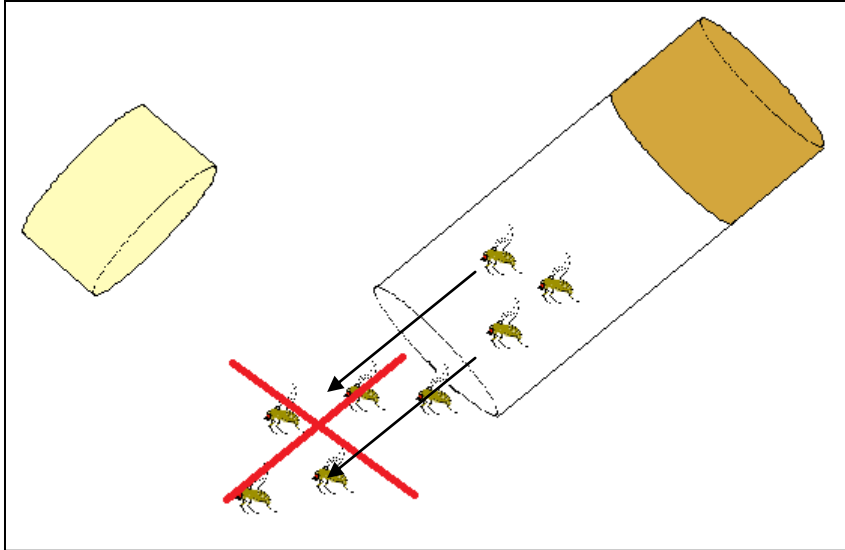


## A General Buridan Protocol

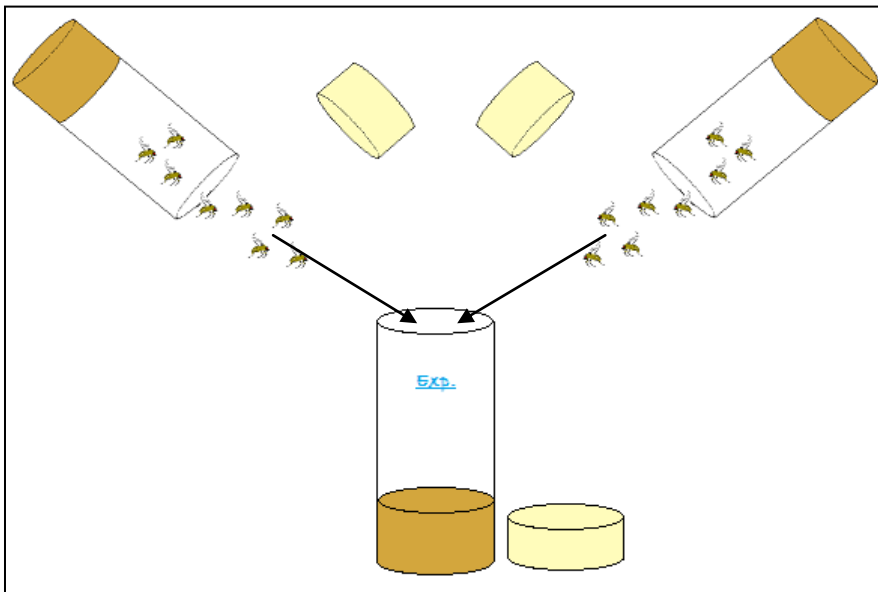
### Day 1 – Pushing the Flies:

Freshly hatched flies of all test groups (e.g. wild type controls, experimental groups) are disposed.



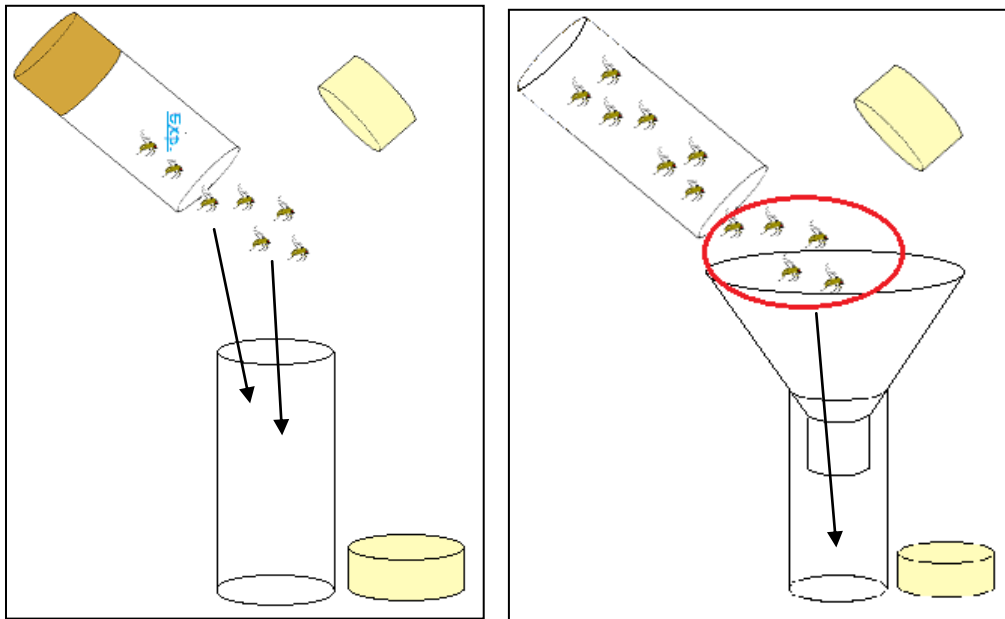
### Day 2 – Collecting the Flies:

The overnight newly hatched flies from all vials of each test group are collected into new experimental vials (“Exp.”). The flies are now 0 to 1 day old.

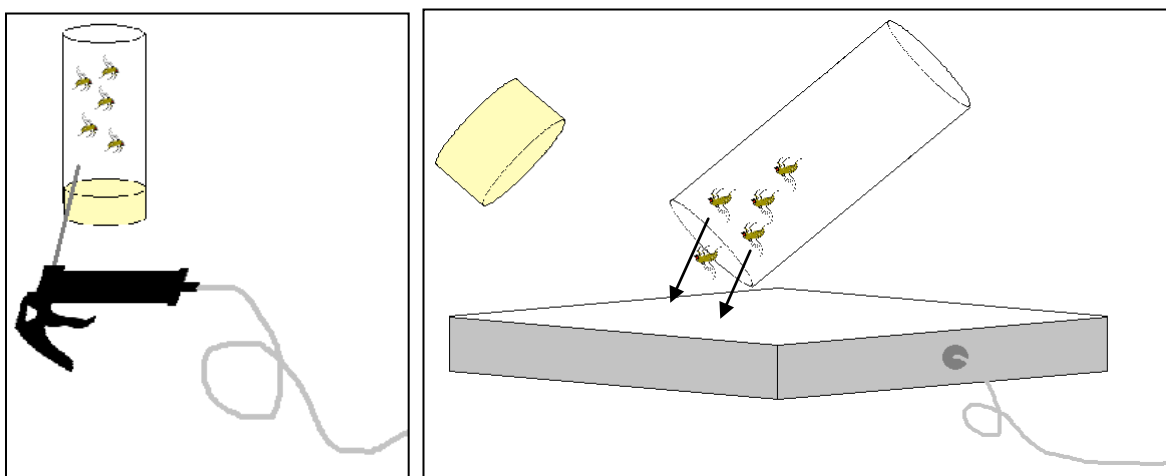


### Day 3 – Wing Clipping:

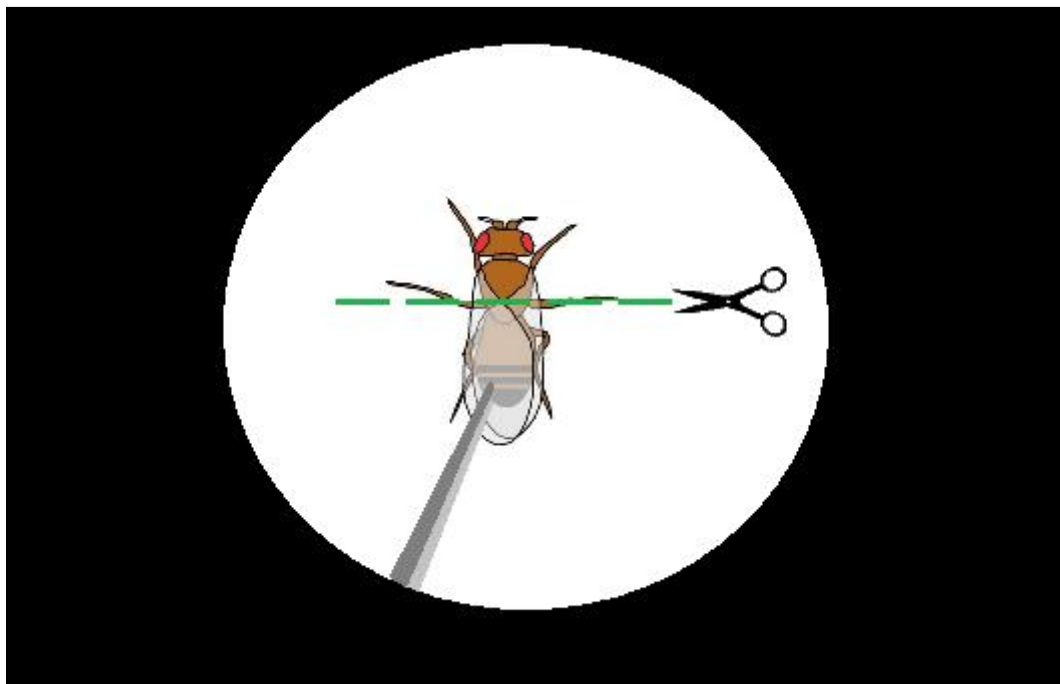
The collected flies from the day before become anesthetized with CO<sub>2</sub> and their wings are shortened. It is important to keep the flies anesthetized for not longer than 5 minutes. Therefore, it is helpful to portion the flies: They get transferred into an empty vial; from this vial single portions of flies can be transferred into another empty vial (via tube or funnel).



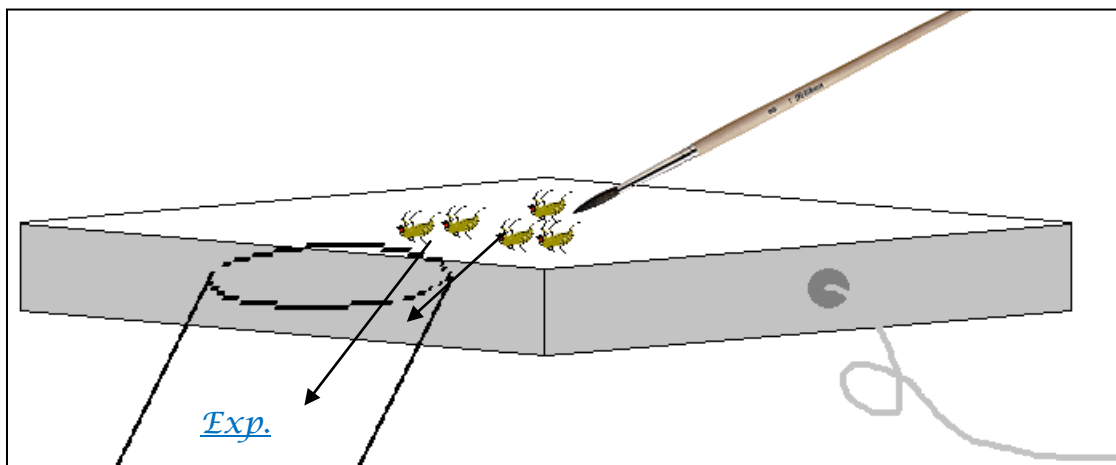
The single fly portions then get anesthetized by holding the vial upside down, inserting the CO<sub>2</sub> pistol and fill in the CO<sub>2</sub> for a few seconds (stop when all flies fell down onto the plug). Afterwards, the vial can be opened and the flies can be placed down the activated CO<sub>2</sub> pad.



Flies need to be sorted in case the experiment is asking for distinct gender or stocks are balanced. The selected flies can be held by the forceps at the very tip of the wings. The wings need to be shortened to one third of their original length and straight through the crossing line of both wings. Too long wings can cause the fly to jump more often; cutting the wings too shortly can injure the fly. If the wings of a fly are spread into different directions it is still possible to cut them straightly, even if hitting the crossing line is more difficult.



After cutting the wings the still anesthetized flies get transferred carefully to the now empty experimental vial by using a brush; following portions of wing clipped flies can be added the same way, as long as the work is done quickly enough.

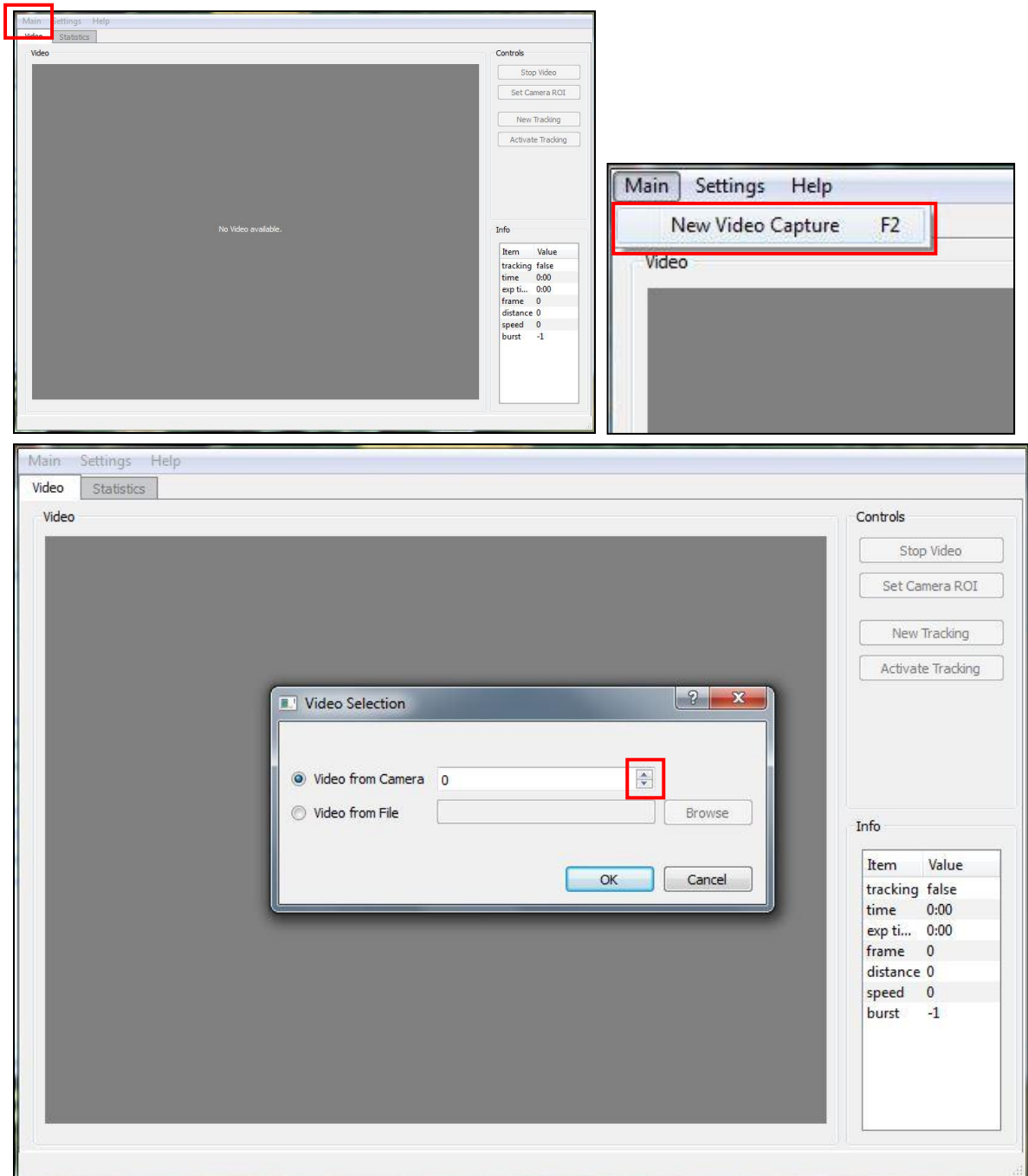


The vial with the freshly wing shortened flies needs to be set aside as long as flies recovered from anesthesia; sleeping flies fall down into the food and stick to it. The completely prepared flies are put back into the incubator.

#### Day 4 – Experiment:

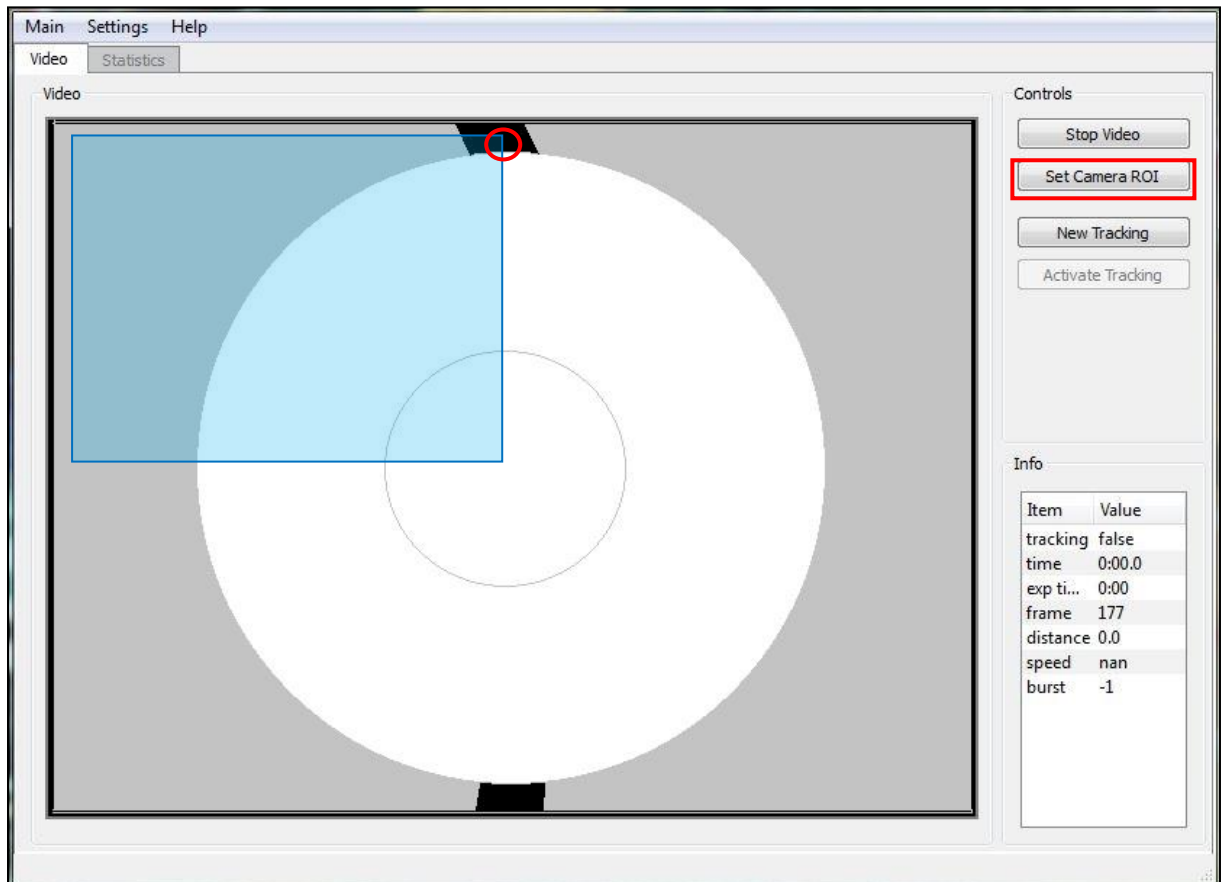
##### Preparations:

- darken the room (close blinds and curtains, switch off lights)
- turn on the air condition (the temperature inside of the Buridan arena is supposed to be 25°C)
- switch on the lights of the Buridan arena
- assemble the bottom of the arena such that the platform perfectly fits into the designated hole (the grommet must seal the hole perfectly)
- fit the bottom into the arena and make sure that the platform is straight in all directions (use water-level)
- check whether the camera is in a straight position above the arena (water level)
- start the computer
- start the program (Buritrack)
- choose “Main”
- choose “New Video Capture”
- choose the camera (if there is only one camera linked to the computer, leave the default camera number “0”)



- adjust the camera such that the platform is clearly visible and the edge of the platform is not too dark but clearly visible as well, make sure that the “Auto Focus” is switched off and focus the platform manually in the web cam software user surface.
- use the function “Set Camera ROI” to check whether the dark stripes are in the exact north and south position of the platform: Therefore click left on “Set Camero ROI”; click left in the middle of the platform (if you misplaced

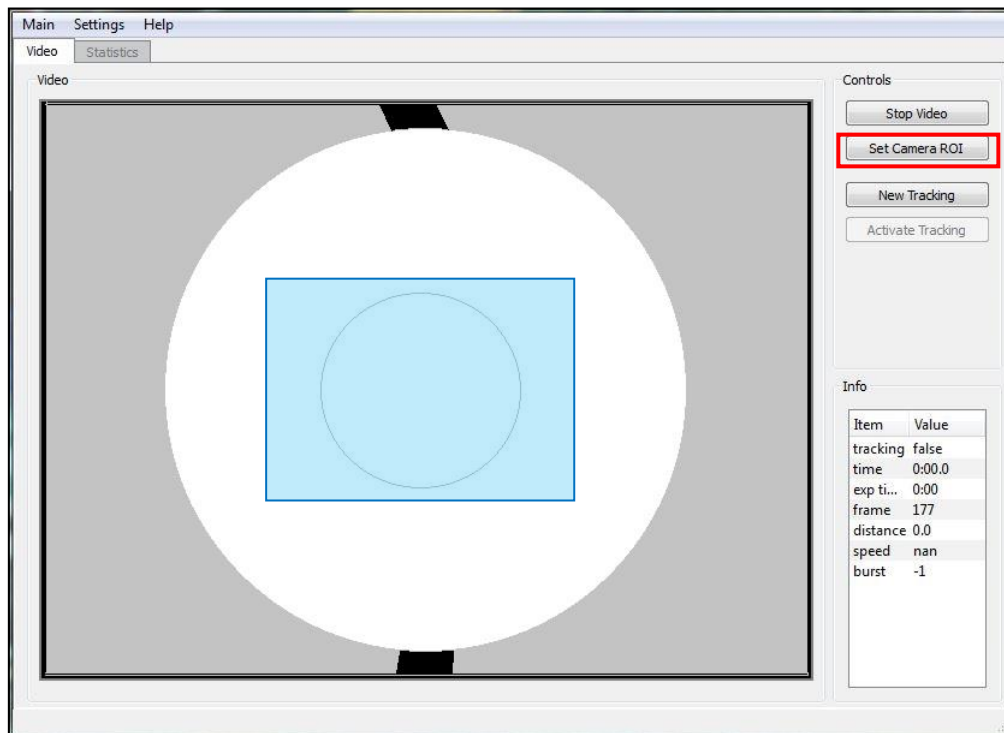
the cursor, click right and choose “Set Camera ROI” again); move the cursor up to the north stripe and down to the south stripe (the edge of the square has to cross the stripe in the middle); move the stripes if necessary



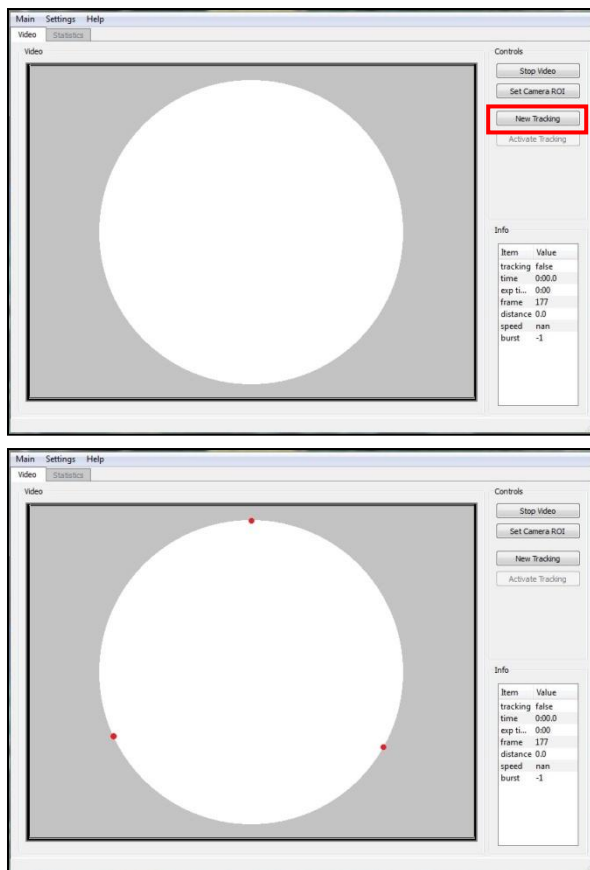
- fill in water (approx. 2,5 big fly vials per arena); the platform is supposed to be surrounded by a “wall” of water so that the fly cannot jump out of the platform
- clean the platform with 70% Ethanol

Experiment:

Choose a tight area around the platform which shall be recorded (“Set Camera ROI”); the stripes or the edge of the arena are not supposed to be recorded.

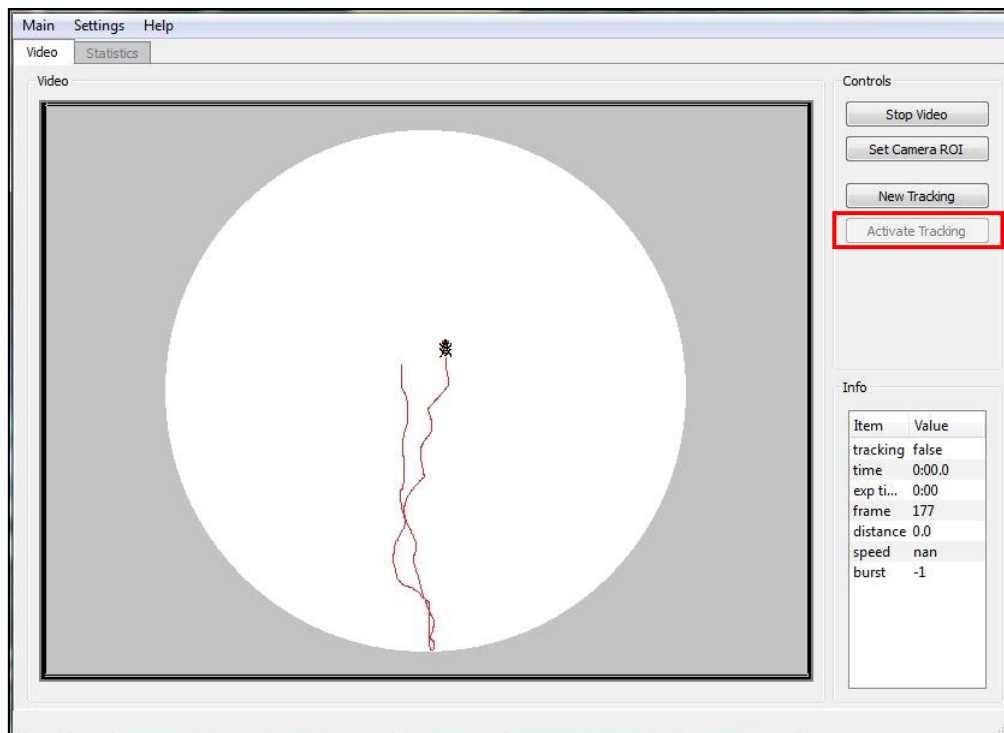


Choose “New Tracking” and browse for your destination folder then type in the name of your file (e.g. **wa3.xml** for **wild type**, arena **A**, sample size (**3**)). Fill in the group of your recording at “fly name” (e.g. **wa** for **wild type**, arena **A**). Choose the duration of your recording (e.g. 900 seconds) then click left on “Set Arena Size” and define the platform by choosing three points on the edge of the platform; the drawn circle has to perfectly cover the platform (if it does not, check whether the camera is really straight above the arena). An incorrectly set arena size will cause imprecise data! Choose “OK”.




If required, measure the temperature in the middle of the platform and document it.

Transfer the fly to the platform by using a tube. Start the recording (“Activate Tracking”). The walking fly is now recorded by the program and its trace will become visible as a red line. In the bottom right corner of the program’s window there is an “Info” box which shows the most important data: tracking (has to be “true” during the recording), time (has to run during the recording, otherwise the tracker stopped and the fly has to be put back to the platform), exp time (the time that was chosen before, e.g. 900 seconds = 15:00:00), frame (starts running as soon as the program is started), distance and speed (walking parameters which are directly analyzed) and bursts (-1 before the tracking is started; 0 as long as the fly didn’t jumped off the platform; one burst = one jump; when the tracker only runs for 5 minutes, the burst number has to be 0!)



Whenever a fly jumps off the platform there will be a sound. For a 15 minutes experiment it suffices to put the fly carefully back on the platform (brush) and press “Activate Tracking” again. For a 5 minutes experiment it is necessary to start the recording all over again, therefore put the fly back on the platform and choose “New Tracking”; keep all of your settings, overwrite the existing file and choose “Activate Tracking” again.

When the recording is done another sound will be audible. Check if the time is the same as the experimental time (and, for a 5 minutes experiment, if the bursts number is 0) and take the fly out (tube). Clean the platform with 70% Ethanol and start a new recording.

It is helpful for the later analysis of the data to write a .txt file while recording. Therefore generate a new .txt file and name it after the experiment (e.g. Mutant xy) then open it and note the exact file name (e.g. wa3.xml) with the file type (.xml!!!), type a tab (  ) and write the group (e.g. wa). Do this step for every recording!

If there is a paper table it is also necessary to write down the details of the recording (date, experimenter name, group, file name, possible treatment) to make sure that another experimenter will be able to reproduce the whole experiment.

After finishing the experiment clean the arena: Get out the water such that the arena won't get wet (pipetting), disassemble the bottom, and dry all pieces, especially the grommet. Clean the arena wall and the stripes carefully. Switch everything off (arena lights, air condition, and computer).