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🌐 Imaging on the multicamera worm tracker-Phenix V.2

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Behavioural Genomics

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Protocol status: Working
We use this protocol and it's working

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Abstract

Caenorhabditis elegans, a small (adults are ~1 mm long), free-living soil nematode that feeds on bacteria, is an ideal organism for applying various live microscopy techniques. The organism is transparent, thus it is possible to microscopically analyze and record the whole animal throughout its entire life. Its complete cell lineage is known, making it possible to follow developmental and differentiation processes in real time. This tracker does real time compression of the videos and also it should be taken care that the worms are in focus while recording.

Troubleshooting

Pre-Imaging set-up

1
- Clean the rectangular glass plate with ethanol and lint free tissue
- Switch ON the rig (Switch is at the back of the machine)
- Align the movable part till desired position and lock it by pressing ON (If you want to move it again press EMO, to lock again twist the EMO knob and press ON again)
- Log in to the 3 PCs- PC1 labelled yellow, PC2 labelled red and PC3 labelled blue
- Set up destination folder on PC1 only as (PC → Data Part 1 → Run script (init_exp.ps1) → Name the folder with the date as (year month date e.g. 20181011)
- Turn the rig ON and leave for atleast 15mins before use for the temperature to stablilize
- Turn on the software GECKO on each pc and check the following: (See Figure 1, 2, 3 & 4)
  i) Recording mode: Select the duration of the recording
  ii) Output Video type: hdf5 or mjpg or others as desired by the experiment
  iii) Output folder: Select the folder created earlier
  iv) Press the drop down arrow beside the RECORD icon to make sure ALL CAMERA option is selected if you want both the cameras in use otherwise select CURRENT CAMERA option

Actual Imaging

2
- After picking the desired number of worms on the imaging plates, place the plates on the rectangular glass plate underneath the cameras
- Place the imaging plates open side down with the lids off (See Figure-5. This helps to reduce condensation and other contaminants falling on the agar while tracking. Also since the imaging is done through the agar it is crucial to have a thin layer of agar in the imaging plates)
- Check the focus on each camera of each PC and make sure the ring of food is at the centre of the field of view (Focus can be checked by zooming in with the mouse)
- Leave worm containing imaging plates on the rig for a certain duration to acclimatize to the surroundings if required by the experiment, otherwise press RECORD on all three PCs after all the important checks have been made

Post Imaging

3
- Turn OFF the rig by turning the EMO knob and then closing the switch at the back
- Close GECKO (This is an important step as failure to do so wearout the cameras eventually, the camera light will turn blue if GECKO is closed successfully)
- Run the script on each PC to copy the data to the network (PC → Data Part 1 → Run script (move_files_anyPC.ps1)

- Discard the plates

Troubleshooting tips/notes

4
- If the air table is not floating then manually lock air in the system by turning the red knob parallel to the silver tube (See Figure-6&7), let air draw in the the system, once the pressure increases as indicated by the meter, turn the red knob perpendicular to the silver tube and isolate the compressed air
- If multiple hours are recorded, it is better to select the autorepeat option in recording mode to prevent Gecko from crashing and also this will make the video in small segments of selected time period instead of a longer one
- PC1 controls Camera 1 and 2, PC2 controls Camera 3 and 4, and PC3 controls Camera 5 and 6. So if needed the two cameras controlled by one PC can be started independently of the rest and if doing so it is better to start from the back ie PC3-Camera 5 and 6 and then move along.
- It is always good to check whether the recorded videos are being saved in the selected destination folder, it can be done by opening up the created destination folder and checking for the video name while or after being recorded
- During recording if GECKO ever shows a message saying 'Grabbing', wait for the on-going recording to finish and then close GECKO and restart again as failure to do so might result in frames being dropped during the recording
- If Windows on any of the three PCs ask to update, do not proceed with the update and immediately contact the responsible person in the group or IT
- If the shared drive-behavgenom gets disconnected then double click on move_files_anyPC.ps1, which will open up a terminal (See Figure-8, 9 & 10), type CSCDOM\your username press enter and type your password in the next line and press enter again. This should connect the shared drive and start moving the files to the network
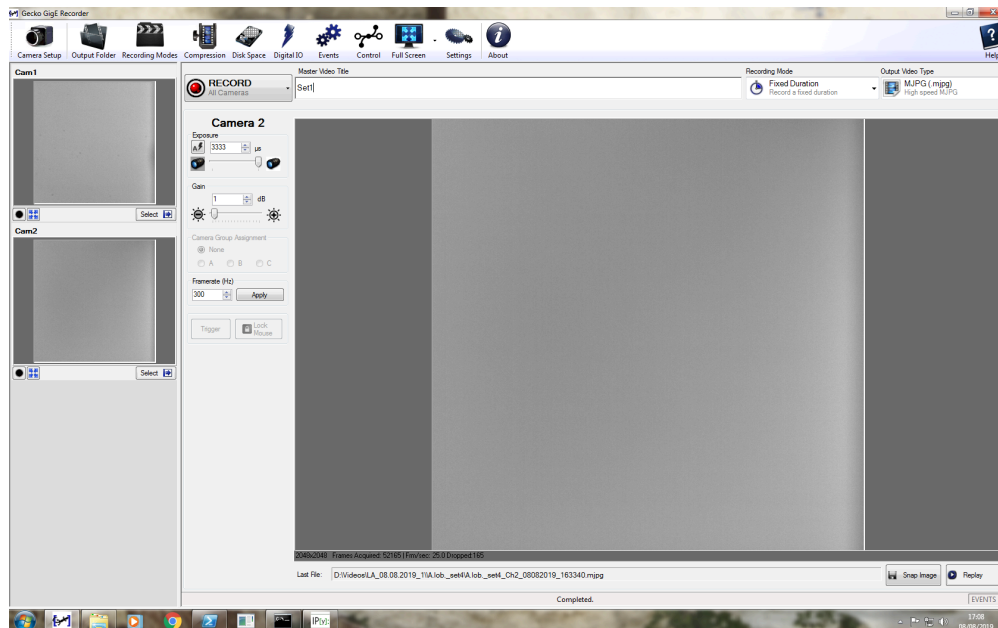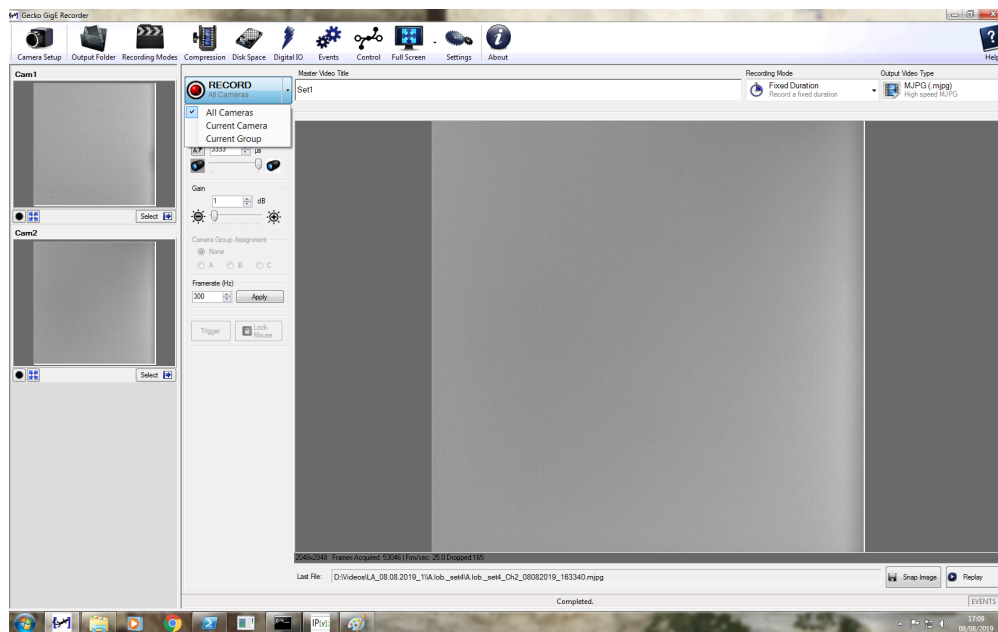
Figure 1: Gecko interface

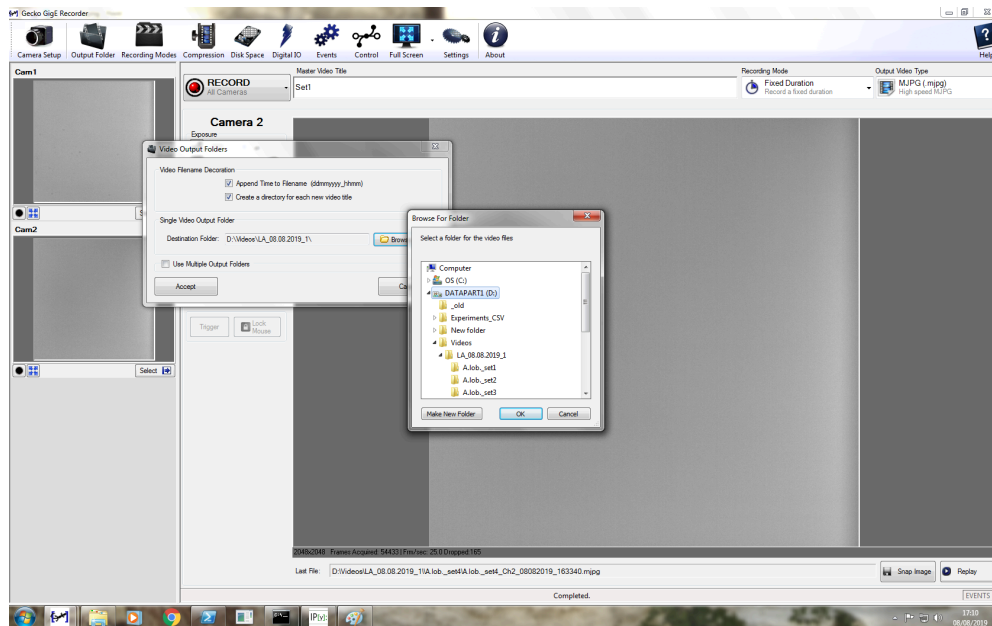Figure 2: Selecting either All or Single camera for recording

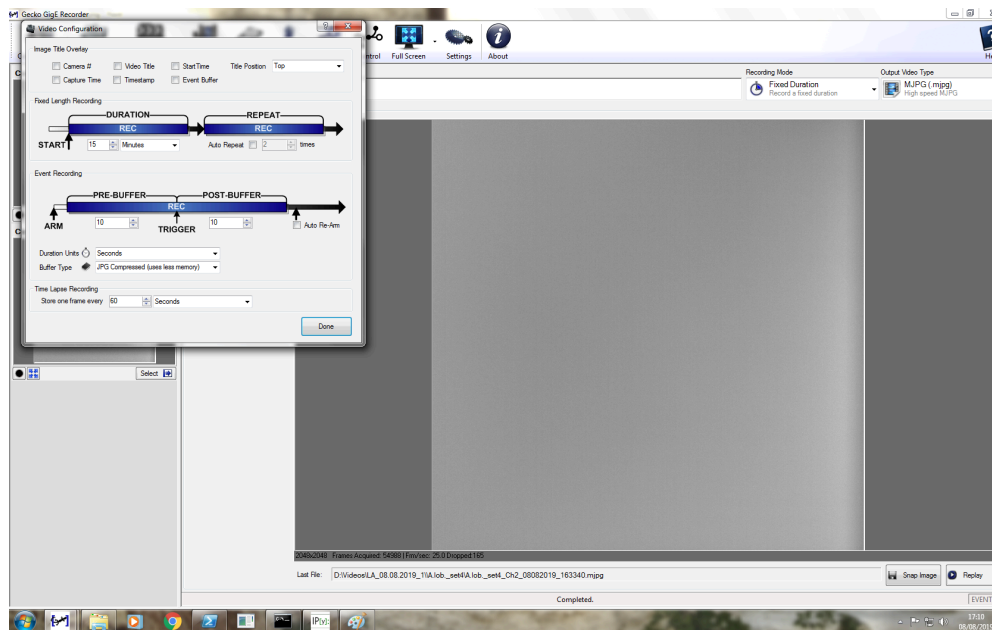Figure 3: Selecting the output/destination folder

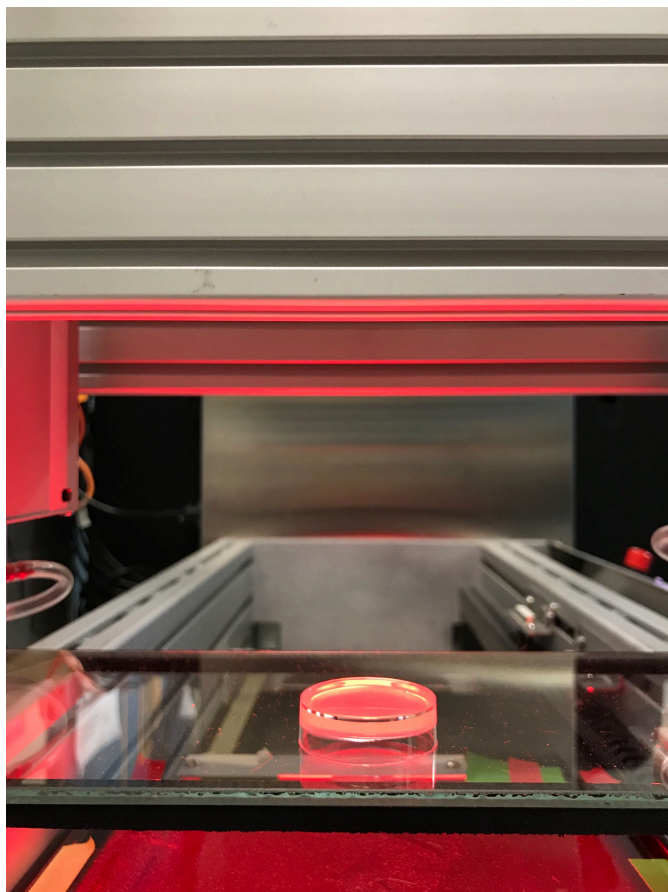Figure 4: Selecting the duration of recording in recording mode

Figure 5: Placing the imaging plate for recording (Open side down with lids off)

Figure 6: Pump for the air table, red knob parallel to the silver tube letting air in the system

Figure 7: Pump for air table, red knob perpendicular to the silver tube isolating the compressed air
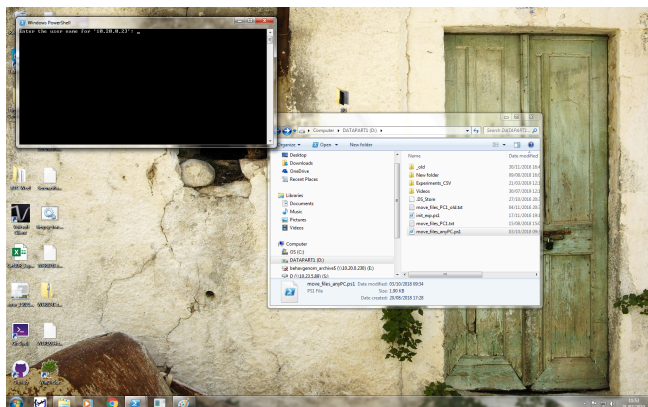

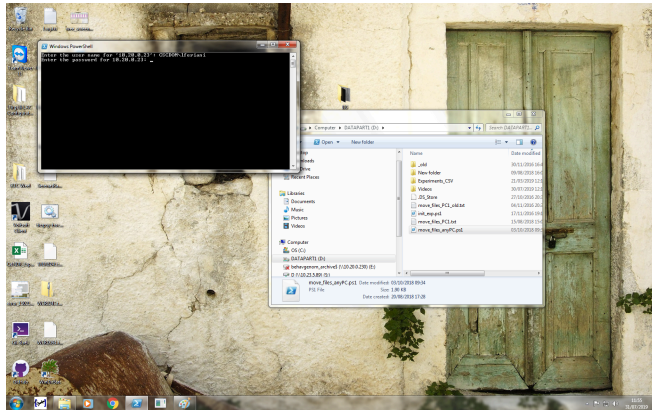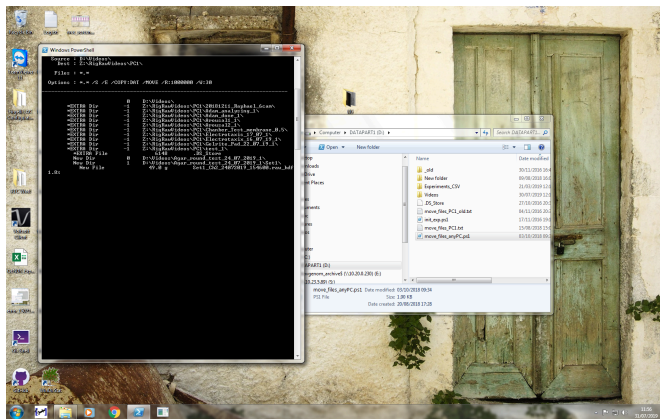
Figure 8: Terminal to connect to behavgenom

Figure 9: Input of credentials

Figure 10: Moving files to behavgenom