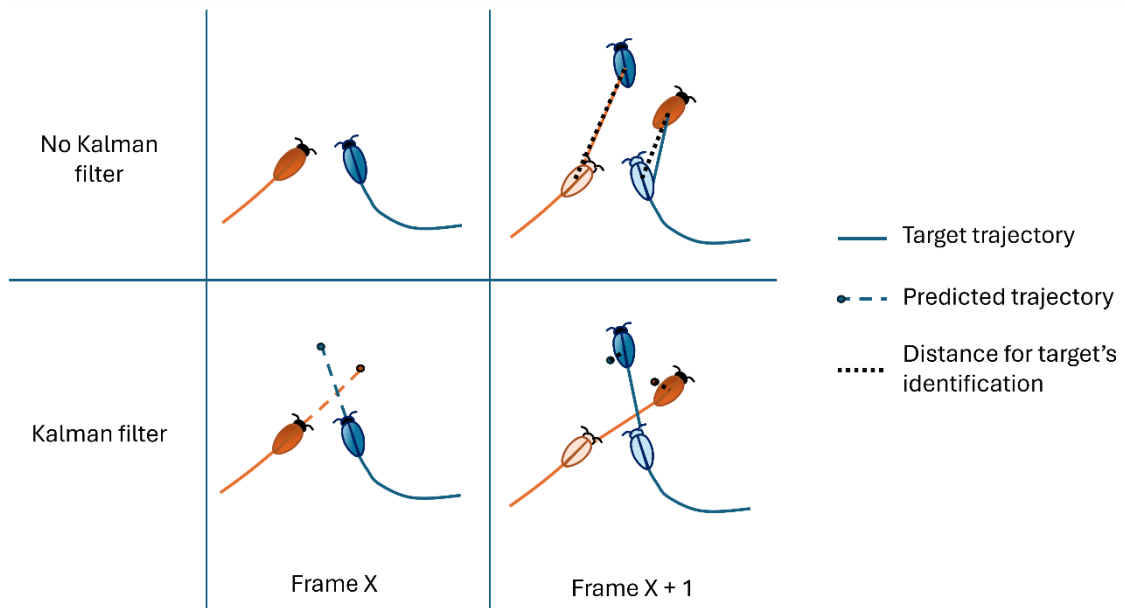


Figure 1: Illustration of how the Kalman filter works. In a similar situation when the Kalman filter is not used the program will make an error of identification.

- Keep entrance data. When tracking a variable number of targets, an “Entrance area” must be define. This area is delimitating from where the targets are expected to join/leave the arena. If this option is not selected the trajectories inside this entrance area are not kept.

#### 4. Create a new project

The notion of project is at the center of AnimalTA functioning. A project may be seen as a collection of videos, each associated with various information. By pressing the “New project” button, you will be able to choose a location on your computer where you want the files linked with your project to be located. Two things will be created in the indicated location: a *.ata* file which is your *project's file* and a folder (*project folder*) with the name of your project that will later contain all the outputs from AnimalTA.

#### 5. Open a project

If you already have created a project before, you can reopen it using the “Open project button”. Then just select the *.ata* project file you want.

#### 6. Save a project

An opened project can be saved using the “Save” Button. The last saved version of the project will be replaced by the current one.

#### 7. Autosave

AnimalTA will automatically save a copy of active project every 15 minutes in a safe location. In case of crash or of an error of manipulation, you can find this security copy in:

“My\_installation\_folder/AnimalTA/Files/Autosave”, after 30 days of non-utilization, autosaved files are destroyed, for this reason if you need to use one of those files, we advise to copy-paste them in another location before using them.

## IV. Gestion of videos

### 1. Add videos

Use the “Add video(s)” button to add some videos to your project. The number of imported videos is not limited. Note that AnimalTA is not doing a copy of these videos, also once a video has been imported in the project if you rename it or change its location you will not be able to use it in AnimalTA anymore.

If one or more of the videos you want to import are not in *.avi* format, a window will appear proposing to convert them. See the next section [Video conversion](#) for more details.

For each imported video, a line will appear in the table in the center of the main project (see section: [Main project table](#)). A video can be selected by clicking on its name, in this case, the rectangle at the left of the video name will appear in green to indicate which video is selected.

### 2. Add image sequence

Since version 4.1.0, AnimalTA permits the creation and tracking of videos made from image sequences. Images to be loaded must be stored in the same folder and be numbered/named in a way that an alphabetical ordering will match with the images order of reproduction. Click the down arrow button at the right of the “Add videos” button so that the button changes for “Add image sequence” then click the button and select the folder in which the images are stored. You will then be asked for the frequency at which the images were taken. You can either indicate the value in images per second (fps) or the time between two images (in sec).

Note that AnimalTA is not doing a copy of these images, also once an image sequence has been imported in the project if you rename the folder or files or change their location you will not be able to use it in AnimalTA anymore.

*Tip:*

If your images are stored in more than one folder, you can import them as two separated videos and later concatenate them (see section [Gestion of videos, Concatenate videos](#)).

### 3. Video conversion

While trying to import videos whose formats are not compatible with AnimalTA, the program will propose to convert them. A window with a list of videos needing conversion will appear. You can select from this list the videos you want to convert. Note that only the converted videos will be added to the project.

Before validating, you can change the “Video quality” value in the bottom right corner of the window. High video quality will result in heavier files with slower tracking process but as the quality improves, the details in the video will be more visible. In contrast, low quality conversion will result in lighter videos, faster to process but with an increased loss of details. The default value of 5 is generally recommended for most of the tracking tasks.

After the conversion is done, the converted videos may be found in the [project folder](#), in the “converted\_vids” subdirectory.

*Special case:*

AnimalTA uses the video file metadata to find the frame rate of the videos. In some cases, this frame rate may be wrong or inexistant (for instance if videos are acquired *via* non-traditional video cameras or have been previously modified by other programs). Such situation will result in videos not being read at a proper frame rate (i.e. videos are slowed down or accelerated), which will result in biased analysis. To compensate for this problem, it is possible to select the “Correct metadata frame rate information” option in the Conversion panel. Then manually specify what is the frame rate of the selected videos before validating.

#### 4. Import videos from another project

To import an existing AnimalTA project into a new one, you can follow the same steps as you would to add videos. Simply click the "Add video(s)" button and then select the AnimalTA file that you want to import (with a *.ata* extension) instead of video files. By doing this, all the videos that were present in the old file will be imported into the new project along with any associated data, such as parameters and tracking data. This will help you to transfer your existing work quickly and easily to a new project without having to manually add all the videos and associated data again.

#### 5. Suppress video

To suppress a video, click the red cross (✖) under the “Video” label, or the “Delete” key after selecting the video. Note that this action will remove the video from the project and cannot be canceled after validation. All the data associated with this video will also be suppressed (deletion of the tracking parameters and coordinates resulting from tracking). The original file of the video will not be impacted by this action.

*Tip:*

You can also delete multiple videos at once using the <Shft + del/supr> key combination on the keyboard.

#### 6. Duplicate video

Click on the double sheets (☰) to duplicate a video. A new video row will appear in your table which the exact same characteristics as the original one, except for its name. This new video is completely independent from the previous one.

#### 7. Concatenate videos

Often, video cameras recording for a long period of time will create several videos of short durations instead of one long video. To add these short videos into one long, you must first import all the parts of the video in the project (see section: [Add videos](#)). Once the importation done, click on the symbol of the first video representing a torn film (🎞️). The video after which other videos will be concatenated now appears in purple. Click on a second video’s name to paste this second video after the first one. The second video will be added after the first one and will disappear from the main project table. You can then repeat this operation as much as you

need. If you press the “concatenate video” button by accident, you can cancel it by selecting again the same video.

## 8. Reorganize the videos

To move a video up or down in the list, select it (click on its row) and click the small arrow buttons (↑ and ↓) in each side of the “See summary table” button under the table.

## V. Main project table

In the main window of the project, each imported video is associated with one line of the table presents in the center of the window. A vertical scrollbar at the right of the table allows you to scroll through the videos if they are too numerous to fit within the window. The mouse wheel or up and down arrow keys can also be used to scroll. A horizontal scrollbar at the bottom of the table allows you to scroll from left to right to see the whole table.

Each row of the table is separated in several sub-elements, described below from left to right. Changing these elements is not mandatory and the program will work even if no changes are done.

A summary of what has been done for each video is available under the table (button “See summary table”).

### 1. Video identity

The first section of each row contains three elements:

- A vertical rectangle whose color indicates whether the video is selected (green) or not (red). It is not possible to select more than one video at a time.
- The video name.
- The eye symbol. You can see the first frame of the video by putting your mouse over this symbol.
- Two arrow buttons to rotate the video.

You can change the name of a video within the project by simply click and write in the video name text field. You cannot have two videos with the same name or with an empty name. AnimalTA will indicate un-accepted names by coloring the video row in red. Changing the name of a video inside the project won’t change the file name of the video out of AnimalTA. The new name will be the one used to refer to this video in AnimalTA and in the data created by the program. After a video has been tracked, it is still possible to change its name by clicking on the “X” button at the right of the video name.

### 2. Video orientation

To apply a 90° rotation of the video, use the two rounded arrow under the video name (↻).

### 3. Frame rate

The fps (frame per second) or frame rate of a video corresponds to the number of images this video is displaying per second. Here reducing the fps means that you will only analyze part of the images of your video. For instance, dividing the fps by two mean that only one out of two images will be analyzed.

By reducing the frame rate, you can greatly improve the speed of the tracking and of the analyses. Decreasing too much the fps will decrease the precision of your measurements and the distances traveled by your subjects will be underestimated. In the opposite, a too high frame rate might result in a confusion between real displacements of the subjects and the noise related to the tracking imprecisions and induce an overestimation of the distances traveled (see Figure 2 for illustration). This last problem can be later corrected by applying a smoothing filter on the coordinates (see section: [Prepare analyses, Smoothing filter](#)).

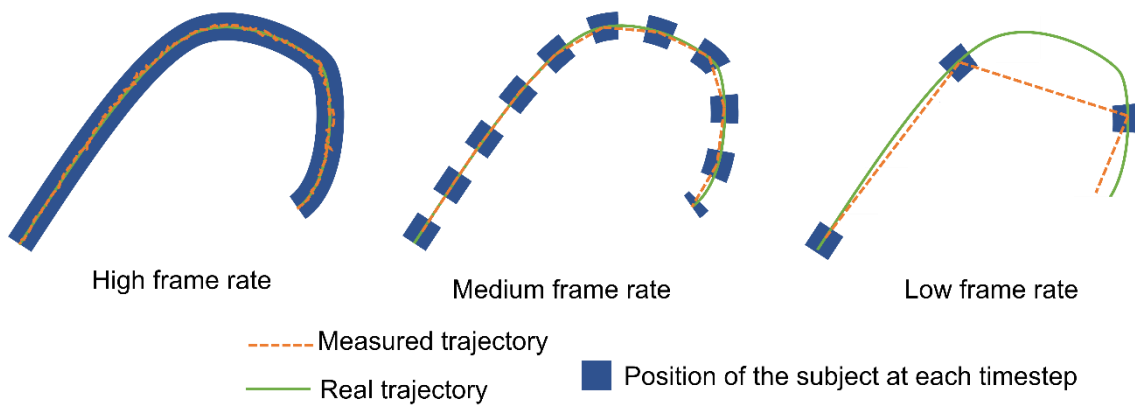


Figure 2: Illustration of the impact of the frame rate on the tracking results.

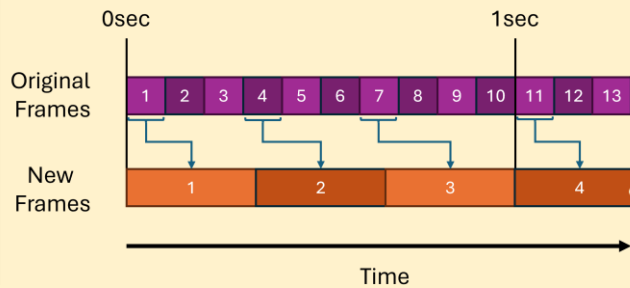
If you are working with more than one video at a time, you can use the “Apply to other videos” button to use the same frame rate reduction to other videos. Note that the frame rate reduction consists in dividing the number of fps by a given number, also if your videos did not share the same frame rate before the transformation, they will also end up with different frame rates.

*If you don't change this option:* the original video frame rate will be used.

*Special case:*

You can also use a personalized frame rate by selecting the “personalized” option instead of a simple frame rate reduction, but in that case the program will have to take existing frames to fill-in the gaps, which might result in inaccuracies. For instance, if you have a 30fps video and you ask for a 60fps frame rate the program will simply duplicate each image of the video. For this reason, it is not advised to choose a higher frame rate than the original one.

To illustrate with another example, here is an illustration of a video with an original frame rate of 10 fps and asked for a frame rate of 3fps.



#### 4. Cropping

##### *Temporal cropping*

Many researchers record longer movies than needed when recording animal behavior, this method ensure that the whole test will be correctly recorded without missing the first or last seconds of the experiment. This second section allows to remove the useless part of the video *via* cropping.

When you press on the “Crop video” or “Modify cropping” buttons, the main project window will be replaced by a new one. In the center of this new window, you will see the video reader (see section: [video reader](#)) which displays your video.

Under the video reader, you have access to the information about your video cropping both in terms of seconds and frames. To indicate where you want your video to begin, you can either write it in the text field under the “start” label or go to the frame you want as the first frame using the video reader and then press the “Select video beginning” button. The time-bar will be immediately updated, and you will see a blue rectangle indicating where your video will begin (after the cropping). To indicate where you want you video to end you can either click on the time-bar and press the “Select video end” button or indicate the duration you want for your video by writing in the text fields below the “Duration” label.

##### *Spatial cropping*

Sometimes, you may also be interested in a reframe of the video. To reframe the video, click on the borders of the displayed image and drag toward the center of it. You will see that a red delimitation will appear, only the part inside these delimitations (which will appear brighter than the rest of the image) will be used for the video tracking. You can then repeat the operation with

*Tip:*

If you are interested in cropping spatially as close as possible to your experimental arenas, you can skip this step and do later an automatic spatial crop while defining the experimental arenas (see [Definition of the arenas](#)).

the three other borders. Note that removing a significant part of the video with this method will accelerate the tracking process, but removing a very small part of the video might in the opposite slow down the process.

#### *Validate cropping*

Once you finished the cropping processes, you can press the “validate” or “validate and go to next video” buttons. The first one will bring you back to the main project table, while the second will open the next video.

You can also move from one video to another using the drop-down menu in the top left corner of the window. Note that in this case, the changes you made regarding the cropping will not be saved.

*If you don't change this option:* the whole video will be tracked.

## **5. Stabilization**

This option allows to correct the video in case of camera tremors during the recording. Shortly, this option will look for points of interest in the first frame (after cropping) of the movie. Then, for each frame of the video these same points are identified, and a transformation is applied to ensure that the points of interest remain in the same place through the video.

You can ensure that the result of the stabilization is satisfying by clicking the “Check stabilization” button. This option will open the video reader (see section: [video reader](#)) that will show which points of interest have been selected on the frame of reference (first frame). You will also see below the original video with the position of the points of interest and the video after stabilization. If your background is highly homogenous, the program might have difficulties in finding good points of interest. Also, if you see that the stabilized video is not satisfactory, you can remove the problematic points by clicking on them in the reference frame from the video reader. A minimum of two points is mandatory. You can also play with different parameters (in the right part of the windows) to change the way the points of interest are found by the program.

Finally, the “Apply/remove stabilization to multiple videos” button in the main project window allows to generalize this option and its parameters to several videos at the same time.

Note that the process of stabilization is time consuming, also we recommend to not apply a video stabilization on videos that are already stable.

For more technical information, see:

[https://docs.opencv.org/3.4/dc/d6b/group\\_video\\_track.html#ga473e4b886d0bcc6b65831eb88ed93323](https://docs.opencv.org/3.4/dc/d6b/group_video_track.html#ga473e4b886d0bcc6b65831eb88ed93323)

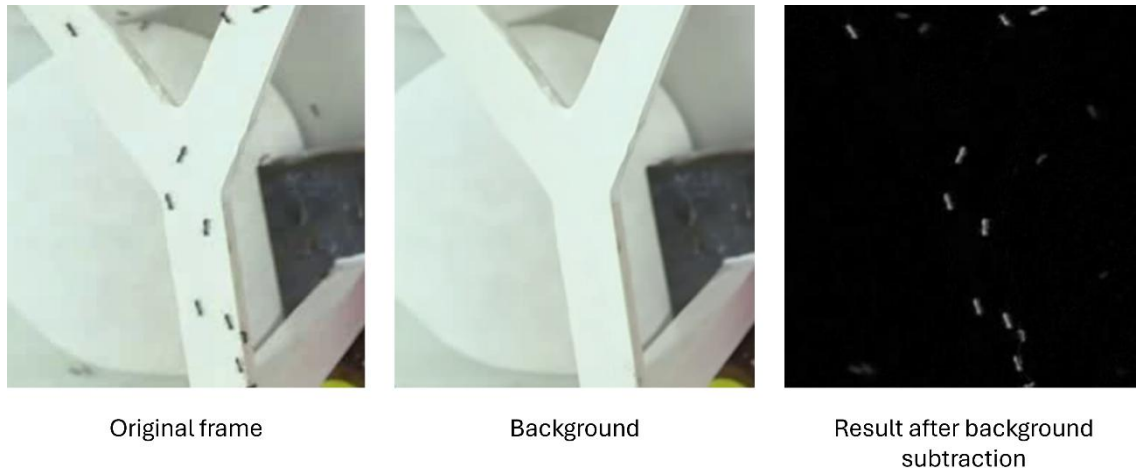
and

<https://learnopencv.com/video-stabilization-using-point-feature-matching-in-opencv/>

*If you don't change this option:* the original cropped video will be used.

## 6. Background

To detect the moving targets in the video, AnimalTA uses a process of subtraction: it compares the frame with and without the targets to find the target's position (see Figure 3).



*Figure 3: Illustration of the process of background subtraction.*

Also, prior to the tracking, it is important to have a background image (image without the targets). To automatically create this background image, you can press the “Create background button”. To create the background, AnimalTA will take a subsample of the images of the video and calculate the median color value of each pixel of the frame. The “Do background for multiple videos” button will open a window asking if you want to reuse the same background for all videos or if you want to recreate a new automatic background for each of the videos.

The automatic background method will successfully remove all the targets from the image, except if these targets remain at the same place for more than half the duration of the video. In this case, the automatic background process will fail. To ensure that such problem did not arise, you can put your mouse over the eye symbol to see the background proposed by AnimalTA. If some targets are still present, even partially, you will need to correct this background. The correction can be simply done using the “Modify background” option.

This option will open a new window in which you will find the current background of the video, the objective is then to draw over the remaining targets to hide them and to recreate an image empty of targets. To do so, you can select a new color by right clicking in the image, the color under your cursor will be taken (the current color is displayed on the bottom right of the window, above the “validate” button. You can then paint over the targets using left click. The size of the painting area can be adjusted using the mouse wheel. If you are unsatisfied with the automatic background proposed by AnimalTA you can also directly use the first frame of the video by using the button in the bottom right corner.

Keys	Actions
Right click	Change the current color
Left click	Paint
Ctrl + left click on the image	Zoom in
Ctrl + right click on the image	Zoom out
Ctrl + left click + drag on the image	Zoom in a particular area
Mouse wheel	Change the size of the tool
Ctrl + Z	Cancel last painting operation

Once you finished the modification of the background, you can press the “validate” or “validate and go to next video” buttons. The first one will bring you back to the main project table, while the second will open the next video.

*If you don't change this option:* The method used to find the targets will be different and an adaptive thresholding or dynamical background will be used instead of a background subtraction. These other methods can be better in case of videos with changes in luminosity, with important perturbations or with slowly changing background. More details regarding these options are available the [Tracking preparation, Background](#) section.

## 7. Definition of the arenas

This option allows you to draw the delimitations or the arenas in which the targets may be found. Drawing the arenas ensures that the targets will not be confused between arenas during the tracking: a target inside an arena cannot leave this arena or appear in another one. It also limits the risks that some elements outside the arena will be confused with the targets. Finally, the defined areas will be later used in the analyses to calculate for instance the proportion of the arena explored by the target, or the time spent close to the border of the arena.

Once you pressed the “Define arenas” button, a new window will appear. In this window you will see the image of your background (or the first image of the video if the background has not been defined) and you will be able to draw the different arenas. To draw an arena, simply click on the background where you want to place a corner of the first arena. At any moment, you can indicate the kind of shape your arena should be (Ellipse/Rectangle or Polygon). Once the corners are placed, you can move them by click and drag, or suppress them using the “supr” key. Once your first arena is correctly defined, you can press the “enter” key to validate it and begin the drawing of a new one. You can modify a precedent shape simply by clicking on one of its points. Arenas can also be moved, rotated, or resized as described in the table below. One area must be completely separated from the others, also if two shapes are overlapping, the combination of these two shapes will be considered as a unique area. You can use the “View” button to see the result. If the arenas have the same location in several videos, you can use the “Apply these arenas to other videos button”. In case of doubt the number of arenas is displayed below the information panel.

You can also create negative shapes using a right click instead of a left click when creating the first point of an arena. This will exclude shapes from an existing arena (see Figure 4).

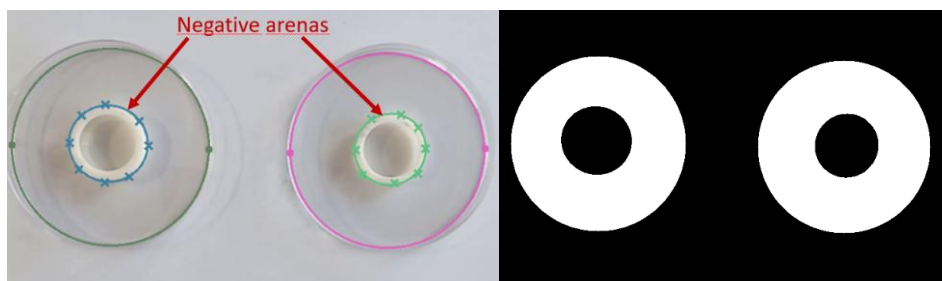


Figure 4: How to create arenas with non-full shapes.

Shortcuts:

Keys	Actions
Left click	Add a corner

Left click on a point	Select/move the corner
Left click and drag on a border	Move the arena
Right click	Add a negative corner
<Del/Supr>	Delete the selected corner
<Return>	Validate a shape
<Shift> + Left click and drag on a border	Duplicate the arena
<Shift> + Right click and drag on a border	Resize the arena
<R> + Right click and drag on a border	Rotate the arena
Ctrl + left click on the image	Zoom in
Ctrl + right click on the image	Zoom out

*Tip:*

If you are interested in cropping spatially as close as possible to your experimental arenas, you can select the More options > Adapt cropping option in the bottom of the window. This will apply a [spatial cropping](#) as close as possible to the arenas for either the current video or all untracked videos of the project with arenas defined.

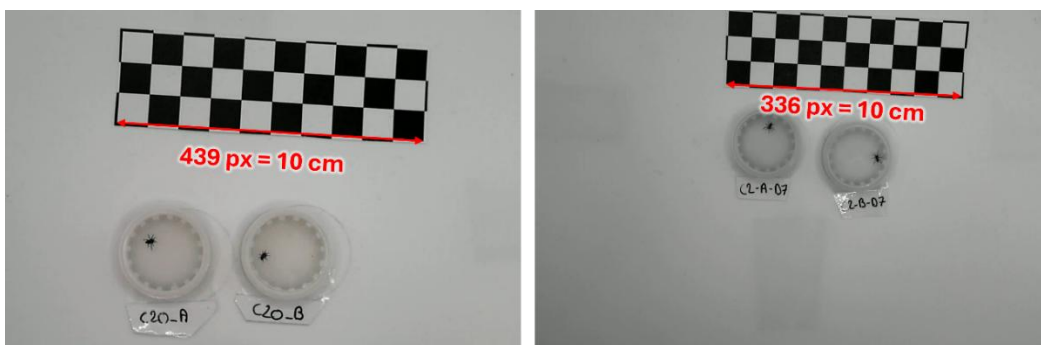
*If you don't change this option:* The program will consider that there is only one arena occupying the whole image.

## 8. Definition of the scale

Defining the scale of your recording will allow you to obtain data expressed in international units instead of pixels. Another advantage of defining the scale of your video is that it will allow to uniformize the tracking parameters between videos which are not recorded at the same scale (e.g. Figure 5).

After clicking the "Define scale" button, a new window will open in which you can see the [video reader](#). Click on reader to place two points separated by a known distance. Then, indicate in the bottom right files the real distance between these two points and which unit you are using. If you already know the scale of your video, you can directly type in the "Ratio" text field.

You can use the "Apply this scale to other video" if several videos have been recorded at the same scale (same distance between the camera and the arenas, same quality of recordings, same zoom).



*Figure 5: Illustration of the importance of the scaling: when the recording is not done from the same point of view, the values in pixels cannot be compared between videos but the distance in international units can be.*

*If you don't change this option:* All the later measurements will be done in pixels.

## VI. Video reader

The video reader will be found in different situations using AnimalTA. It will show you the video you are working with (Fig 4, A). Under the video, you can see a time-bar (Fig4, B) that will show you the duration of the video and which indicate which frame of the video is visible on the screen (indicated by a rectangle, Fig4 C). The red/purple part of the time-bar is the part of the video you are working with (i.e. video after cropping, see section: [Main project table, Cropping](#); Fig 4, D), it is delimited by a blue and a red rectangles who indicate respectively the beginning and the end of the working part of the video. You can click directly on this time-bar to go to a particular moment of the video.

Four buttons are available under the time-bar:

Play/Stop buttons: play/stop the video

Go to begin/end buttons: Make you jump at the beginning/end of the video

A slider at the right of these buttons allows you to increase/decrease the speed at which the video is displayed while playing. A speed of 0 usually corresponds to the original video speed, but in cases of videos with a frame rate lower than 1 fps, a speed of zero correspond to playing one frame of the video per second (i.e. video will be accelerated).

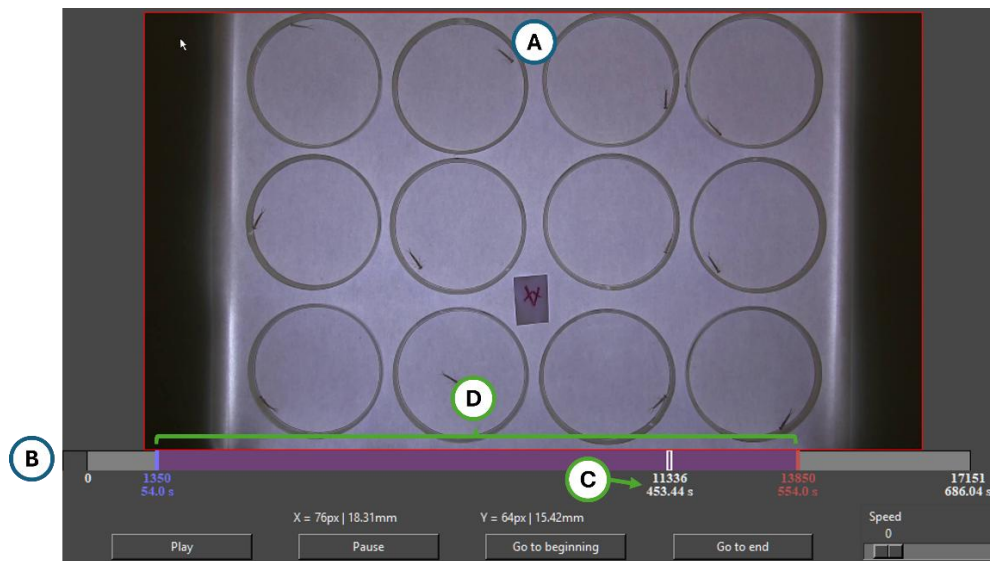


Figure 6: Illustration of the video reader.

Shortcuts:

Keys	Actions
Ctrl + left click on the image	Zoom in
Ctrl + right click on the image	Zoom out
Ctrl + left click on the image + drag	Zoom in a particular area
Spacebar	Play/Stop the video
Left arrow	Move backward of one frame
Right arrow	Move forward of one frame

## VII. Data importation

If you already have the tracking data of a video and want to import them from an external source, you can do so by selecting the corresponding video in the main table and clicking the “Import data” button on the top right part of the panel. Note that you can still define a scale (see section [Main project table, Scale](#)) and arenas (see section [Main project table, Definition of the arenas](#)) before importing the tracking data. The scale and arenas will later be used for tracking analysis and correction.

### *Pre-registered programs*

If your dataset comes from one of the programs already implemented (DeepLabCut, SLEAP, ToxTrack, or AnimalTA) click on the corresponding button and indicate the path toward the file with the tracking data. Note that in case of ToxTrack, you will be asked to indicate the Folder where the RealSpace tracking data are saved.

### *Other dataset*

If your dataset does not come from one of those programs, click the “Other” button and indicate the path toward your coordinates file. Note that only files in “.csv” format will be accepted. A new panel will open, asking for you to fill-in some data regarding the original table structure.

- Separator: The character used in the original file to separate the columns of the original table.
- Header: Check this option if the original table includes a header row (i.e. column names).
- Frame column: Indicate here the name or index of the column in the original table that summaries at which frame of the video the data corresponds to. Note that if the “Time column” option is filled, this option is not mandatory.
- Time column: Indicate here the name or index of the column in the original table that summaries at which time of the video the data corresponds to. Note that if the “Frame column” option is filled, this option is not mandatory.
- Individuals as columns/Individuals as rows: Check the “Individuals as columns” if the original table structure is made in a way that each row is associated with a single targets/individuals are associated with one specific columns (e.g. <columns names: Frame, Target1\_X, Target 1\_Y, Target2\_X, Target2\_Y>). Check the “Individuals as rows” option if the original table structure is made in a way that each row is associated with a single target/individual (e.g. <columns names: Frame, Target\_ID, X, Y> OR <columns names: Frame, Target\_ID, leg1\_X, leg1\_Y, leg2\_X, leg2\_Y>).
  - o ID column: To be filled only if the “Individuals as rows” option is selected. Indicate here the column name or index of the column giving the target identification.
- Column X/Y coordinates: Indicate the name or index of the column(s) where the X/Y coordinates is(are) located. If there is more than one column concerned, you can also list those columns between brackets and separated by a coma (e.g.: [3,5,7]), or indicate a specific chain of character always found (e.g. If the columns you are interested in are Ind0\_X, Ind1\_X, Ind2\_X, you can simply write \_X here).

Once all the data are filled, click the “Build table” button and ensure that the converted table is correctly done. Then click the validate button.

## VIII. Tracking preparation

Once you finished with the preparation of the videos (see section [Main project table](#)), you will be able to define the parameters of the tracking. To that aim, select the video you are interested in and press the “Prepare tracking” button in the bottom left of the main project window. This will redirect you toward a new window in which you will see the video reader (see section [video reader](#)) and several parameters on the right of the window. Each parameter corresponds to a transformation made into the video that will allow detecting the targets in the image. The transformations will be applied from top to bottom, also if you select the 5<sup>th</sup> checkbox (Erosion) it means that the image displayed in the video reader is the image that went through the 4 other transformations (Original video importation, Color mode, Background subtraction and Thresholding).

From the main project window, you can see the tracking preparation’s parameters of a selected video in the bottom right of the window. You can also apply these parameters to other videos using the “Apply these parameters to other videos” button.

### *Original video*

Show the video cropped and stabilized if this option was selected.

### *Color mode*

You can either work with color video or convert the video to grayscale. If you keep colored background, it easier to track targets whose value (how dark/light they are) is similar to the background’s one (see Figure 7). However, colored videos need more time to be processed than grayscale ones, inducing a slower tracking.

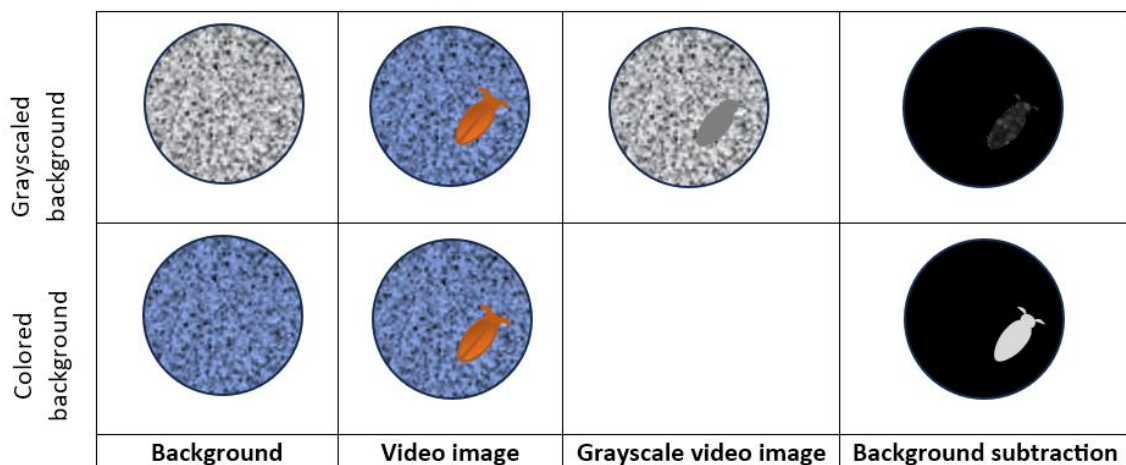


Figure 7: Illustration of the difference between color and grayscale modes.

### *Apply corrections*

If activated, the “correct brightness” button induces a correction of the brightness. To that aim, the brightness values of the current frame are modified according to the distribution of a

reference image brightness values. The reference image is the background, if there is no background, the reference image is the first frame of the video.

### *Background*

If a background has been defined (see section [Main project table, Background](#)), this option shows the video after the background subtraction.

If there was no background created during the video preparation (see section [Main project table, Background](#)) the program will propose to use either a dynamical background or an adaptive background.

**The dynamical background** is a background build and adapted little by little through the video. To simplify, this method identifies movement in the image. Such method has the advantage to work event if the background is changing (if this change in background is slow enough). The window used to build the background can be changed using the “P” button on the right of this row. The inconvenience of dynamical background is that targets immobile during the selected time window will be integrated in this background and therefore will be lost. This might be compensated later during tracking corrections by a global interpolation (see section [Visualization and correction of the tracking, Corrections, Interpolation](#)).

**The adaptive threshold** is identifying changes in the contrast of the video to identify spots darker/lighter than the background.

You can click the “P” button available in this row to unlock more options.

The first option allows to specify if the targets are darker/lighter than the background. Selecting one of those option will remove all potential noise linked to the non-selected category. For instance, if you select the “Dark targets” option, potential light reflections will be ignored and won’t impair the tracking.

The second option allows to use a relative background instead of an absolute background (see Figure 8 for illustration). This option is particularly useful if you have non homogenous background with important changes in darkness.

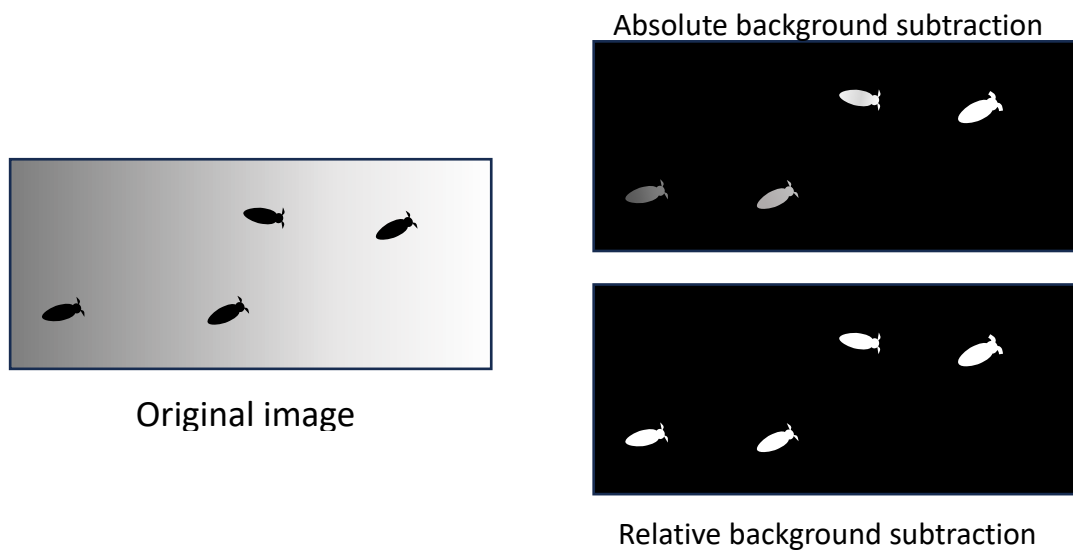


Figure 8: Illustration of the concept of relative background subtraction.

### Threshold

If a background (classic or dynamical) has been defined, this parameter transforms the image resulting from the background subtraction process to create a binary image (only composed of white and black pixels, no grey anymore). The threshold parameter determines the lightness above which a grey pixel will be converted to a white one and below which it will be converted to a black one (Figure 9). For a good quality tracking, the result (visible in the video reader) must be the closest possible of the targets in white and the rest of the image in black.

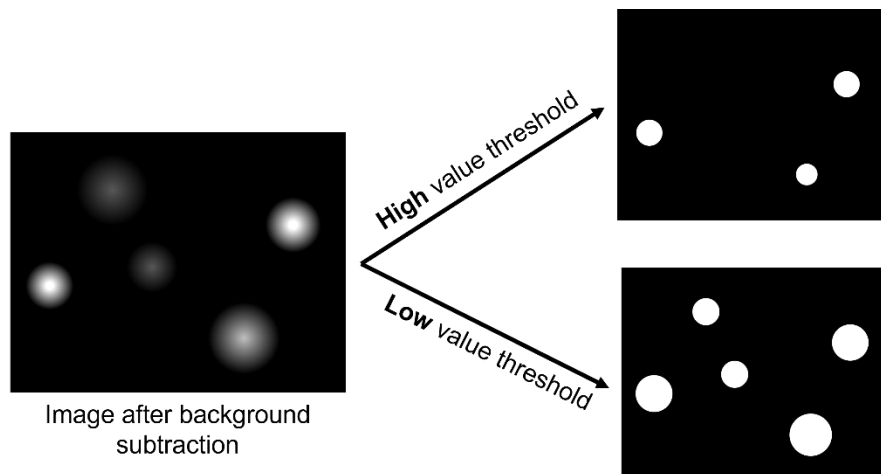
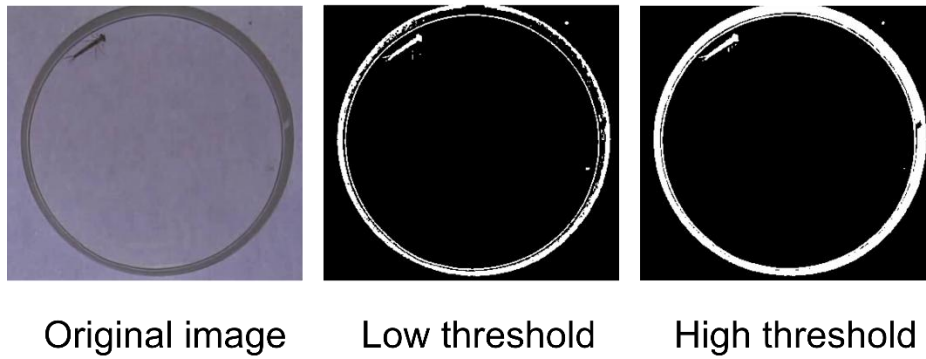


Figure 9: Illustration of the process of thresholding.

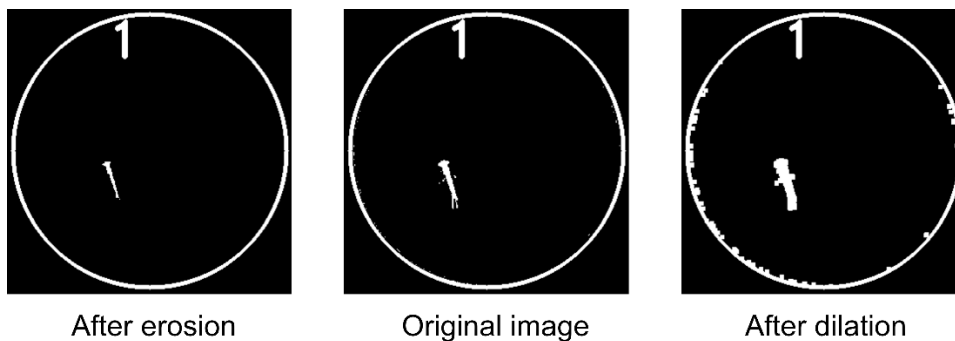
If there was no background defined (adaptive thresholding) for the selected video, this process will be replaced by an adaptive thresholding: the program will identify spots that are darker than their close environment. The first parameter can be changed to change the range of considered environment (Figure 10). The second parameter corresponds to the detection sensibility.



*Figure 10: Illustration of the process of adaptive thresholding.*

### *Erosion/Dilation*

This allows to increase/decrease the size of the white areas resulting from the threshold process. While erode will remove white pixels from the borders of the white shapes, dilate will add some. These parameters allow to remove potential noise from the background and improve the visibility of the targets (see Figure 11).



*Figure 11: Illustration of the processes of erosion and dilation.*

### *Area selection*

This parameter allows to determine the minimum and maximum area of your targets. All white shapes that are smaller/bigger than the indicated value will be removed from the image (Figure 12). This way you will limit the risk that another element is considered as a target.

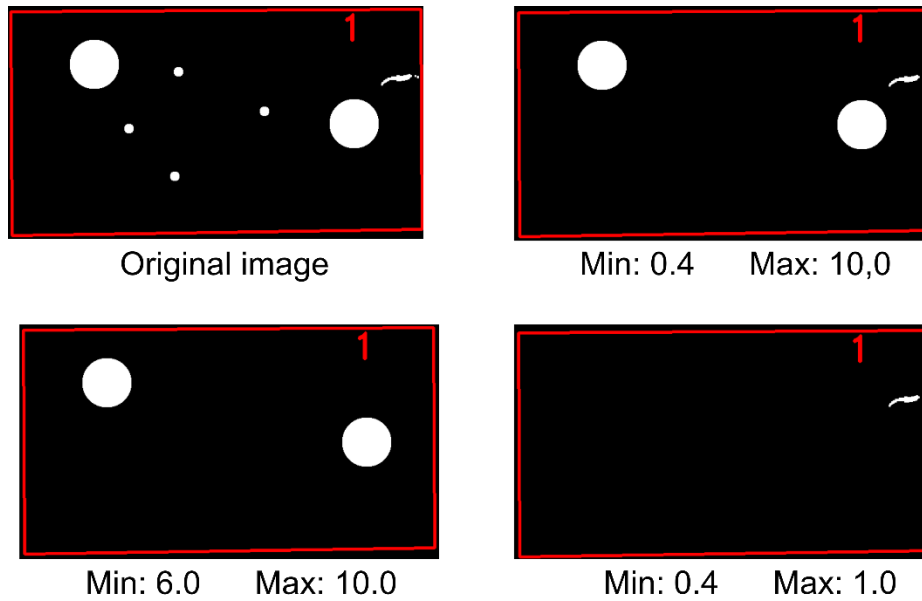


Figure 12: Illustration of the process of area selection. Under each image is the value set for minimum and maximum areas.

#### Distance threshold

This parameter may be used to indicate the maximum distance a target can move between two frames. During the tracking process, the distance between the target at a time  $t$  and the potentials targets at  $t+1$  is calculated, and all the potential targets whose distance from the target position at time  $t$  is higher than the threshold distance will be excluded. The best value for this parameter is not easy to find as it will depend on a lot of parameters like the maximum moving speed of the targets or the frame rate of the video.

To facilitate this process, after clicking the “Distance threshold” checkbox, you will see in the video reader light blue points: they indicate where the potential targets are located. You will also see dark blue disks whose center is the position of the potential targets at the previous image and whose radius is your distance threshold. Assuming that a target was correctly located at time  $t-1$ , when at  $t$  the target light point is inside the corresponding dark circle, it means that AnimalTA will consider this light point as a potential position of the target for time  $t$  (Figure 13). We advise to look for moments of the video where the targets are moving fast and to ensure the distance threshold is not too low. It will always be better to use a too high than a too low distance threshold.

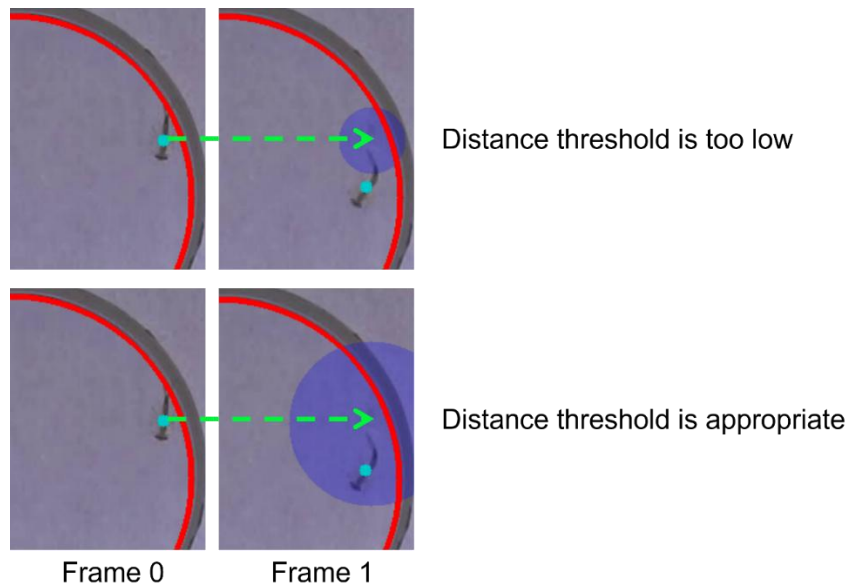


Figure 13: Illustration of the distance threshold.

#### *Number of individuals per arena*

AnimalTA proposes to track two kinds of videos. The first kind are videos with fixed number of targets. In these videos, the number of targets is known from the beginning and won't change through the video. It means that the target cannot leave, enter or hide inside their arena.

The second kind of video are videos for which the number of targets is unknown and for which targets can enter and leave the arenas. It is for instance the case when recording the flow of individuals crossing a bridge or the entrance of a nest.

**Fixed number of targets.** According to your experimental design, you might have more than one target per arena, in that case you must indicate how much targets are to be found in each area. If this number is the same for all the arenas, you can just change the slider to adapt it to your needs. On the image, each arena is drawn in red, and the number of targets expected to be found appear as a red number close to the arena border.

If the number of targets per arena is variable, press the “P” button at the right of the “Number of individuals per arena” section to manually change the number of individuals for each arena.

Note that AnimalTA does not allow to track the identity of the different targets within a same area. For such kind of analyses, please see: <https://www.idtracker.es/> from Pérez-Escudero et al., 2014.

A. Pérez-Escudero, J. Vicente-Page, R.C. Hinz, S. Arganda, G.G. de Polavieja, *Nature Methods* 11(7):743-748 (2014)

**Variable number of targets.** To indicate to AnimalTA that the number of targets is not fixed, you must select the “Variable number of targets” option. Then click the “Redraw entrance area” button. You will then have to identify which part of the video corresponds to the entrance/leaving area (i.e. where do you expect the targets to appear/disappear). You can either directly paint on the video (tool size can be adjusted with the mouse wheel), or select the border of interest and increase the “width” parameter. Note that an entrance area must be touching an arena and cannot be touching two different arenas at the same time. See Figure 14 for illustration.



*Figure 14: Illustrations of the entrance areas (here in yellow). In the left image ants are walking up and down a tree, we will track their displacements inside the red rectangle, yellow rectangles are used as entrance area (i.e. the program will use this space to identify incoming individuals but won't keep their trajectories). In the right image, cockroaches can leave the shelter by going through the yellow area.*

## IX. Tracking process

### 1. Automatic tracking

Once that at least one of the videos is ready for the tracking, you will be able to click the “Begin tracking” button and to select the videos you want to track. According to the number of videos, their length, their quality, and the options you selected, this process duration can be highly variable.

*Tip:*

If you experience a slowdown in your computer's performance while AnimalTA is running the tracking, you can set AnimalTA to low priority in the main options (see section [Generalities and project management, Settings](#)). Tracking will take longer, but more system resources will remain available for other tasks.

*Head/tail separation option.* If you are interested in getting data of both the head and the tail of a target and that this target is alone in an arena, you can use the “Separate head from tail” option before running the tracking.

## 2. Manual tracking

In some cases, you might be interested in performing a manual tracking instead of relying on the tracking algorithm of AnimalTA. To that aim, you can prepare your videos as explained in the [Main project table](#) section. There is no need to go through the [preparation of the tracking](#), even if you can use this step to indicate the [number of individuals per arenas](#) (but you will always have the possibility to add as much targets as you want later). Once you finished with these first steps, you can press the “Begin tracking” button. You will then have the possibility to select the videos you are interested in tracking manually, just ensure that the “Manual tracking” box is checked. This will prepare your videos for manual tracking.

Once you are back in the [Main project table](#), you can select the video you want to track manually and press the “View and correct track” button. Read the section [Visualization and correction of the tracking](#) (especially the section [Punctual errors](#)) to get more information about how to proceed with the manual tracking.

## X. Visualization and correction of the trackings

### 1. Visualization

You can check the accuracy of the trackings using the “View and correct track” button in the bottom right part of the main window. A new window will then appear with the video reader on the left on which you can see the results of the tracking. The coordinates of the targets are marked by a colored point and the tails behind these points represent the previous positions of the targets. At the same time, you can see the coordinates on a table at the right of the window (Figure 15). The length of the tail may be increased using a slider which represents the tail length in seconds (Figure 15, A). You can also make appear all the trajectories recorded during the video by pressing the “see all trajectories” button (Figure 15, E).

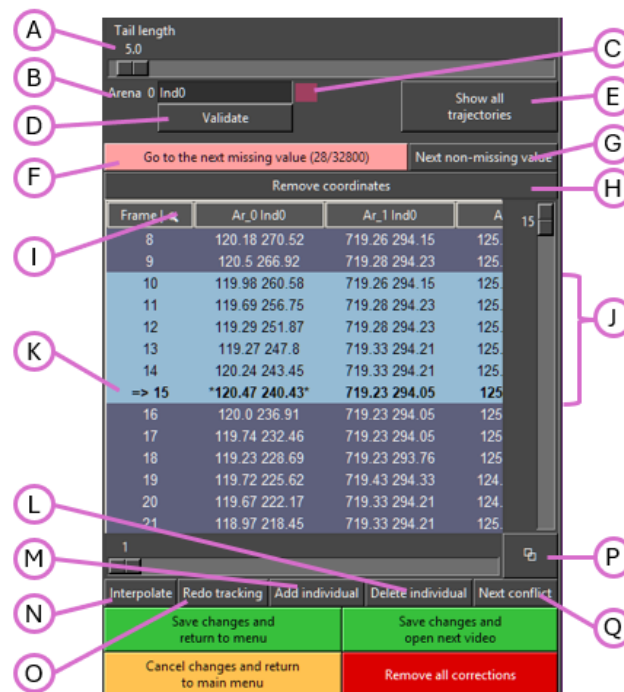



Figure 15: Correction tracking panel. A) length of the tail displayed, B) Name of the target, C) Color of the target, D) Validate the name of the target, E) Draw all trajectories on the frame, F) Move the video up to the next missing value, G) Move high-speed up to the next non-missing value, H) Replace coordinates by NA values, I) Search for a specific column/target, J) Selected

frames, K) Current frame, L) Remove selected individual, M) Add a new individual, N) Interpolate the coordinates for the selected frames and individual, O) Redo the tracking for the selected frames, P) Open the coordinates table as a separate window, Q) Move the video up to the next unexpected high speed value.

In the table at the right of the window, you can see the coordinates corresponding to what is displayed in the video reader. The frame displayed is indicated by “=>” preceding its number (Figure 15, K). Each column of the table corresponds to one target; each cell contains the X and Y coordinates of these targets. In addition to using the features of the video reader (see section [Video Reader](#)), you can set the video reader to a particular frame by clicking on a row from the table.

*Tips:*

- After Zooming in the video, you can lock the view on the selected target using the “L” key on the keyboard to facilitate manual correction.
- The Coordinates table can be dissociated from the main window using the “” button (Figure 15, P)
- You can search for a specific column/target in the table by clicking on the magnifier symbol in the top left cell of the table (Figure 15, I)

## 2. Set the identities of the targets

By default, the targets are identified with an unrepeated within area label, going from “Ind0” to “IndN”, N+1 being the number of targets expected inside the arena (see section [Tracking preparation, Number of individuals per arena](#)). When you select an individual (by pressing on it in the image or by pressing on its column in the trajectories table), its identity will be displayed in the text field on the top of the trajectories table (Figure 15, B). You can change the label of the selected individual by changing the text in the corresponding text field and then press the validate button (Figure 15, D). The new label will later be used when performing data analyses. The color used to display the position and trajectory of the selected target can also be modified by clicking the colored rectangle at the right of the text field (Figure 15, C). A color chart will then appear, just click on the color you want to use, and this new color will be associated with the target.

## 3. Corrections

There are three different ways of correcting tracking errors that must be used according to the duration of the according error.

### *Find missing values*

If the targets are lost during a part of the tracking, their coordinates will appear as “NA” values. To correct rapidly the video, you can press the “Go to the next missing value” button (Figure 15, F), which will transport you directly to the next problematic frame. If your dataset has no missing value, this button will appear green and cannot be clicked.

*Tip:*

- Pressing “N” on the keyboard will also transport you to the next missing frame.

In the opposite, if you want to reach the next non-missing value, you can use the “Next non-missing value” button (Figure 15, G).

#### *Identify abnormal speed*

The “Next conflict” button (Figure 15, Q) we move the video timeline to the moments during which the targets’ speed is above the expected range.

#### *Punctual error*

To correct a punctual error which happened during only one frame, you can directly change the position of the points on the video reader by clicking and dragging it, the table will be updated accordingly. To avoid the dragging part, you can also directly click on where you want the point to be while holding the “Alt” key on the keyboard.

If there are no points at all (i.e. NA value), you can directly click right on the frame where you want to add the point, and the coordinates of the target will be added to the table. To facilitate these manual corrections, after each right click the video will be moved forward by one frame, allowing only right clicks to make frame by frame corrections.

#### *Interpolation*

**A single target, a given group of frames.** If the target has been lost during a short duration and that its trajectory during this period can be resumed by a straight line, you can interpolate the coordinates between two frames (see Figure 16). To do so, you must first select the concerned target. You can either click on it on the video reader or click on one corresponding cell of the table. The selected individual may be identified on the video as the point will be highlighted, on the table, the corresponding coordinates will appear between “ \* ”. You must then select the frames on which you want to apply the interpolation (Figure 15, N). To that aim, you can either press <Shift> and click on another frame of the table or play the video maintaining the <Shift> key pressed. Once you made this selection, press the “Interpolate” button to apply the changes to the trajectories.

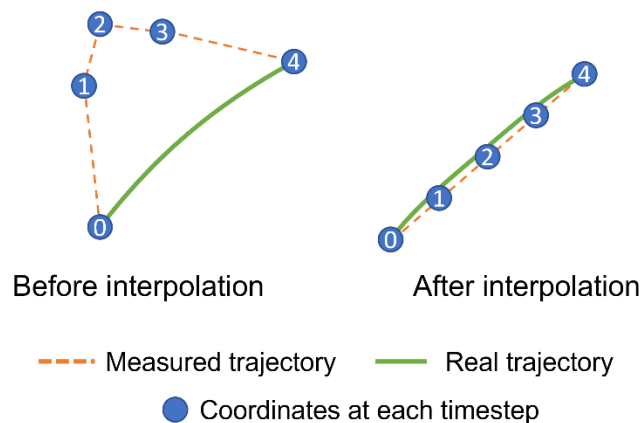


Figure 16: Illustration of the process of interpolation. In this case, an interpolation is realized between frame 0 and 4.

**Grouped interpolation.** If you want to fill all the missing values of: i) one target, ii) all the targets, or iii) all the targets for all the videos, you can press the “Interpolate” button when less than two frames are selected in the data frame (see Fig 15, J; section *Interpolation*). This will open a window, asking you which of these three options you want to apply. Note that in case of variable

number of targets, only the missing values between the first and last appearance of the target will be filled.

**Other.** More options are available in the upper menu “Interpolation”. Interpolation can be done for selected frames but more than one target, or for all the frames and all the targets of a same arena.

#### *Redo part of the tracking*

If you need to correct some bigger issues, you can simply redo a part of the tracking with different parameters. You can redo the tracking of either all the targets from all arenas of the video or only the targets from the selected arena. To do so, select the frames you want to change (see section *Interpolation*) and press the “Redo tracking button” (Figure 15, O). This will open a new window in which you will be able to choose the pre-tracking parameters for this portion of video (see sections [Main project table](#) and [Tracking preparations](#)). After changing the parameters, press the “Redo the track” button to see the result. Once you are satisfied with the result, press the “Validate these changes” button to apply them within the rest of the video.

#### *Invert coordinates*

If the identities of two targets have been inverted, you can swap it back by selecting one of the two targets and then click on the second one (either on the image or in the table) while holding the “Shift” key. The coordinates of these two targets will be inverted from the current frame until the end of the video.

#### *Delete existing values*

If you want to replace some existing coordinates by NA values, you can select them (Figure 15, J; section *Interpolation*) and press the “Remove coordinates” button (Figure 15, H). If you want to remove coordinates for selected frames for more than one individual at the time, you can use the “Delete coordinates” option in the upper menu.

It is also possible to remove all the coordinates data present in a specific spatial part of the video using the “E” key of the keyboard and then selecting a rectangle while maintaining the “Ctrl” key pressed. To leave this data removal mode, press “E” again. Also available in the upper menu (“Delete coordinates” > “Spatial selection”).

#### *Add/Remove an individual*

In case of variable number of targets, AnimalTA might have missed an individual or in the contrary might have added a non-existing individual. The “Add individual” and “Remove individual” buttons (Figure 15, M-L) are here to solve those problems. In case of adding individuals, note that the new individual will be added to the same arena as the currently selected individual. By default, removed individual will be the one selected. If you want to remove more than one individual at a time, use the upper menu “Delete individuals”.

#### *Fusion trajectories*

If two targets were treated as different individuals but are in reality a single one, you can fusion the coordinates to form a single target out of the two. To do so, select the first one and then hold “ctrl” + “shift” on your keyboard while selecting the other one (by clicking directly on the image or on the table column). Be careful as the fusion process may result in data loss (see Figure 17).

Frame	Ind1	Ind2		Frame	Ind1
100	105, 55	NA, NA		100	105, 55
101	104, 56	NA, NA		101	104, 56
102	103, 57	NA, NA		102	103, 57
103	102, 58	110, 57		103	102, 58
104	102, 59	110, 58	----->	104	102, 59
105	NA, NA	110, 59	----->	105	110, 59
106	NA, NA	110, 60	----->	106	110, 60
107	NA, NA	110, 61		107	110, 61
108	NA, NA	110, 62		108	110, 62
109	NA, NA	110, 63		109	110, 63
110	NA, NA	110, 64		110	110, 64

Figure 17: In that case, after fusion, the coordinates of individual 2 highlighted in red will be lost.

### Validate corrections

Once you pressed one of the two validate button, a new coordinates file will be created inside the [project folder](#) (see section [Generalities and project management](#)) under the "corrected coordinates" subdirectory. You can also remove all the corrections you did by pressing the "Remove all corrections" button.

Shortcuts:

Keys	Actions
Ctrl + Z	Cancel last action
Shift + Ctrl + select individual	Fusion trajectories of two individuals
Shift + select individual	Swap identities of two individuals
Ctrl + C	Copy coordinates
Ctrl + V	Paste coordinates
A	Show arenas position and identity
Tabulation	Select next individual
Return	Validate the name of the target / focus the Entry
Alt + C	Copy targets' names and color to other video(s)
Right click on table header	Hide column
Ctrl + W	Reset column size
Ctrl + F	Search a specific column
Spacebar	Play/Stop video
Left/Right arrows	Move 1 frame forward/backward
Ctrl + Left click	Zoom-in
Ctrl + Right click	Zoom-out
Ctrl + drag and drop	Zoom in a particular arena

## XI. Prepare analyses

From the main Project window, you can press the "Prepare analyses" button in the bottom right of the window after selecting a video.

### Warning:

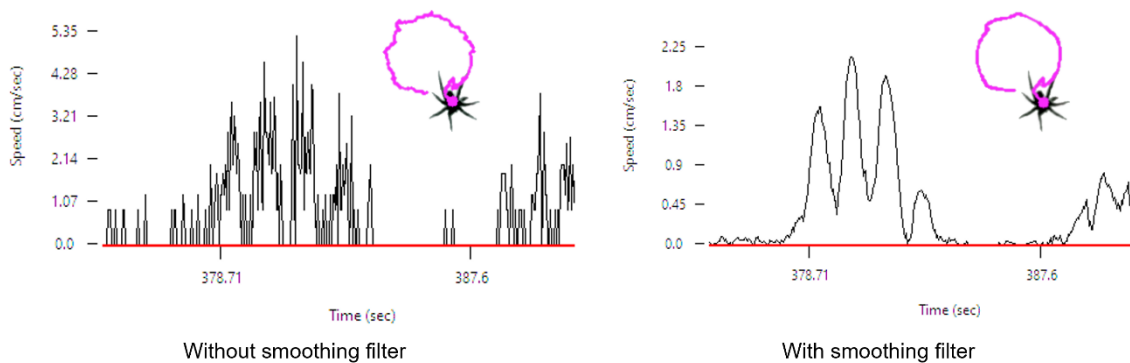
The results displayed in the "Prepare analyses" module of AnimalTA may be an estimation of the real results based on a subsample of the data. Using an estimation instead of the real data ensure a fast functioning of the program and limit the risk of bugs. Data which are estimated are displayed with a "△" symbol. See section [Run analysis](#) to get the real data analysis.

## 1. Smoothing filter

You can smooth the trajectories of your targets by checking the “Smooth trajectories” checkbox. This parameter applies a Savitzky-Golay filter (function `savgol_filter` from the `scipy` python package) on the coordinates. The application of this filter allows to facilitate the identification of the movement phase and to correct the noises associated with the imprecision of the tracking (Figure 18, see also section [Main project table, Frame rate](#)). The parameters of the smoothing filter can be controlled by pressing the “P” button at the right of the checkbox. To compare the between video’s information, you might want to apply the same smoothing filter to all of them at the same time. To do so, you can use the “Apply smoothing to other video(s)” button to apply the smoothing parameters defined for this video to other ones. If no smoothing was applied at this moment, the smoothing filter will be removed in the other designated videos.

For more information, see:

[https://docs.scipy.org/doc/scipy/reference/generated/scipy.signal.savgol\\_filter.html](https://docs.scipy.org/doc/scipy/reference/generated/scipy.signal.savgol_filter.html)



*Figure 18: Illustration of two trajectories before and after the application of the smoothing filter. The graphs show the measured speed of the individual as a function of time. We can see that the periods of movement are easier to identify after the application of the filter.*

## 2. Perspective correction

This option allows to correct for camera deformation and/or the effect of perspective. If you press this button, a new window will appear. In this window, you will see a frame of reference (Figure 19, A), a schematic representation of your environment (Figure 19, B), and the result of the deformation (Figure 19, C). You must first click on the reference frame (A) and define at least 4 points of reference. These points will appear in the schematic representation. Move the points by click and drop inside the schematic representation so that all distances and angles between points are correct. You can delete an existing point by pressing the <Delete> key of your keyboard after selecting it. After validating, you can apply the same movement threshold to all videos using the “Apply the exploration parameters to other video(s)” button. Note that the transformations of the images and trajectories are done after the tracking process, so they won’t impact the tracking quality.

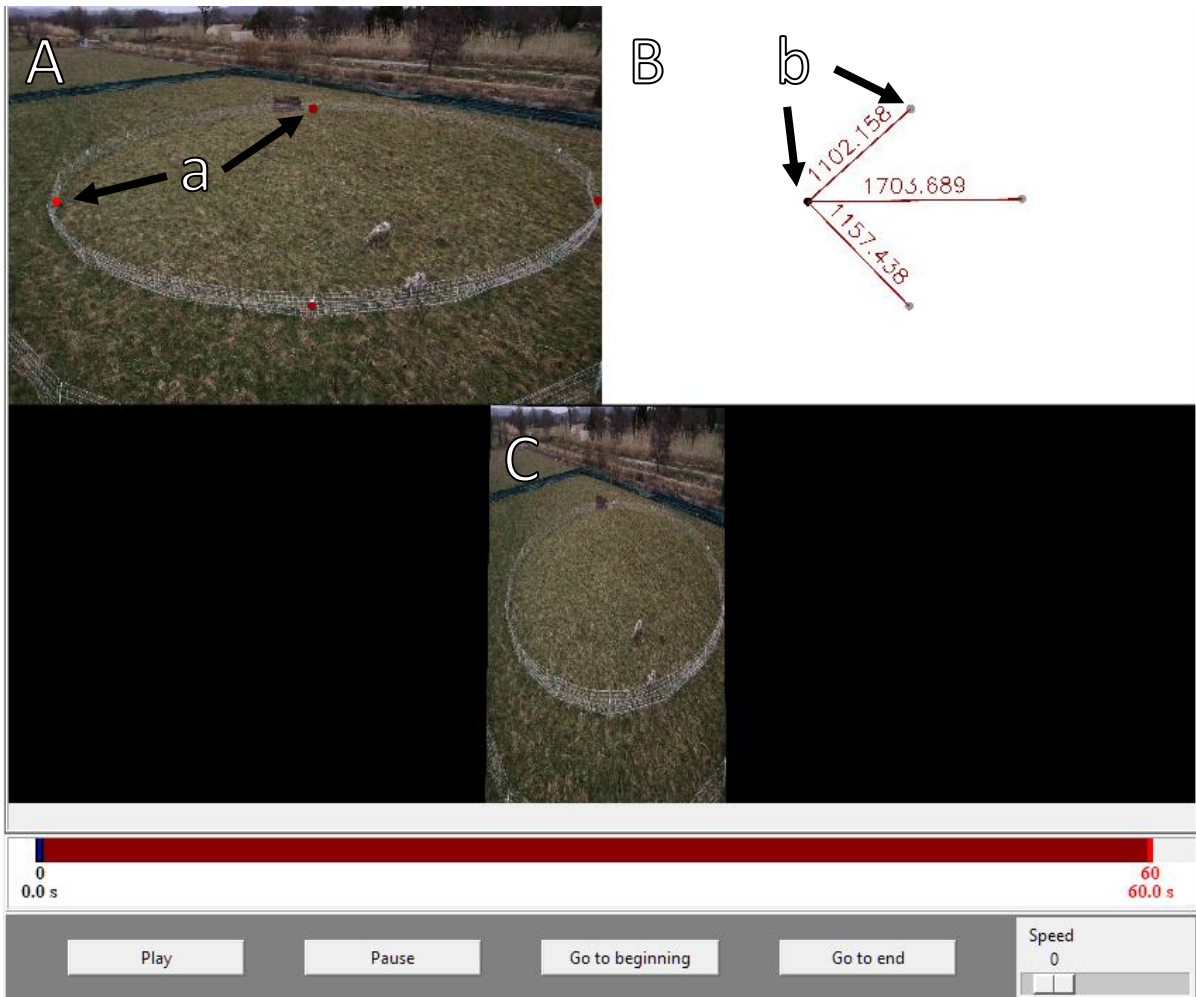


Figure 19: Panel used to correct the effect of perspective. We can see in the reference frame (A) the points of reference (a). A schematic representation of the scene (B) in which the points are also drawn (b). Finally, the result can be seen in the last image (C).

### 3. Analyses of individual trajectories

If you click the corresponding checkbox, you will see on the video reader the movement speed of each target (except for the first frame). The speed value will appear in green/red if the target is considered as moving/stopped respectively. By default, a target will be considered as moving if the distance traveled between two frames is higher than 0. However, this method does not consider the tracking imprecisions which normally results in little trembling of the target.

To change the threshold of movement above which you want to consider that a target is moving, you can press the "P" button. This will open a new window in which you can see a graph of the speed of the selected target as a function of the time. You can press the drop menu on the top of the window to change the selected target, the selected target will also be highlighted on the video reader. A blue vertical line on the graph indicates the currently displayed frame in the video reader, you can move this line by simply clicking somewhere on the graph; the video reader will be immediately updated. A red horizontal line indicates the movement threshold: the speed above which an individual will be considered as moving. You can change this value by click and drag the red line.

Finally, in the bottom right corner of the window, you will have a summary of the measured data for the selected target:

- Proportion of time lost: Count the proportion of frames for which the target was not detected (appears as “NA NA” in the data tables).
- Average speed: The displacement speed between each frame will be calculated and averaged for the whole video.
- Average speed when moving: The displacement speed between each frame where the target is considered as moving (i.e. speed above the red movement threshold) will be calculated and averaged for the whole video.
- Proportion of time moving: Proportion of time the target is considered as moving.
- Total distance traveled: Sum the total distance traveled by the target.
- Total distance traveled when moving: Sum the total distance traveled by the target when we only consider the frame during which the target is considered as moving.

After validating, you can apply the same movement threshold to all videos using the “Apply the exploration parameters to other video(s)” button.

#### **4. Spatial repartition**

This section of AnimalTA allows to define some points or areas of interest. Click the corresponding checkbox to see the elements that are already defined (non by default). Click the “P” button to add some new elements. This will open a new window in which you can see the current frame of the video with the selected area highlighted. Use the drop menu in the top of the window to change the selected area. You can then press the “Add element” button (top left of the window) to add new elements.

##### *Elements manipulation*

The elements placed on the image can be easily moved by click and drag the associated points (except for borders, see below). When created, each element will appear in the right part of the window where you can see its name (by default the kind of element and an identity number) and the measurements associated with this element. You can rename an element by simply writing in its name text field. Note that you cannot have two elements with the same name. You can also suppress an element by pressing the “Suppr” button at the right of its name.

##### *Types of elements*

- Point

A point can be either freely placed (just click on the frame where you want to place it) or placed in the center of the area.

A point is associated with three different measurements: i) the average distance to this point, ii) the latency to approach at less than a given distance to this point (to be defined by the user by simply writing in the corresponding text field), iii) the proportion of time the target spent inside this at less than this distance from the point.

From version 2.3.1, if the user defined a distance threshold to the point, new variables will be calculated referring to the movement of the target inside the corresponding circle (see [Results per individual](#) for more details).

- Number of entries (number of times the target entered the circle. Note that if the target was already inside the circle at the beginning of the video, it will be considered that the target already did a first entry).
- Proportion of time lost
- Proportion of time moving
- Traveled distance
- Traveled distance while moving
- Averaged speed
- Averaged speed while moving
- Meander
- Meander while moving

Note that the program will only consider displacements that are beginning and ending inside this circle. See Figure 20 for illustration.

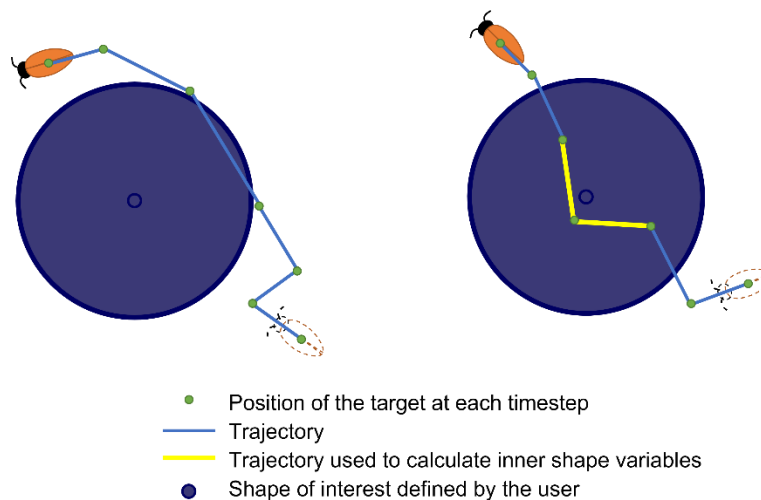


Figure 20: Illustration of how AnimalTA will calculate the inner-shape variables.

- Segment

A segment is defined by two points. These two points can be placed freely (click anywhere on the frame) or be associated with a border. If you choose the second option, you must click on the border/corner of the area where you want to place the first point of your segment. If you select a corner, the point will be immediately placed on the frame. If you choose a border, a small window will appear that will ask you what position you want this point to take on the border. Then repeat the same operation for the second point.

Three measurements are associated with a segment. i) The mean distance to the segment which is the average value measured for all frames as the shortest distance between the target and any point of the segment. ii) The number of times the target crossed the segment. iii) The latency before crossing the segment for the first time.

- Borders (All)

This will immediately select all the borders of the area (i.e. the whole contour of the area) and add it as a new element.

Two measurements are associated with this. i) The mean distance to the border is the averaged shortest distance between the target and the border. ii) The proportion of time the target spent

at less than a given distance from the border (to be defined by the user by simply writing in the correspondent text field).

- Borders (selection)

You can also select specific borders. In this case, you must indicate on the frame which border(s) you want to consider. To do so, click on the border(s) you want to consider (their color will change from purple to red if they are selected). Once you selected all the borders you want, press the “Return” key to validate and the borders will appear in the list of elements.

Three measurements are associated with this. i) The mean distance to the border is the averaged shortest distance between the target and the selected border(s). ii) The proportion of time the target spent at less than a given distance from the border (to be defined by the user by simply writing in the correspondent text field). iii) The latency to approach the border(s) at less than the indicated distance.

- Area

To add an elliptical, circular or polygonal area, you must click on the frame where you want the corners of your area to be placed. Once all the corners are placed, press the “Return” key to validate the shape, which will appear in the list of elements.

Two measurements are associated with shapes: i) the proportion of time the target spent inside this area and ii) the latency to enter this area.

#### *Expend elements to other arenas*

Once you at least one element defined, you can duplicate this element and apply it in other arenas. To that aim, click the “Expend to other arena(s)” button. This will open a new window with two lists on it. The left list is the list of elements present in your arena; from this list you can select the elements you want to duplicate in other arenas. The second list is the list of arenas (all videos confounded). If you want to visualize an arena, just put your cursor over its name in the list, an image of the video with the arena drawn in blue will appear. Select in this list the arenas toward which you want to duplicate the elements. Simply click the “Validate” button to apply these duplications. It is important to note that except for the “All borders” elements the other elements will be duplicated only if the arena of origin and of reception are of the same shape (same number of corners, square/circle, ...). If the shapes are too different or do not share the same number of corners, the duplication of elements will not be applied. Moreover, two elements in the same arena cannot have the same name. Also, if you try to add a new element in an arena which already possessed an element with the same name, the old element will be replaced by the new one.

### **5. Interindividual distance**

The interindividual distance is simply measured as the sum of all distances between all the targets inside an arena. By clicking this checkbox, you will see on the video reader the calculated distances for the current frame.

You can also define a threshold distance by using the “P” button at the right of the checkbox. This will open a new window in which you can set a threshold either by moving a slider or by directly writing in a text field. If the distance between two targets at a given timestep is lower

than this threshold, these targets will be considered as “neighbors” when you will be running the analyses (see section [Run analyses, Results of interindividual distance](#)).

## 6. Exploration

This option allows to calculate the proportion of the arena explored by each target. To change the parameters associated with the measurement of exploration, you can choose between them clicking the “P” button at the right of the checkbox. This will open a new window in which you can select a particular individual (you can change the current individual using the drop menu at the top of the window). AnimalTA proposes two different methods to measure the exploration. After defining the method of exploration, you can apply it to other videos using the “Apply the exploration parameters to other video(s)” button.

### *Individual centered*

This method allows a fine measurement of the explored area. With this method, we consider that a target explores a circular area around it at each frame. Each pixel that falls within this circular area at least once during the video is considered as visited while the others are considered as unvisited. The measure of exploration returned are either the total visited surface (absolute value), or the proportion of pixels visited within the arena (relative value). You can change the surface of the circular area around the target using the slider or by directly writing in the corresponding text field.

### *Meshes*

This method is more traditional as it has been primarily invented to analyze videos by hand. It consists in drawing a mesh on the top of the arena and counting the number of visited cells of the mesh. The program will then report either the proportion of cells visited (relative value) or the proportion of cells visited (absolute value). We propose here two types of mesh: a rectangular or a circular one. In both cases, the surface of each cell of the mesh can be determined by the user. Regarding the circular mesh, a supplementary parameter can be defined (aspect parameter) which determine the shape of the mesh cells. The method used to draw the circular mesh are based on the work of Beckers and Beckers: *Beckers, B., & Beckers, P. (2012). A general rule for disk and hemisphere partition into equal-area cells. Computational Geometry, 45(7), 275-283.*

For both methods, you can create an heatmap of the target’s presence using the “Do heatmap” buttons. Individual heatmap means that only the selected individual trajectories will be used to do the heatmap (selected individual can be changed from the drop-down menu). Combined heatmap will allow you to select numerous targets so that their trajectories are combined to create the heatmap. Note that this process will only be efficient if the arenas of the arenas in which the targets are similar (Same size and shape).

#### *Tip:*

It is possible to create a heatmap based on the segmented image in the “Export video” panel (see section [Export a video](#)).

## 7. Sequences

The sequences panel allows you to analyze separately different parts of the video. For instance, analyzing what happens in the first 5 minutes of the video in a first time and the rest of the video in a second time. In AnimalTA, these portions of video are called sequences. By default, all the individuals are assigned one sequence called "General" which corresponds to the entire video. In the Sequences panel, you can either change this first sequence and/or add new sequences with the "Add a new sequence" button. The sequences panel will allow you to create as many sequences as you want.

Each sequence is characterized by: i) its name in the first column of the sequences table, ii) the time it will begin in the second column of the sequences table, iii) the time it will finish in the third column of the sequences table.

The sequences duration can also be seen in the video reader above the sequences table. The timeline will be cropped accordingly to the options selected and the trajectories displayed will be the ones part of the selected sequence. Selected sequences are highlighted by a colored frame.


To change a sequence name, simply write in the Entry in the first column. There are several options to choose when the sequences will begin/end:


**Begin/End.** The sequence will begin or finish at the first or last frame of the video.


**Time.** Write down at which moment of the video you want the sequence to begin/end. This value must be in seconds, time 0 sec corresponds to the first frame of the video (after cropping).

**Exploration.** The sequence will begin/end the moment that a particular exploration value (which can be chosen using the scale in the corresponding column) has been reached by the target. How to set exploration parameters is described in section [Prepare analyses, Exploration](#).

**Spatial event.** The sequence will begin/end the moment that the target enters/leaves a particular element of interest or crosses a segment for the Xth time (defined in the last entry box). To see how to add elements of interest, see the section [Prepare analyses, Spatial repartition](#). Note that you can also apply a delay before/after the given interaction.

Sequences with potential problems are indicated with the symbol: . If this symbol appears in the first column (under the sequence name), it means that the sequence does not contain any frame. It might be the case for instance if the selected parameters imply that the end of the sequence happens before its beginning.

If the symbol  appears in the second column of the table, it means that the asked beginning of the sequence is before the beginning of the video. In that case the sequence will begin at 0 sec.

Finally, if the symbol  appears in the third column of the table, it means that the asked end of the sequence is never reached during the video. In that case the sequence's end will be the end of the video.

Note that the sequences are attached to a single individual (you can change the individual of interest in using the drop-down menu in the top of the panel). If you want to apply these sequences to several individuals, you can use the “Copy for other individuals” button.

*Tip:*

If you change the exploration parameters or modify the characteristics of the elements of interest the sequences will be immediately updated, you do not need to come back to the Sequence menu. If an element of interest has been deleted after a sequence linked to that element was created, the sequence will be ignored.

*Auto sequence range option*

If you wish to divide your video into repetitive sequences (for example, each time an event occurs or at fixed time intervals), you can enable the “Auto sequence range” option. Three types of sequences can be created:

**Time.** This option divides the video into smaller chunks defined by From and To (the start and end of the overall range) and Steps, which represent the duration of each segment.

**Exploration.** This option divides the video timeline into chunks based on the proportion of the area explored (see section [Prepare analyses, Exploration](#)).

**Element of interest.** This option divides the video into chunks based on the animal’s interaction with an element of interest, grouping frames from Entrance to Exit or from Exit to Entrance.

## 8. More analysis

The “more analysis” panel proposes to prepare less commonly used analysis and to determine which part of the data should be saved in the [Run analysis, Detailed data](#) file.

*Save detailed data regarding segments crosses*

If this option is selected, a file will be created while running the analysis that will summarize all the event of the targets crossing the segments that were defined in the [Spatial repartition](#) section. When Running the analysis (see section [Run analysis](#)), AnimalTA will create a new file: Results > Spatial > Summary\_crosses.csv which will summarized which targets crossed which segment and when.

*Durations of movements and stops*

The program will identify the periods of movements and stops of the selected target. To do so, the program will first assign for each frame of the video whether the target is moving or not, based on the moving threshold set by the user (see section [Prepare analyses, Analyses of individual trajectories](#)). It will also remove potential accidental changes in the moving/stop status by removing any group of frame showing the same status with a duration lower to the one defined by the user in the text field “Ignoring breaks of duration lower then:” (see Figure 21). Finally, it will identify each period of continuous movement or stop and calculate the duration of these events. If the option(s) “Mean\_dur\_moves / Mean\_dur\_stops” is (are) selected, the program will summarize for each individual and sequence the average durations

of all the movements / stops periods of a considered individual, and add it to the final “Results\_by\_Ind.csv” file (see section [Run analyses, Individual results](#)). If the option(s) “Events\_dur\_moves / Events\_dur\_stops” is selected, all the events will be summarized in a new result(s) file(s) (respectively Results>Moves.csv and Results>Stops.csv).

Table illustrating how the Movements/Stops periods are identified. In that case, the user chooses to ignore breaks duration of less than three frames (0.15s) and the moving threshold is set to 80mm/sec.

Frame	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Time (sec)	0.5	0.55	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95	1	1.05	1.1	1.15	1.2	1.25	1.3
Speed (mm/sec)	2	3	118	20	44	57	115	125	95	49	66	90	85	20	4	6	10
Moving	0	0	1	0	0	0	1	1	1	0	0	1	1	1	0	0	0
After correction	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0
Event period	Stop (6 frames, 0.3 sec)						Movement (8 frames, 0.4 sec)						Stop (3 frames, 0.15 sec)				

### Detailed data columns

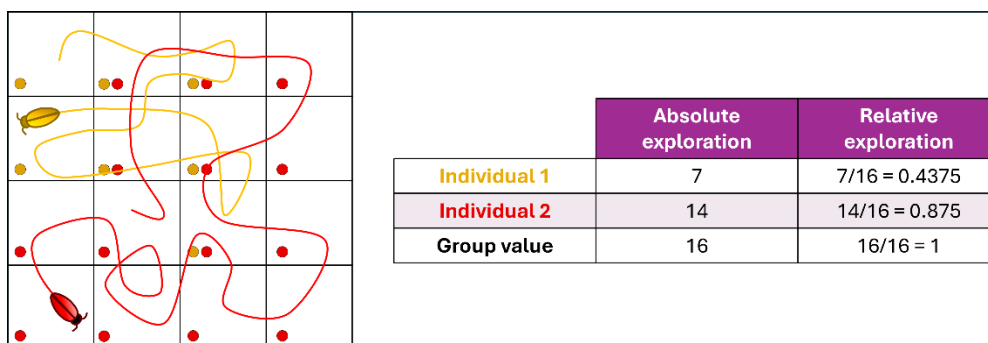
With this option, it is possible to select which columns you want to appear in the Detailed data files (see section [Run Analysis, Detailed data](#)).

### Group level analysis

Two options can be selected here to define the variables included in the data frame saved in My\_project\_folder > Results > Results\_InterInd.csv.

The “Interind\_data” option saves three columns corresponding to the mean, minimum, and maximum inter-individual distances measured throughout the video. For each frame, the inter-individual distance is calculated as the sum of all pairwise distances between individuals detected within the same arena.

The “Exploration” option adds two additional columns to the same file: the absolute and relative group-level exploration of the arena (see section [Prepare analysis, Exploration](#)). In contrast to the individual exploration values, these metrics are computed by combining the trajectories of all individuals present in the same arena.



**Figure 21.** Illustration of the calculation of individual and group exploration values. Absolute exploration corresponds to the number of arena zones visited, whereas relative exploration is obtained by dividing this value by the total number of zones available in the arena. Group exploration values are calculated by combining the trajectories of all individuals within the arena.

## XII. Run analyses

After you prepared the analyses, you can run them using the “Run analyses” button in the main project window. The results will be summarized as .csv files in the [project folder](#), in the “Results” subfolder. In this subfolder, you will find two .csv files.

### *Results per individual*

The file “Results\_by\_ind” is constituted of 16 columns and of one row per tracked target and [sequence](#). In the following table, the “unit” is the [unit defined by user](#).

Column name	Content	Units	Supplementary information
Video	The file name of the video	NA	NA
Arena	The ID number of the arena in which the target was (the arenas are numerated from left to right and from top to bottom).	NA	<a href="#">Section Main project table, Definition of the arenas</a>
Individual	The ID of the target, if not modified by the user: from Ind0 to IndN, unique for each arena. The user can also personalize the target’s ID (see section <a href="#">Visualization and correction of trackings, set the identities of the targets</a> ).	NA	If two targets have been confounded during the tracking process, you can restore the target’s identity following the steps described in section <a href="#">Visualization and correction of trackings, Corrections, Invert coordinates</a>
Sequence	From which sequence are those results. See the <a href="#">Prepare analyses, Sequences</a> section to change/create new sequences.	NA	If you did not create any sequence, a “General” sequence is automatically created that corresponds to the whole video.
Prop_time_lost	Calculate the proportion of frames for which the target’s coordinates were unknown.	Ratio (from 0 to 1)	In case of tracking errors or lost targets, you can correct the trajectories (section <a href="#">Visualization and correction of trackings, Corrections</a> ).
Smoothing_filter_window	The window parameter set for the smoothing filter. If the value is 0, it means that no smoothing filter was applied.	Frames	See section <a href="#">Prepare analyses, Smoothing filter</a> .

Smoothing_Polyorder	The polyorder parameter set for the smoothing filter. If the value is 0, it means that no smoothing filter was applied.	NA	
Moving_threshold	The speed threshold set by the user above which an individual is considered in movement.	unit/sec	See section <a href="#">Prepare analyses, Analyses of individual trajectories.</a>
Prop_time_moving	Proportion of frames the target is moving faster than the moving threshold.	Ratio (from 0 to 1)	
Average_Speed	The averaged displacement speed of the target between each frame.	unit/sec	
Average_Speed_Moving	Similar to "Average_Speed" value but considering only the frames where the target was moving faster than the moving threshold.	unit/sec	
Traveled_Dist	Total distance traveled by the target.	unit	
Meander	Estimation of the meander of the trajectories. This value is the average of the change in direction (turning angle) divided by the distance traveled for each frame.	degrees/unit	
Traveled_Dist_Moving	Similar to "Traveled_Dist" value but considering only the frames where the target was moving faster than the moving threshold.	unit	
Meander_moving	Similar to "Meander" value but considering only the frames where the target was moving faster than the moving threshold.	degrees/unit	
Exploration_absolute_value	The absolute exploration score of the target. The nature of this value will change according to the method of exploration measurement choose by the user. It can be expressed in units <sup>2</sup> , or in number of cells visited.	unit <sup>2</sup>  OR number of cells	See section <a href="#">Prepare analyses, Exploration.</a>
Exploration_relative_value	The proportion of the arena explored. Ranging between 0 and 1. The way this value is calculated will change according to the method of exploration measurement choose by the user. It can be either the	Ratio (from 0 to 1)	

	proportion of pixels visited or the proportion of cells visited.		
Exploration_method	The method of exploration measurement set by the user.	NA	
Exploration_area	The area used as an exploration unit. If the method used is squared or circular mesh, this value is the surface of each cell of the mesh. If the method is the individual centered one, this value indicates the surface a target cover around it at each moment.	unit <sup>2</sup>	
Exploration_aspect_param	The aspect parameter used in case of circular mesh method. This value will be NA if another method was used.	NA	See section <a href="#">Prepare analyses, Exploration, Meshes.</a>
Mean_nb_neighbours	Calculate the number of neighbors the target has for every frame. These values are then averaged for the whole video. The distance threshold to consider two targets as neighbors is defined by the user.	NA	
Prop_time_with_at_least one_neighbour	Calculate the proportion of frames for which the target has at least one neighbor.	Ratio (from 0 to 1)	
Mean_shortest_dist_neighbour	For each frame, the distance between the target and the closest neighbor is calculated. These values are then averaged for the whole video.	unit	See section <a href="#">Prepare analyses, Interindividual distance.</a>
Mean_sum_distances to_neighbours	For each frame, the distances between the target and all the other targets (within the same arena) are summed. These sums are then averaged for the whole video.	unit	

### *Results of interindividual distances*

This file is a summary of the inter-individual distances, it contains one row per arena which contained more than one target. The data found here are the mean inter-individual distance (see section [Prepare analyses, interindividual distance](#)). It also computed the smallest interindividual distance measured and the biggest one. Note that the identity of different targets within a same area may be confused when these targets cross each other's, in such situation, the user can manually correct such mistakes (see section [Visualization and correction of the trackings, Corrections, Invert coordinates](#)).

In the "InterInd" folder, you will find one folder per video which contains detailed results for each sequence and each arena (with at least two targets and sequences common to all the targets). For these results, a contact event is defined as continuous period during which two targets are neighbors (separated by a distance lower than the threshold defined by the user; see section [Prepare analyses, Interindividual distance](#)). The results are separated in four .csv files:

- Contact occurrences: a table of contingency recording the number of contact events between the different targets.
- Contact events: a table listing all contacts (see the above definition of contact). Each row of this table corresponds to one contact event and summarize which were the two targets involved (columns "Ind\_P1" and "Ind\_P2"), the duration of the contact event (column "Duration"), and the moment at which the contact began (column "Beginning").
- Distances: a cross table in which the distances between targets are summarized (averaged for the whole video).
- PropTime: a cross table with the proportions of frames the two targets spent as neighbors.

### *Spatial*

In the spatial folder, you will find one file per element of interest (see section [Prepare analyses, Spatial repartition](#)). The structure of these table is the same as the "Results per individual" ones, the measured variables are described in section [Prepare analyses, Spatial repartition](#).

Another file called "General" can also be found. This file summarizes for each element the minimum, maximum and average number of targets present inside the element during the video. The coordinates of the elements of interest are also saved in this table. Finally, a "Arenas\_coordinates.csv" file is create in which the arena's coordinates are saved.

### *Detailed data*

If you are interested in more detailed analysis, you will find in this directory the details of each target situation per frame. Files will be organized in subfolders whose names are the ones of the videos. The files names refer to the name of the arena in which target is situated and then the name of the target itself. These .csv files contain one row per frame. The columns that will be part of this file can be selected from the More analysis option (see section [Prepare Analysis, More analysis, Detailed data columns](#)).

Here is a summary of the possible columns:

*Frame*: from 0 to the total number of frames of the video.

*Time*: from 0 to the total duration of the video, time is expressed in seconds.

*X/X\_smoothed and Y/Y\_Smoothed*: The coordinates of the target after transformation (after being scaled and after [smooth-filtering](#) if applicable). The unit of these values is the one defined by the user for the [scale of the video](#). If no unit was defined, the values are expressed in pixels.

*Distance*: The distance traveled by the target between the last and the current frame. The unit is the one defined by the user for the [scale of the video](#). If no unit was defined, this value is expressed in pixels.

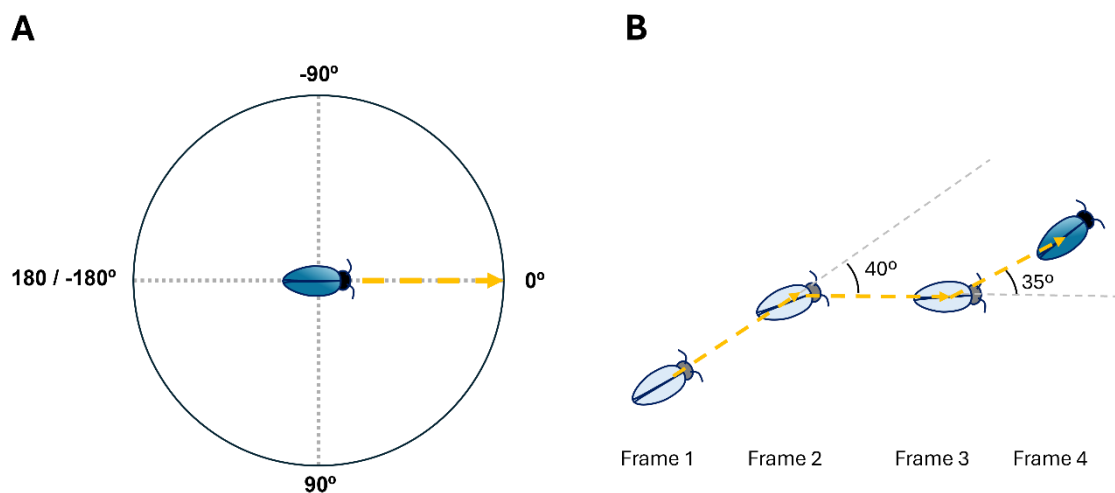
*Speed*: How fast the target moved between the last frame and the current one (in [unit defined by user](#) per seconds).

*Moving*: Whether the target is considered as moving or not. A target is considered as moving if its speed is higher than the [movement threshold](#) defined by the user (default value is 0).

*Corrected\_Moving*: Whether the target is considered as moving or not after the small periods breaks have been corrected (see [Prepare Analysis, More analysis, Durations of movements and stops](#)).

*Acceleration*: The acceleration is measured as the difference in speed between two frames per unit of time. These values are expressed in units/s<sup>2</sup>, with units being the one defined by the user for the [scale of the video](#). If no unit was defined, the values are expressed in pixels/s<sup>2</sup>.

*Orientation*: The orientation of the target expressed in degrees, **based on its movement trajectory**. A value of 0° indicates that the target is moving horizontally toward the right part of the video (see *Figure 21 A*).



*Figure 21. A) Illustration of the way the orientation is calculated according to the individual movement. B) Illustration of the angular change between two frames.*

*Angle\_change*: This value corresponds to the smallest difference between the current target movement orientation and the previous one (see *Figure 21 B*), it is expressed in degrees.

*Angular\_speed*: These values represent how fast the target’s orientation changes. It is measured as the angle changes per unit of time (degrees/s).

*Meander*: This value is the Angle\_change divided by the distance traveled for each frame. It is expressed in degrees/unit defined by user.

*Inter-individual\_distances*: In the case of arenas with more than one individual, this column gives the distance (in unit defined by user) between the considered target and the target X.

*Distances\_to\_elem\_interest*: In the case of arenas with elements of interest defined by user, this column gives the shortest distance (in unit defined by user) between the considered target and the element Y. If the element is a shape, a negative value means that the target is inside the element of interest.

*Exploration\_data\_per\_frame*: If using a “Mesh” exploration method (see section Prepare analyses, Exploration, Meshes), this option will return the Mesh cell ID (X and Y coordinates for rectangular mesh, Ring and Division for the circular one, see Figure 22). If using a “Individual centered” exploration method, this option will return the proportion of already explored area of the circle around the target at the last frame.

**A**

X0 Y0	X1 Y0	X2 Y0	X3 Y0	X4 Y0	X5 Y0
X0 Y1	X1 Y1	X2 Y1	X3 Y1	X4 Y1	X5 Y1
X0 Y2	X1 Y2	X2 Y2	X3 Y2	X4 Y2	X5 Y2
X0 Y3	X1 Y3	X2 Y3	X3 Y3	X4 Y3	X5 Y3

**B**

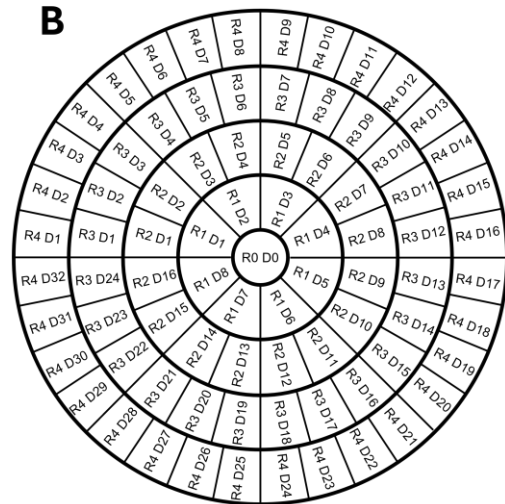


Figure 22. Illustration of how cells of meshes rectangular (A) and circular (B) are named.

### XIII. Export a video

As AnimalTA indirectly allows video-editing, you can export the videos you used during the program using the “Export the video” button in the main project table. This will open a new window in which you can select at which step of the process you want your video to be saved. You can choose the length of the tail to be displayed, smoothed or raw trajectories and with perspective correction or not. Finally, the target’s identities can be displayed.

You can also export the current frame using the “Export image” option.

The “Export heatmap option” will allow you to export an heatmap based on the segmented image (steps “threshold”, “erosion”, or “dilation”). Contrary to the heatmap based on animals’ tracking coordinates available in section Analyses, Exploration, this heatmap is only based on the segmented image.

#### **XIV. Confidentiality**

AnimalTA automatically contacts our server to check for available updates. During this process, certain technical data such as the IP address may be temporarily recorded for purely statistical purposes (e.g., number of daily connections). This data is not shared and is not used for individual tracking.