Tracking Rodents in L'Aquila, TrAQ

MISPIN Lab.
University of L'Aquila
2018-10-12
Version 1

Abstract

TrAQ (Tracking rodents in L'Aquila) is a new MATLAB-based tracking software for off-line video analysis, developed to minimize the user interventions and allow single-click multiple-videos processing. TrAQ allows automatic recognition of the animal and arena, providing the position of the animal's centroid, head and tail. It is based on MATLAB and uses standard routines of its Image Processing toolbox. The MATLAB environment allows easy software management as well as any kind of data analysis useful in characterising freely moving animals.

Contents

1 Introduction

TrAQ is a MATLAB based tracking software developed to analyse animal's positions in a behavioural arena. TrAQ is developed as a free software to allow the user to fully exploit it's potential. In this stage of development TrAQ is a reliable and versatile platform for data extraction and analysis from videos of behavioural tests.

1.1 Requirements

TrAQ is developed in MATLAB 2017a, therefore it requires the MATLAB base installation, as well as the image processing toolbox. It could be not compatible with MATLAB versions older than 2015b. We tested the software on a Windows based computer, however the MATLAB environment should avoid any OS incompatibilities. The minimal PC requirements to work with TrAQ are the same as MATLAB 2017a, that could be found here.

1.2 Installation

In order to use TrAQ the **TrAQ** folder must be added to the MATLAB path. Once the folder is added to the MATLAB path TrAQ could be launched typing
>>**TrAQ**
in the MATLAB command prompt. This command will open the graphical user interface (GUI) of the software in which the user can modify all the settings to set-up a new project.

2 Interface

In this section we start from the description of the two main software modules of TrAQ (Project Set-up Window and Results Viewer Window), and the Graphical User Interfaces (GUI) that allow the operator to control the analysis workflow and data output.

2.1 Project Set-up Window

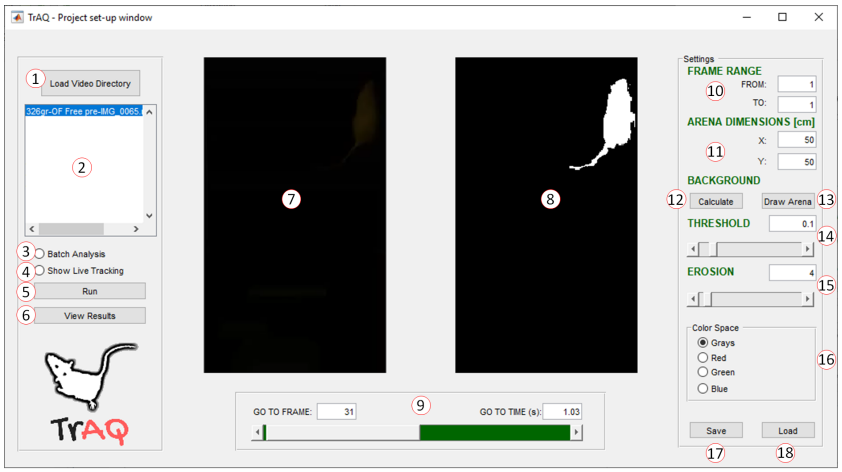


Figure 1: Interface

1. **Load Video Directory**: Specify the directory containing all the video files.

2. **Videos List**: After selecting the directory the video files will be shown here. Then click on the name of a video file in the list to select the video to analyse.

3. **Batch Analysis**: Selecting this button the run function will analyse all the files in the videos list. If the function finds some user defined settings then it will use this to track. Otherwise the function will use standard settings.

4. **Show Live Tracking**: Select this button to see in real time the tracking results displayed on on 7.

5. **Run**: This function will analyse only the selected file using user defined settings.

6. **View Results**: Click here to open the Results viewer window, described in section 2.2.

7. **Video Viewer**: Here will be displayed the selected frame of the video.

8. **Settings Preview**: Here will be displayed the settings preview on the same frame of 6.

9. **Frame Slider**: Use this slider to select the frame to show in 6 and 7.

10. **Frame Range Settings**: here it is possible to select the frame windows to analyse.

11. **Arena Dimensions Settings**: Here it is possible to define the x and y dimensions (in cm) of the behavioural arena.

12. **Background Calculator**: This function will calculate the background image of the video used to enhance the signal to noise ratio of the video. Unlike other software TrAQ does not calculate the background image averaging the video frames, but uses a probabilistic algorithm that assigns for each pixel the most probable value that can be assumed by the pixel during the video. Since the background is calculated in the frame interval defined in 9, if the background image presents artefacts the user can define a new interval used to calculate the background image. This step is completely optional.

13. **Draw Arena**: With this function it is possible to define the shape of the behavioural arena. Note, this function require a background image of the arena, if the background is not defined it will use the first frame of the video.

14. **Threshold Slider**: With this slider it i possible to select the threshold level applied to the video. It is important to select the best value to avoid false positives or false negatives during the tracking step.

15. **Erosion Slider**: With this slider it i possible to select the erosion level applied to the video. it is important to select the best value according to the user's needs (e.g. tail erosion).

16. **Colour Space Selector**: Here the user can select the colour space (by default gray) that presents the best contrast.

17. **Save Settings**: Save all the settings in a MATLAB (.mat) file.

18. **Load Settings**: Load the settings for a video from a MATLAB (.mat) file.

2.2 Results Viewer Window

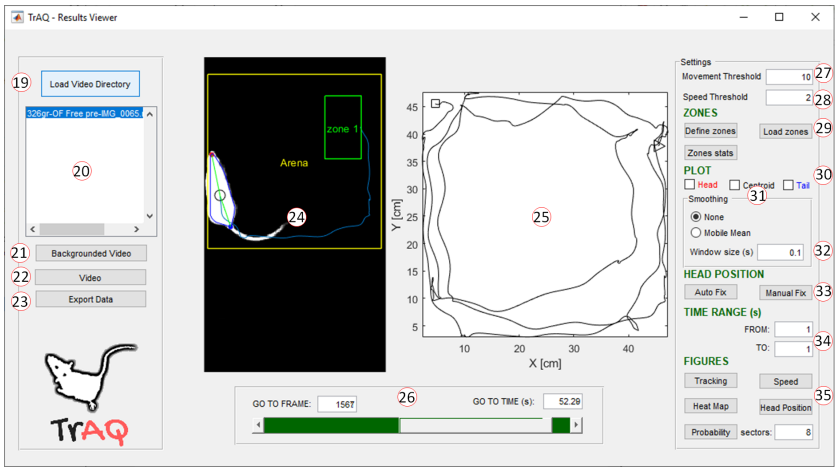


Figure 2: Interface

20. **Load Video Directory**: Same as 1.
21. **Videos List**: Same as 2.
22. **Backgrounded Video**: This function realizes an enhanced video by subtracting frame by frame the background reference image from the original video.
23. **Video**: This function realizes a video of the tracking output.
24. **Export Data**: This function exports all the data provided by the software (*e.g.* total travelled distance, average speed, mean of the instantaneous speed and frame by frame positions of centroid, head and tail in a MATLAB file (.mat) and in Microsoft Excel format (.xlsx).)
25. **Tracking Preview**: In this windows is shown a preview of the tracking output superimposed to the video.
26. **Tracking Plot**: In this windows are shown the plots of the position of Centroid, Head and Tail.
27. **Frame Slider**: Use this slider to select the frame to show in 23
28. **Movement Threshold**: This is the minimal distance (in pixels) that the animal has to cover between 2 consecutive frames used to automatically correct the head position after the tracking.
29. **Speed Threshold**: This parameter is used to define a threshold speed used to characterize the stopping behaviour of the animal.
30. **Zone Definitions and Analysis**: This section allows the user to define regions of interest within the arena and then analyse the animal's interactions with this zones (*e.g.* number of visits, time spent in each zone, distance between the nose and the zone perimeter)
31. **Plot Selector**: This flags are used to select what to plot on 24

32. **Trajectory Smoothing**: This function will smooth the trajectory using a mobile mean algorithm with a time window selected by the user (32).

33. **Window size**: Mobile time window used for trajectory smoothing.

34. **Head Position**: This section allows the user to review and correct the head position. The **Auto Fix** algorithm uses as selection criteria the actual movement of the centroid and assuming a head-ahead motion. The **Manual Fix** function allows the user to correct the head position in a given frame.

35. **Time Range of Analysis**: Here the user can select the time range (in seconds) of the video to analyse.

36. **Data Analysis**: This functions will plot in separate windows the analysis results.

2.3 Changing Default Parameters

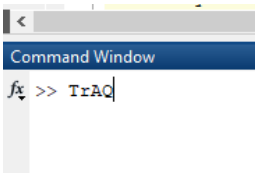
All the operative parameters in the Project Set-up window are fully customizable. By default they are setted according to our experimental design, but if the user needs to change the default parameters he can edit the UserSettings.txt file located in the TrAQ folder. This file has to be written in this form to work:

```
arena_x(cm) = 50
arena_y(cm) = 50
Threshold(0-1) = 0.01
Erosion(pixels) = 4
Movement_Threshold(pixels) = 1
Movement_Threshold(frames) = 10
```

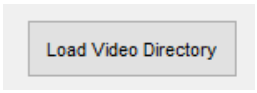
Where arena x and y are the arena dimensions in cm, Threshold is used to generate the binary image, erosion is the neighbourhood size (in pixels) used to erode the image (used *e.g.* to remove the tail), the two movement parameters are used in the tracking review step to fix the head tail position when the animal is moving forward; the first one is the minimum movement (in pixel) that the animal has to do in the time window given by the second parameter. Simply edit the numbers to modify the software default parameters.

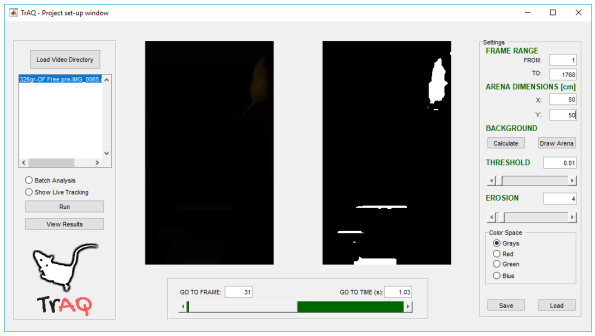
3 Example

Here we report a work flow example of the software. For the first analysis we recommend to use the attached examples video and the default parameters.
Type TrAQ in the MATLAB command prompt.

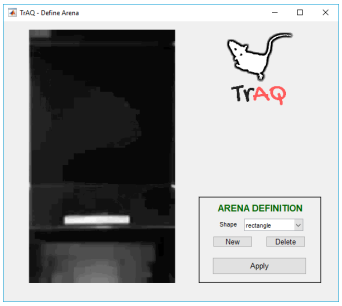


Press the Load Video Directory Button and select the folder containing the video files.
Use the frame slider to navigate the video.

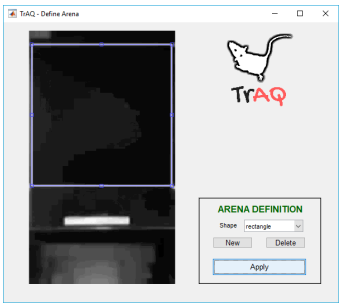




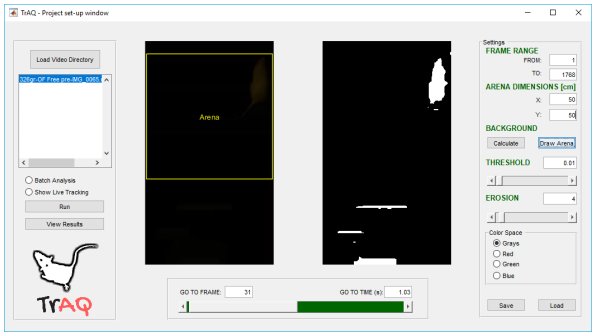
Set the first and last frame to analyse. In this example we decided to set the first frame value to 31 and the last frame to 1768.
Set the arena x and y dimensions to 50 cm.
To enhance the tracker performances for the video calculate the background image of the arena by clicking on "Calculate".
Click on "Draw the arena".



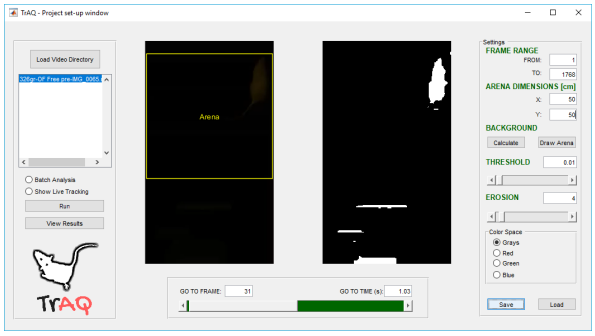
Click on "Apply".



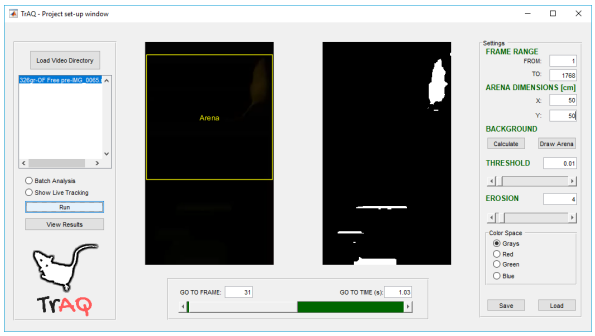
Adjust the threshold and erosion levels (0.01 and 4 respectively).
Select the grays colour space.



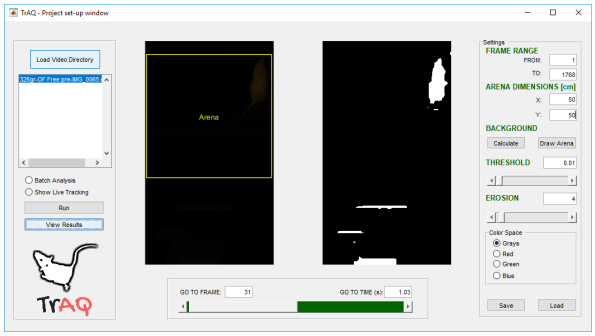
Save all the settings.



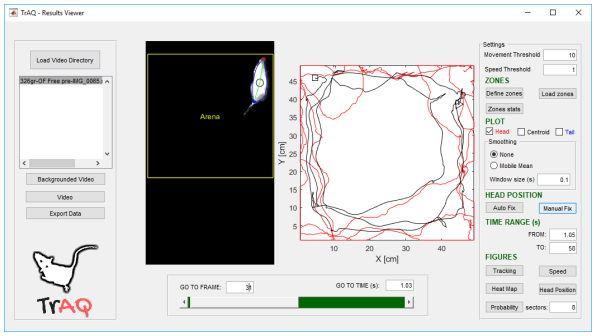
Press the "Run" button.



Wait until the end of the tracking analysis.
Press the "View Results" button.



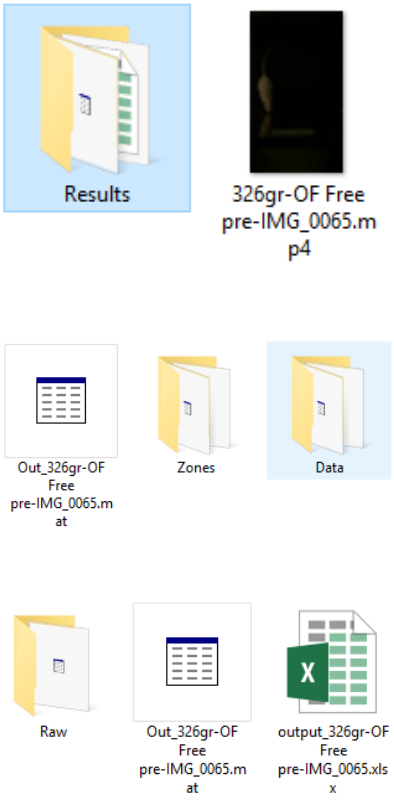
This will open the Results Viewer window described in section 2.
Here the user can view the tracking output and analyse the data. First the user need to load the video directory and select the video from the



list.
After a while the tracking preview will be displayed on 24.
Now the user can review and analyse the tracking output selecting various settings. After the analysis the user can export all the data in MATLAB and in excel format by clicking on the "Export Data" button.

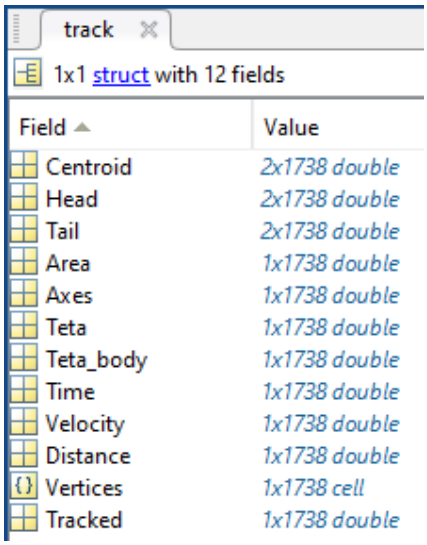
4 Tracking Output

When setting up a new project the software creates a new folder called Results in the main videos folder, here the software stores the raw and the processed data.



The processed data can be used for further analysis simply loading the .mat file in the MATLAB workspace.

Opening the track Structure the user can easily access all the tracking output data



The Centroid, Head and Tail arrays contains the x and y coordinates of the animal's centre of mass, head and tail in cm calculated frame by frame.
The Area array contains the animal's area in cm^2.
The Axes array contains the length (in cm) of the major and minor axes of the ellipse that has the same normalized second central moments as the region, returned as a scalar
Teta contains the angle (in rad) between the head and the centroid. Time contains the time stamp array.
Velocity and Distance arrays contain all the frame by frame instantaneous speed and distance calculated from the Centroid's coordinates.
The Vertices cell array contains the convex hull of the animal, defined as the smallest convex polygon that can contain the animal, returned as a p-by-2 matrix. Each row of the matrix contains the x- and y-coordinates of one vertex of the polygon.
The Tracked array contains a frame by frame flag used to identify where the software failed to track, if the value is 1 then the frame was tracked; otherwise the frame is considered as untracked and the coordinates are linearly interpolated.