SOP: Open Field Test

Notes

- Any type of novelty or stress may modify exploratory behaviour. It is very important to reduce novelty, for instance, by changing the cage after behavioural experiments are done.
- The room in which recording takes place must be isolated from unexpected sounds, as much as possible. It is essential that no one else is in the room, enters the room, or leaves the room during filming. Place a sign on the door that indicates an experiment is in progress.
- There must be consistent lighting across testing days:
    - **N.B.** Brighter light can decrease locomotion.
    - The light level used for experiments must be recorded (using a light meter) and reported in any publication. The lighting level in 1U21 is 130-160 lux.

Materials

- Room: 1U21D
- Open Field Arena (2 arenas, 50cm*50cm*50cm each)
- 70% EtOH spray bottle, paper towels, small towels
- Light meter (CAF)
- Extra cages with food on the floor and water for transfer

Filming

- Video Camera **NB:** Check that all of the equipment is charged, and you have enough memory
- Retort stand
- Phone in silent mode

Open Field Protocol

Transferring animals and set-up (7:30):

1. Transfer animal to testing location 1 hour prior to the start of the experiment. Leave the room, allow animals to acclimatize to the new environment.
2. Record light intensity, ensure there are no shadows in the arena.
3. Place 'Do Not Disturb Sign'. **NB:** All materials and equipment should be set up prior to mice' arrival to the OR.

Recording (8:30-11:30):

1. Designate a specific corner or the centre of the arena as the "Start" point. For each individual trial, place the mouse into this designated spot.
2. Start timer immediately as mouse is placed into arena. Record 10 mins for each mouse.
3. Move as far away from the arena as possible. Remain silent until the end of the trial.
4. At the end of the trial, place a paper with mouse written ID in front of the camera. You will use it to identify individual mouse during analysis. **NB:** Record ids in your lab book in order of test sessions.
5. Put mouse into 'Transfer' cage. Returning mouse into original cage will stress the rest of the mice. Monitor for fighting.
6. Clean the arena thoroughly with 70% EtOH. Mouse can detect odour of the previous mice if not done thoroughly.
7. Wait until the arena is completely dry before placing the next mouse in the arena.
8. Repeat with all animals.

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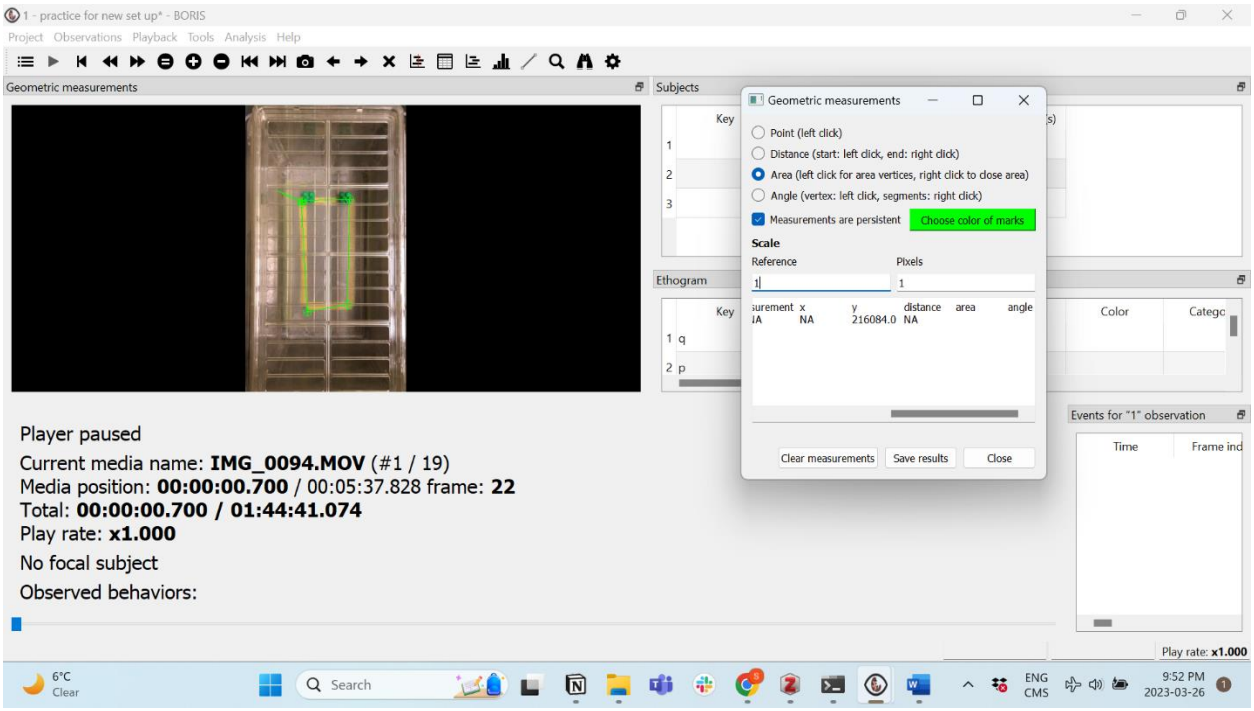
SOP: Open Field Test

Clean up (11:30-12:30):

1. Transfer mice back to the original cage, then transfer them to the animal holding room. Monitor for fighting.
2. Clean OR.
3. Transfer videos to the hard drive and cloud.
4. Charge recording equipment.
5. Prepare equipment for Novel Object Recognition Test.

Instructions for Manual Analysis using BORIS

1. Analysis of the video should be performed by personnel blinded to the animal IDs.
2. Use 'Geometric Measurements' tool to find scaling factor (e.g. 1 side of the arena is 50 cm).
3. Draw center of the arena (30 cm*30 cm). The stickers on the side will help to identify it. The example below used different arena.



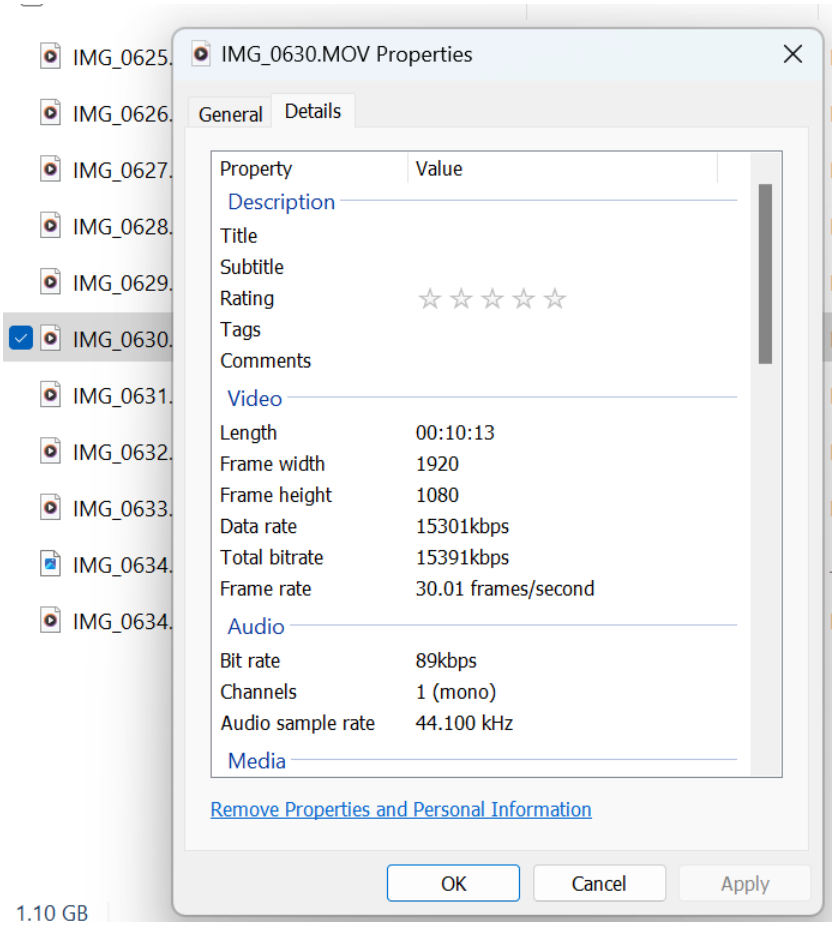
4. Record the following behaviours:
    1. Entries to the center. State Behaviour. Entry starts when the mouse is facing the center and has two paws in the center. Entry ends when the mouse is not facing the center and has only one paw inside the center.
    2. Rearing. Point Behaviour.Rearing is defined as a mouse eighter free standing on its hind legs or using a wall to stand on its hind legs. Look out for the mouse actively moving its vibrissae. Grooming or being completely immobile while on hind legs is not considered rearing. Rearing is considered exploratory behaviour.
    3. Grooming. Point Behaviour. Grooming is defined as mouse scratching and cleaning itself. If the mouse has not moved at least 1 cm or taken the

break in between, several scratches are considered as 1 Grooming Behaviour. Grooming is a sign of adaptation to the new environment.

4. Defecation. Point Behaviour. Some researchers use defecation as anxiety-like behaviour in mice.

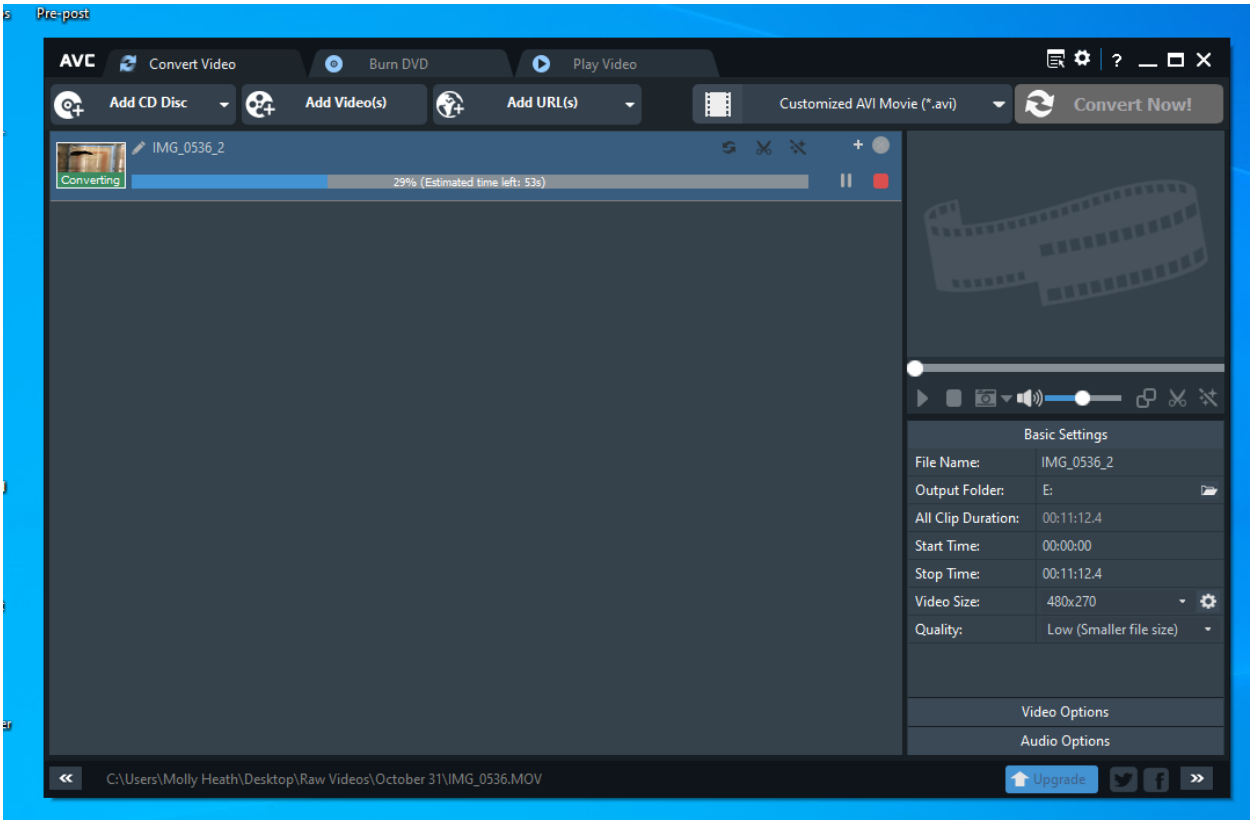
Instructions for ImageJ AnaimalTracker VideoTracking

1. Check video file properties, including framerate and frame width and height.

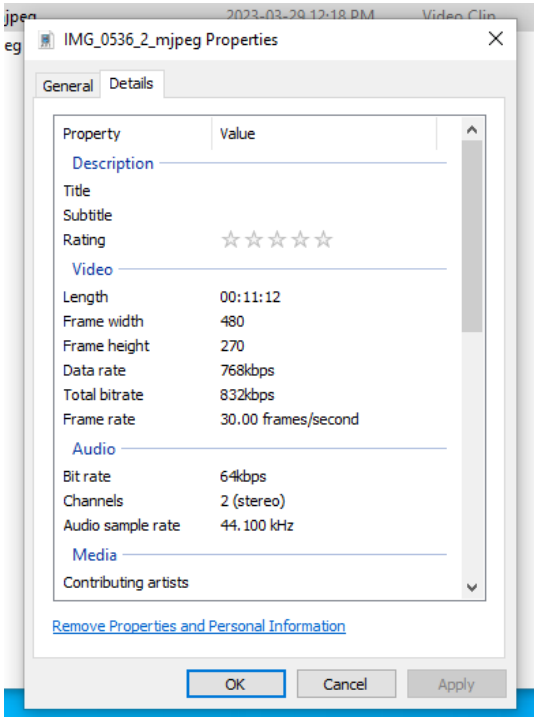


2. Upload to AVC program.
3. Check ‘Basic Settings’. Change Video Size to desired one. Ensure that the aspect ratio is maintained as in the original video (here 16:9). Changing video size will increase the upload speed to ImageJ.

SOP: Open Field Test

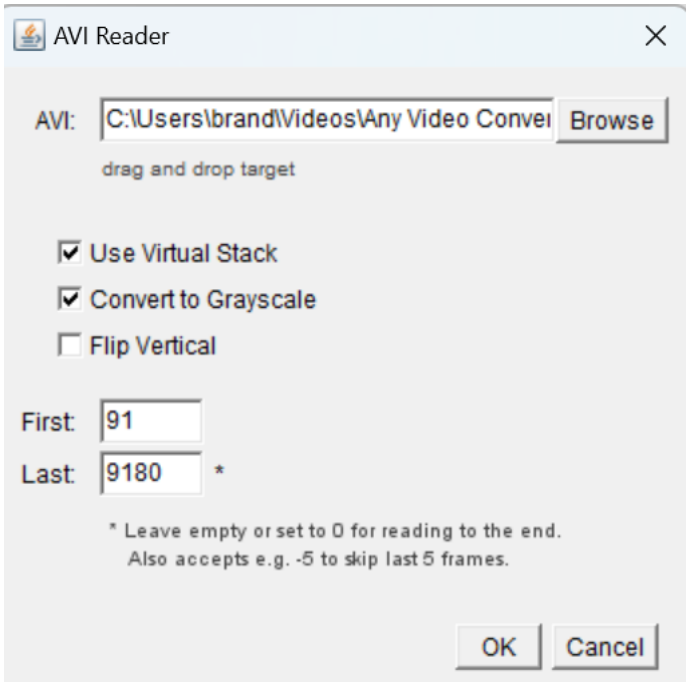


4. Check 'Video Settings'. Change Video Codec to mjpeg. Ensure that the Frame Rate is the same as in the original video (30 in this example).
5. Press 'Convert Now'.
6. Check the converted video to ensure that the frame rate and aspect ratio stays the same (30 fps and 16:9 ratio, respectively in this example of vertical video).



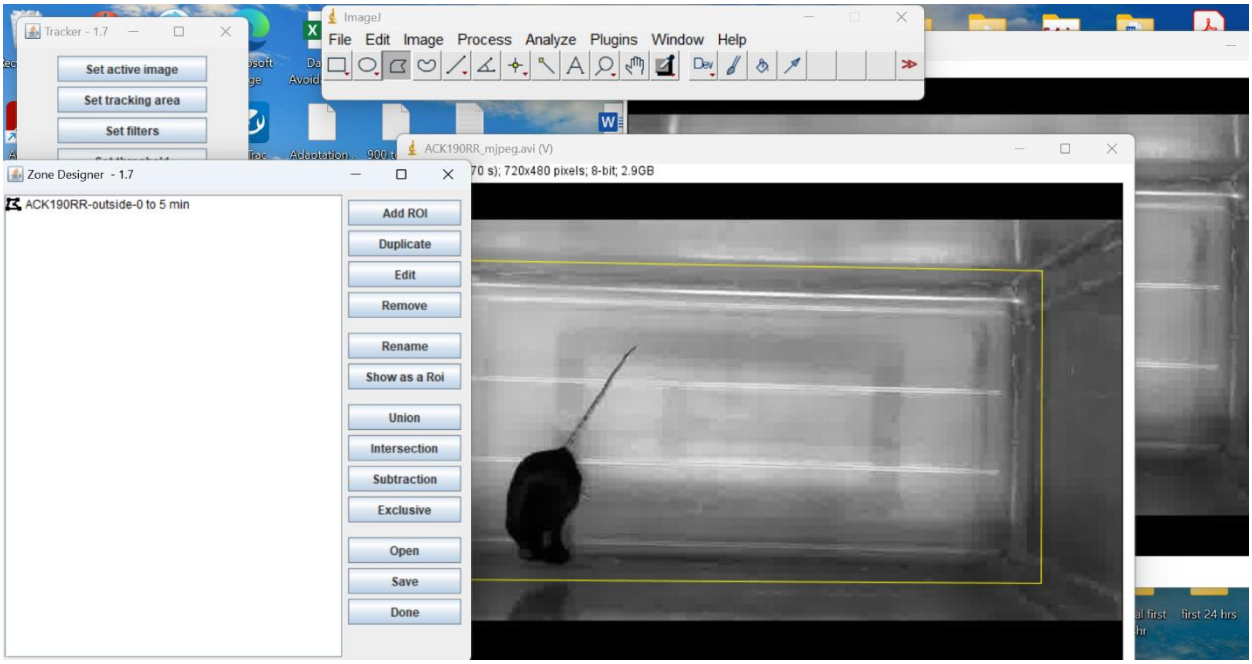
7. Open the new video to check when the mouse was placed in the 'Start Corner'. In this example, the first frame of interest will be after 3 seconds, or 90 frames, of the video passed. This step is done to account for any delays during filming.

8. Start ImageJ, go to File->Import->Avi. Select the video to import. Select 'Use Virtual Stack' and 'Convert to Grayscale'. Type in the first frame of interest (here first frame after 3 seconds of video is 91. Type in last frame (here 9180: 90 frames of the mouse not present in arena, +9000 frames=5min of test + 90 frames to adjust for cover placement in the beginning of the test).



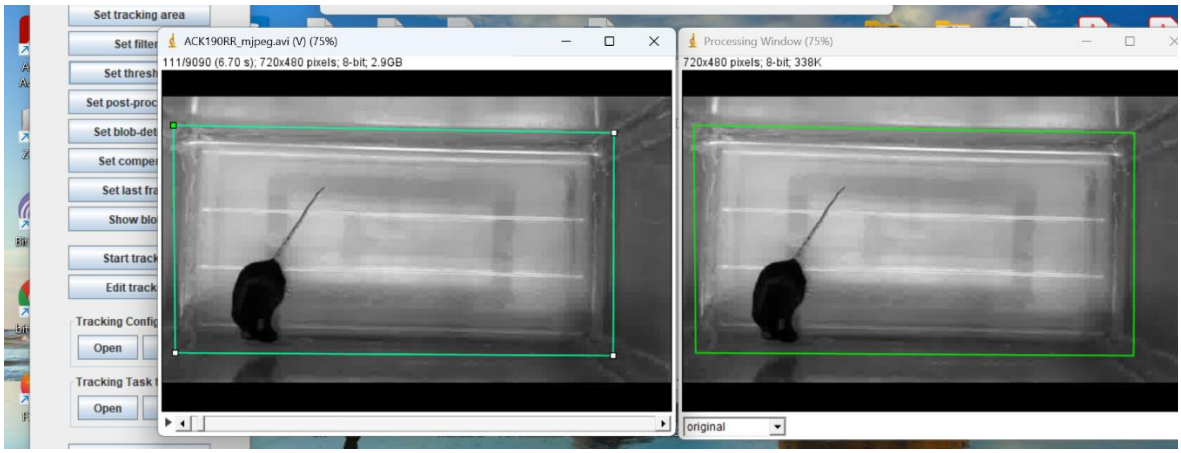
SOP: Open Field Test

9. Once the video is loaded, select Plugins->AnimalTracker->Tracker. Press ‘ Set active Video’ on the left sidebar. You will have free windows open: sidebar on the left, stacks of images, and processing window.
10. Using the line tool, create a line on the outer border of the arena, select Analyze->Set scale. Do not change the number of pixels, this is indicated by the length of the line you created, set known distance to 50 cm. Record the scale factor in your lab book, this will later be used to determine how far the mouse travels.
11. In your stacks of images select a frame where you can clearly identify borders of the arena and the center. Select the Polygon tool to draw borders of arena (outside borders of the arena can be larger because mice stand on their hind legs and support themselves with front paws when they investigated the walls of the arena). Then press ‘Add ROI’ in ‘Zone Designer’ window, rename your zone to identify during future analysis (eg. Mouse ID, Zone of interest, time during investigation).

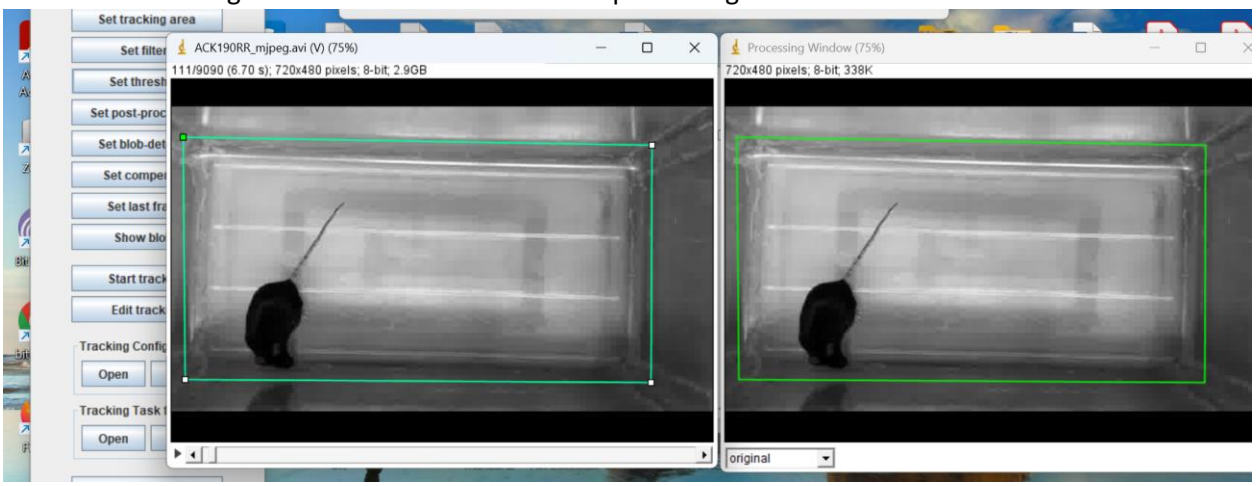


12. Repeat Step 11. for the center of the arena. Remember, the center of the arena is more precise (here we are selecting the inside border lines of the center arena for preliminary video, use a scale tool to confirm the size of arena). Save your arenas in the designated folders, and select the outside zone as your roi (press ‘Show as Roi’). In a standard 50 cm*50 cm*50 cm arena, the inside border should be 10 cm from the outside border (making it a 30 cm*30 cm square).

SOP: Open Field Test

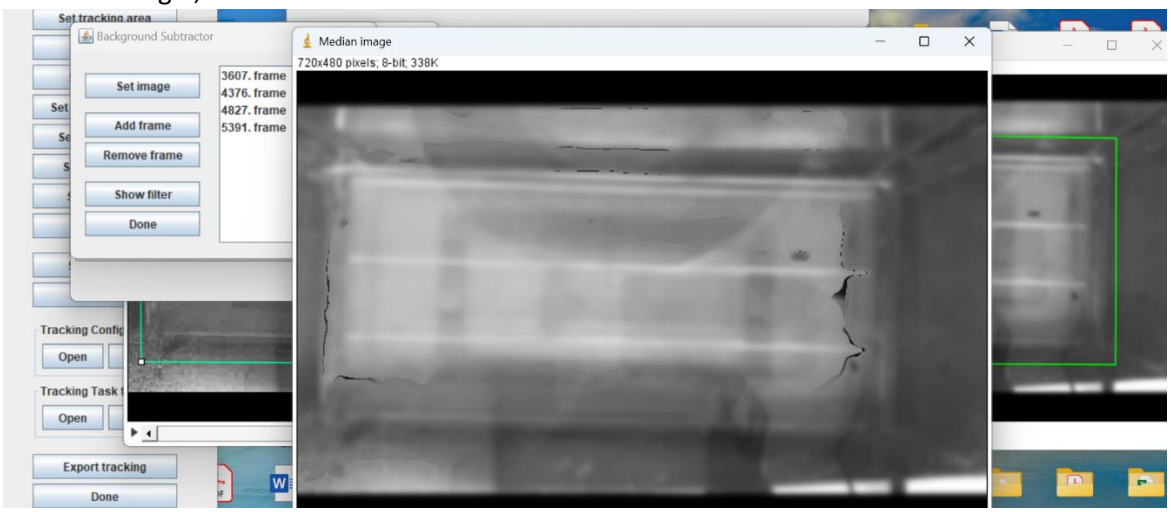


13. Next, select ‘Set Filters’ on sidebar, add ‘Gaussian Blur’. For this video blur of 3.0 was selected. You can track changes in the filtered section of the processing window.

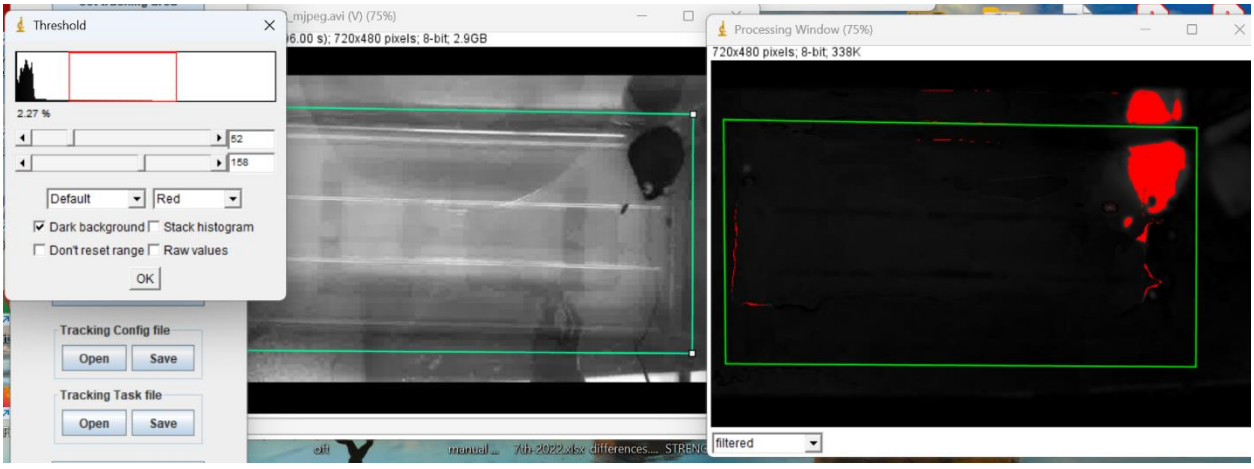


14. Add ‘Background Subtractor’ in your filter setting. Select your image stack window and scroll towards the middle of the stack. Select a frame with the mouse close to the center, press ‘Set Image’, then select 3 frames (or more) with the mouse located in different parts of the arena to subtract the background. You can press ‘Show Results’ to check your progress, when you can no longer see the mouse, the filter is ready. **Alternatively:** choose a frame with mouse present and

click ‘Set Image’, then choose a frame with mouse absent and click ‘add frame.

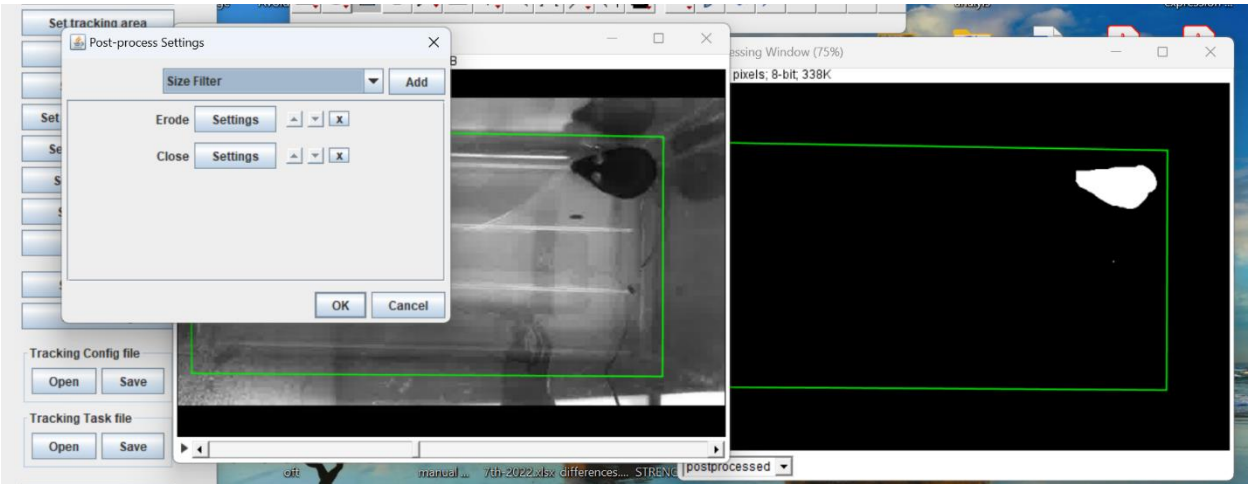


15. Once you are satisfied with your background, press ‘Done’. Your filtered image in the processing window will be updated to the outline of the white mouse on black background.
16. Press on the processing window first, then select ‘Set threshold’ on the left sidebar. Your mouse should appear red in the processing window. Select the appropriate range of threshold, so your mouse would be visible in all of the frames. Press ‘OK’. You can look through images using bar in image stack window, the ‘thresholded’ section of your processing window will reflect new changes. If you notice small artifacts in the frames, they can be edited in the post-processing. It is important to ensure that the mouse is presented as similar size blob in every frame of the video.



17. Press ‘Set Post-processing’ on the left sidebar. Add ‘Erode’ and ‘Close’ settings (iterations 3 and 3 were chosen for this video. Check your progress in the post-processing section of the processing window. You can add additional settings for your video analysis. Make sure there is only one white ‘blob’ present in the frame.

SOP: Open Field Test



18. Choose ‘Set blob-detector’ on the left sidebar.
19. Scroll to the beginning of the video. Choose the frame where the arm of the researcher is not present, and the mouse is the only white ‘blob’ in the frame. Press ‘Show blobs’ on the left sidebar. You will have one or more rectangles surrounding ‘blobs’ present in the arena. In the image stack window, select the rectangle surrounding your mouse. It should be coloured red.
20. Select 'Set last frame’, for 5 minutes of analysis, you will track 30 fps*300 seconds = 9000 frames. The first frame where the researcher is not present is your starting point, this + 9000 frames will be your last frame. For your second 5 minutes of analysis, start on the last frame of the previous track, and add 9000 frames to find your new last frame.
21. Press ‘Start Tracking’.
22. Check the results by moving the bar in the image stack window. Ensure that tack follows the mouse and that there are no artifacts.
23. If there are gaps or errors in the tracking, you can use the ‘Edit Tracking option’ to delete the portion of the track formed due to small artifact, interpolate unconnected tracks, and add a portion of the track at the beginning of the video. Use the ‘Point’ tool from the upper menu bar to add the mouse’s center to the track. If there are still gaps after selecting Edit tracking->Interpolate gaps, return to step 13.
24. Once you are satisfied with how your track looks, press ‘Export Tracking’ on the left sidebar. Save your tracking file in designated folder, add ID and time to the title.
25. Select Animal Tracker-> Tracking Analyzer. FINISH THIS

References

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4. Gulyás, M., Bencsik, N., Pusztai, S., Liliom, H., & Schlett, K. (2016). AnimalTracker: an ImageJ-based tracking API to create a customized behaviour analyser program.

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