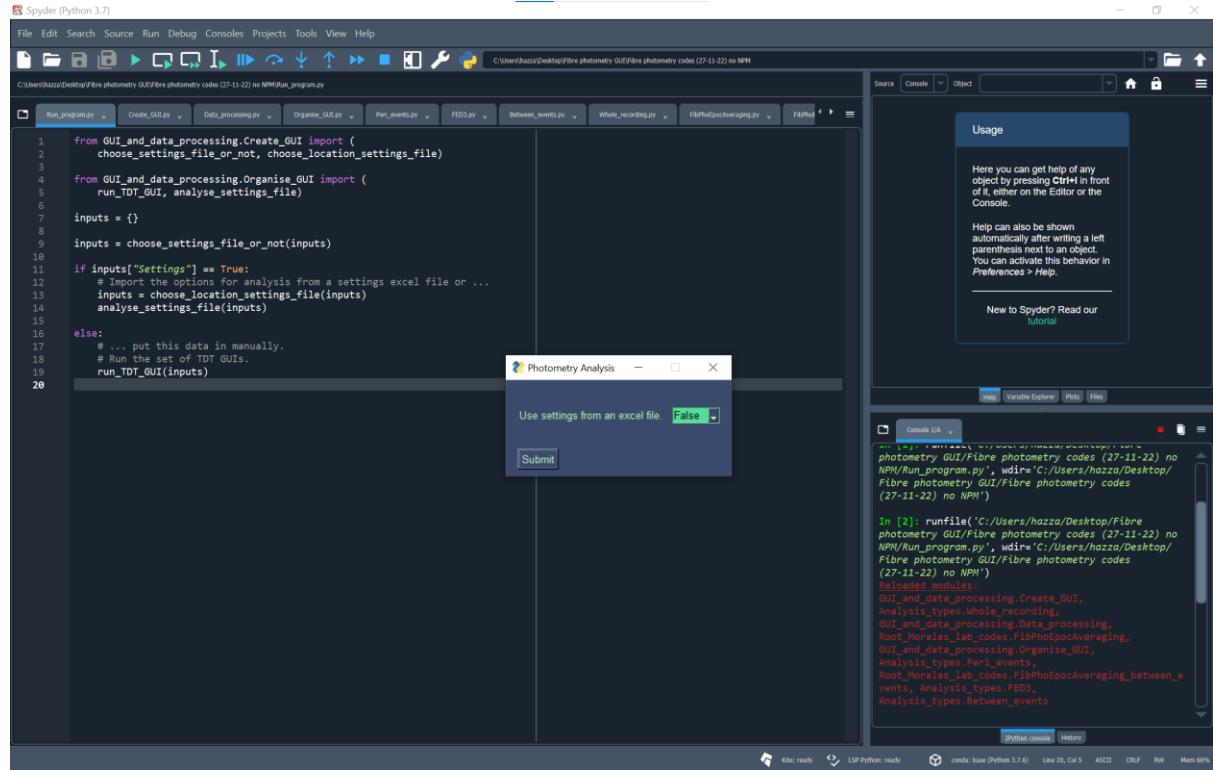


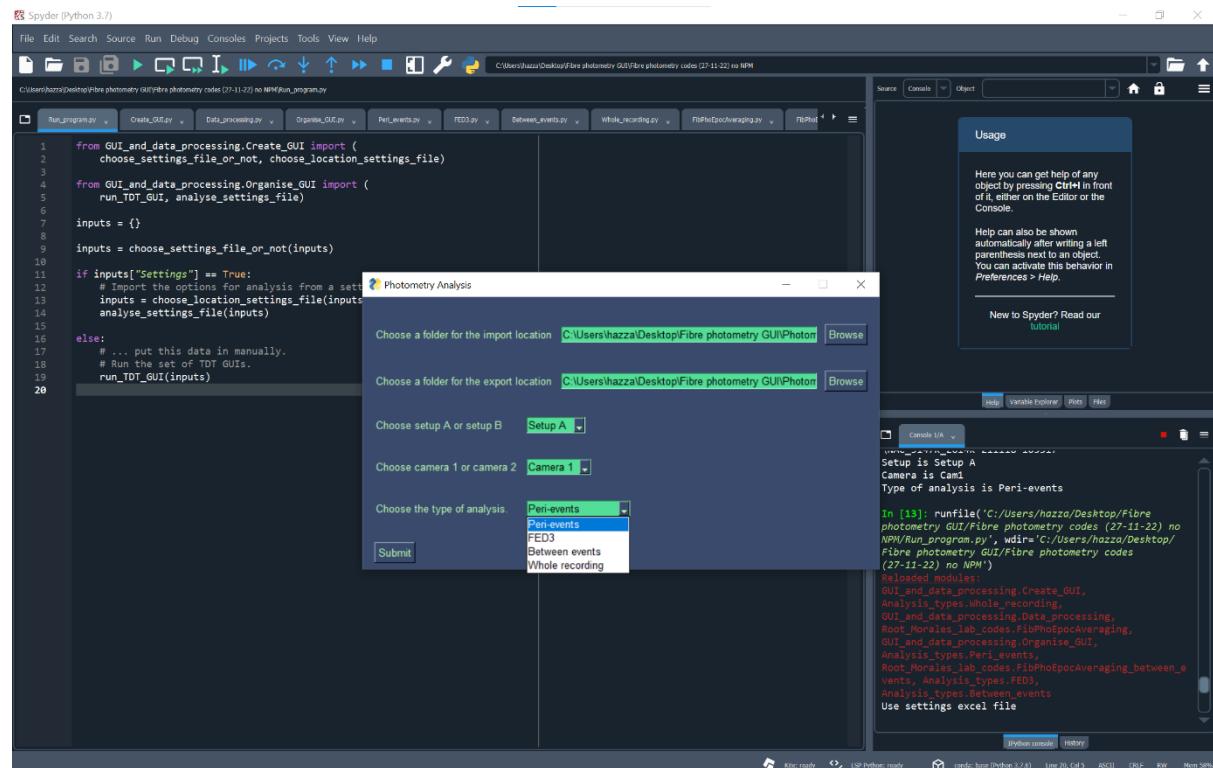
# How to use TDT Fibre Photometry GUI

## Basic options

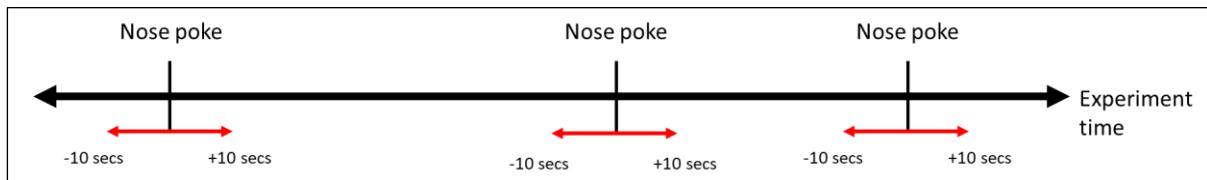
- Leave this as false for now (it will be explained from step 16).



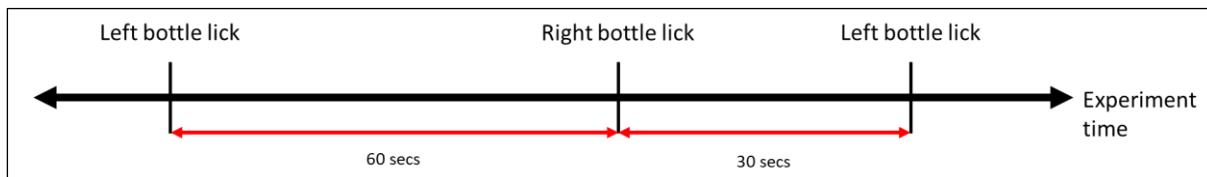
- Select basic options for analysis.



- **Import location:** the folder of the TDT tank.
- **Export location:** the folder to export the analysed data to
- **Setup:** choose the ISOS and GCaMP stream names.
  - Setup A: the ISOS signal is \_405A and the GCaMP signal is \_465A
  - Setup B: the ISOS signal is \_415A and the GCaMP signal is \_475A
  - Custom: this creates a new window where you can select these signals yourself.
- **Camera:** choose the video in the TDT tank that corresponds to the setup used. If “Camera 1” is selected, it will look for the video with “Cam1” in the filename and similarly for “Camera 2”.
- **Type of analysis:**

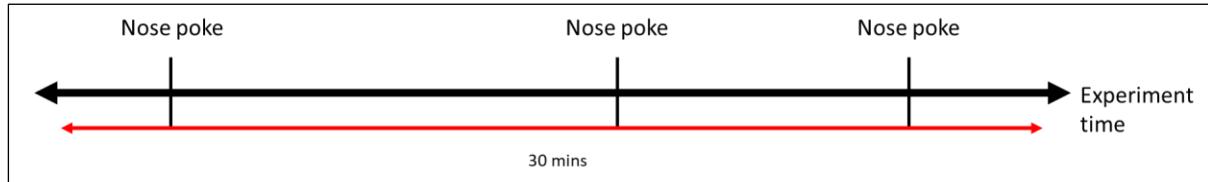


- Peri-events:
  - Analyse a time window around event onsets. For example, analyse the 10 seconds before and after all nose pokes.
- FED3:
  - This is the same analysis as peri-events, just personalised for the Feeding Experimentation Devices.
  - This records 3 events at the same time (left poke, right poke and pellet retrieval).
  - This will indicate whether nose pokes are rewarded with pellet drops in the analysed data.
  - It also assumes that these events are recorded automatically and stored within the “epochs” section of the TDT tank.



- Between events:
  - Analyse the time window between event onsets. For example, analyse the 60 second interval between 2 nose poke events.
  - This is also personalised for 2 bottle choice, open field and elevated plus maze tests.

- The automatically identified events for “left bottle” and “right bottle”, or “outer zone” and “inner zone” can also be customised to any 2 events.

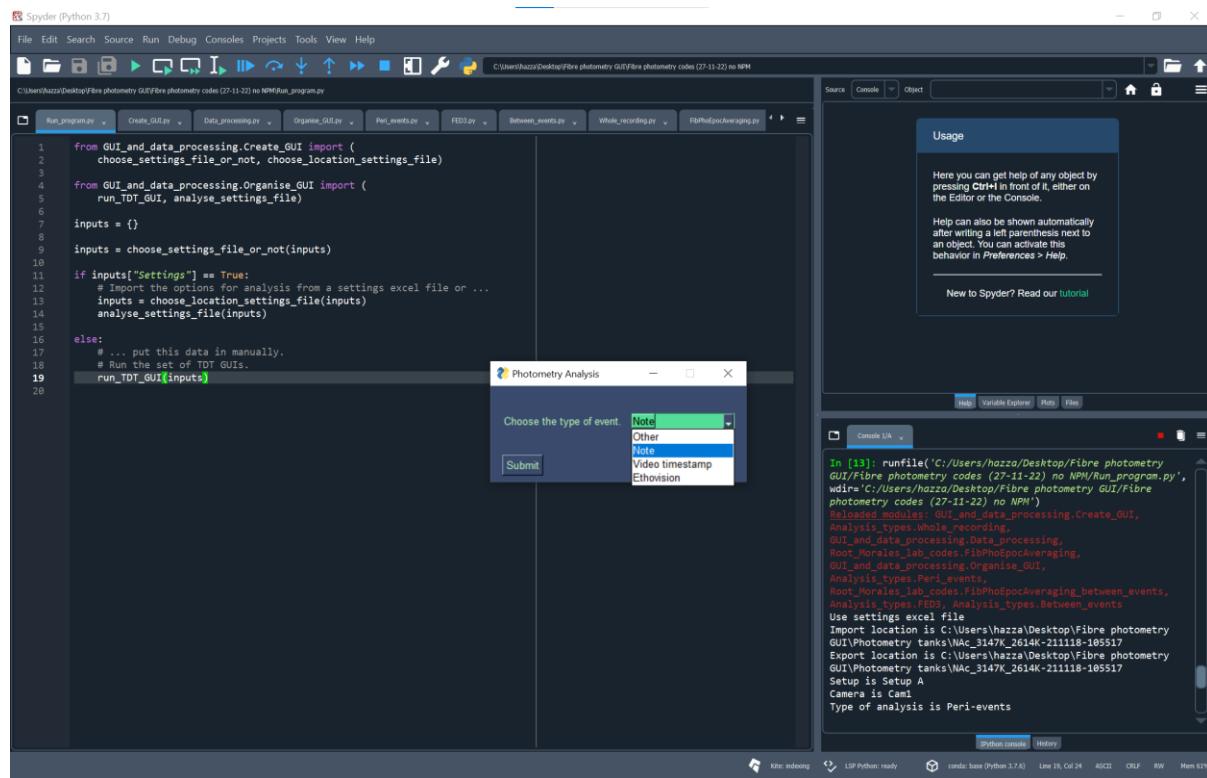


- Whole recording:

- Analyse the whole recording for the experiment and label events that you are interested in.
- This will create a plot with the events colour-coded and an optional raw data file.

## Peri-events

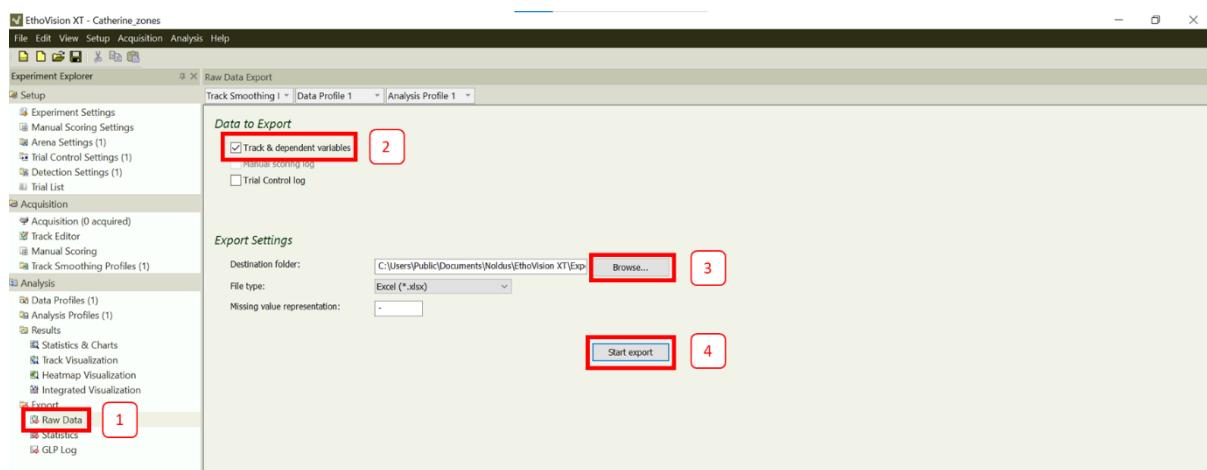
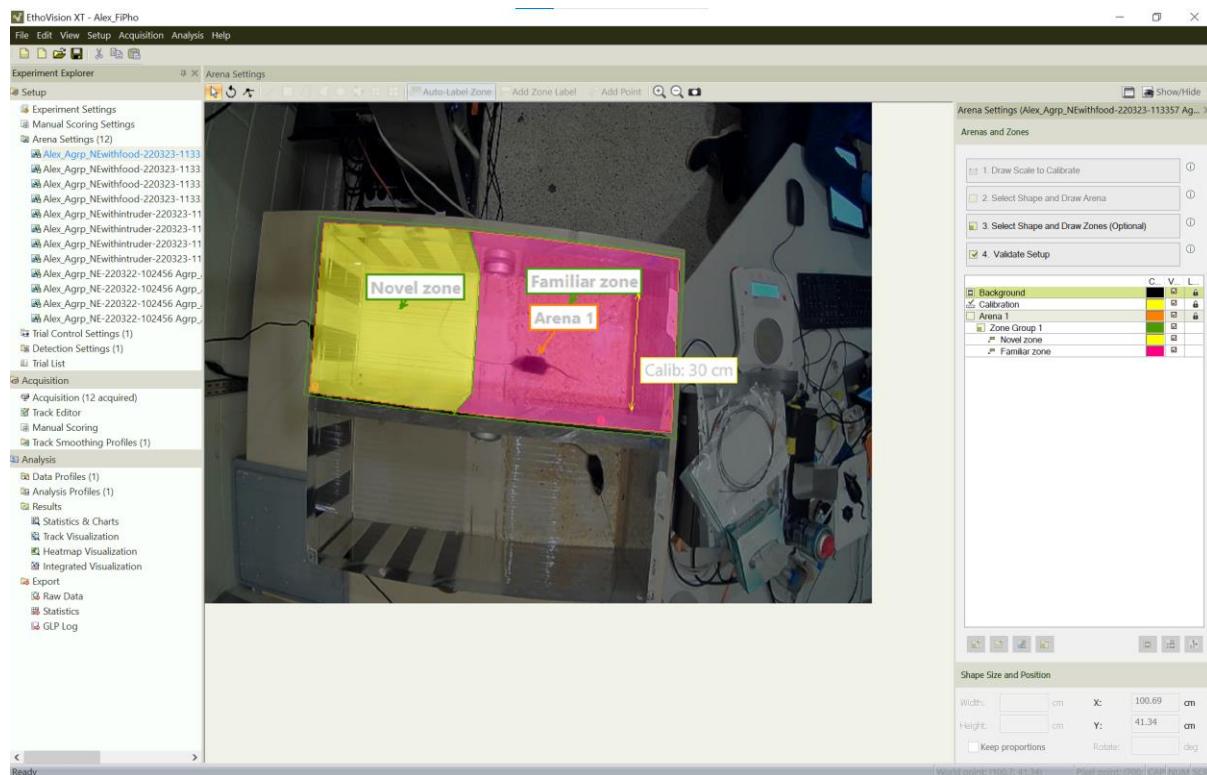
### 3. Choose the type of event for analysis.

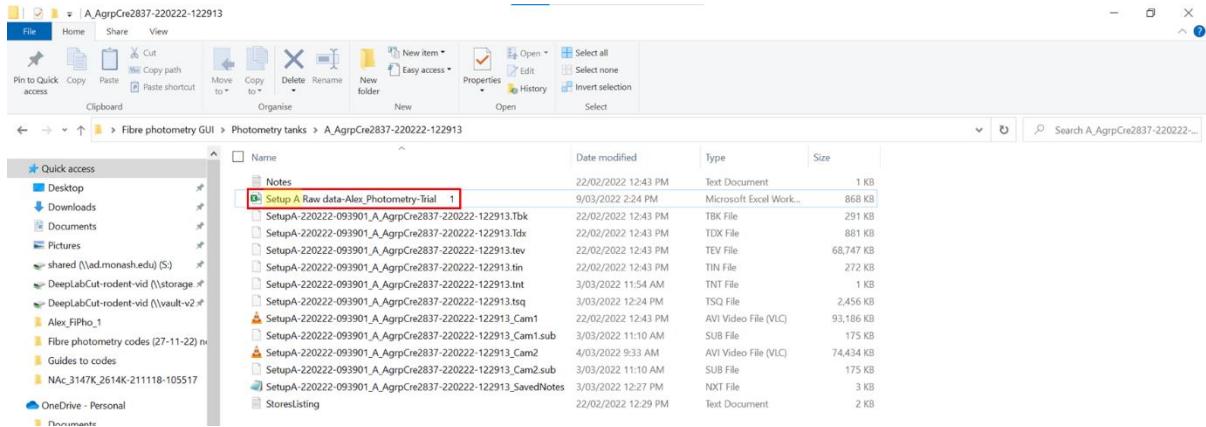


“Other”, “Note” and “Video timestamp” are all sourced from the “epoch” section of the TDT tank.

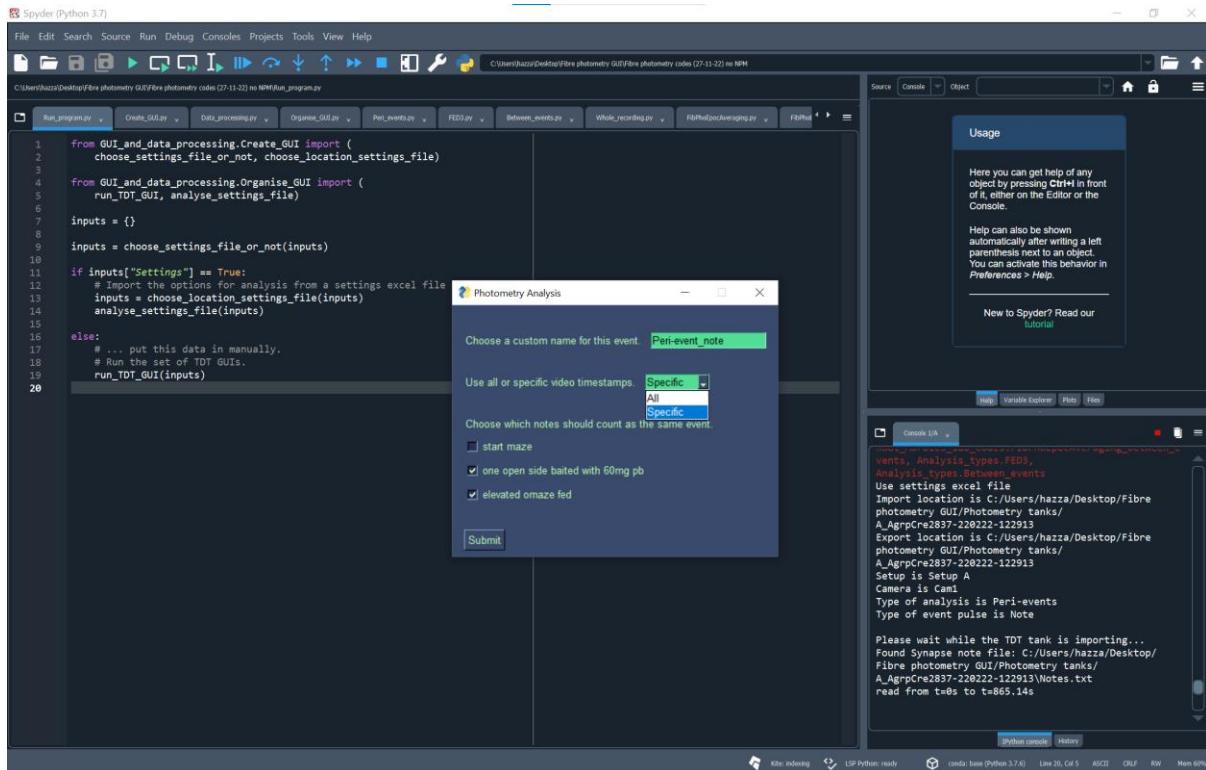
- Other:** the automatically recorded events other than “Note”, “Cam1” or “Cam2”.
- Note:** the manually recorded events stored as “Note”.
- Video timestamp:** the manually recorded events stored as either “Cam1” or “Cam2”.

- **Ethovision:** the manually recorded events from the Ethovision raw excel files.
  - These files are obtained from EthoVision XT 14.0. This is a video tracking software, which can be used to record entries of the mouse into zones.
  - The videos from the TDT tanks can be analysed using this program. Zones can be drawn as in the 1<sup>st</sup> image below.
  - This zone data can be exported as in the 2<sup>nd</sup> image below and aligned with the fibre photometry data using this GUI. Please note that the whole video should be recorded (not just when the mouse enters).
  - The raw excel files should be added to the TDT tank folder as in the 3<sup>rd</sup> image below. The start of the filename should also be edited to the selected setup (either “Setup A”, “Setup B” or “Custom”).

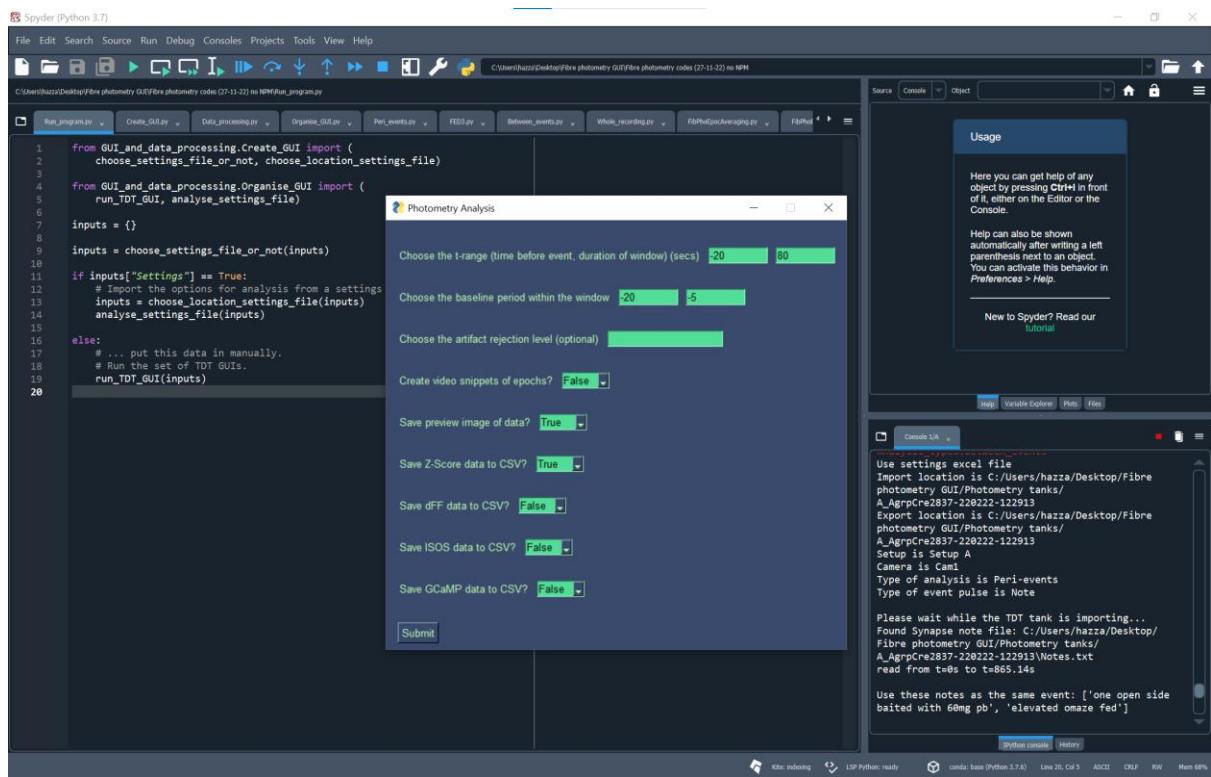




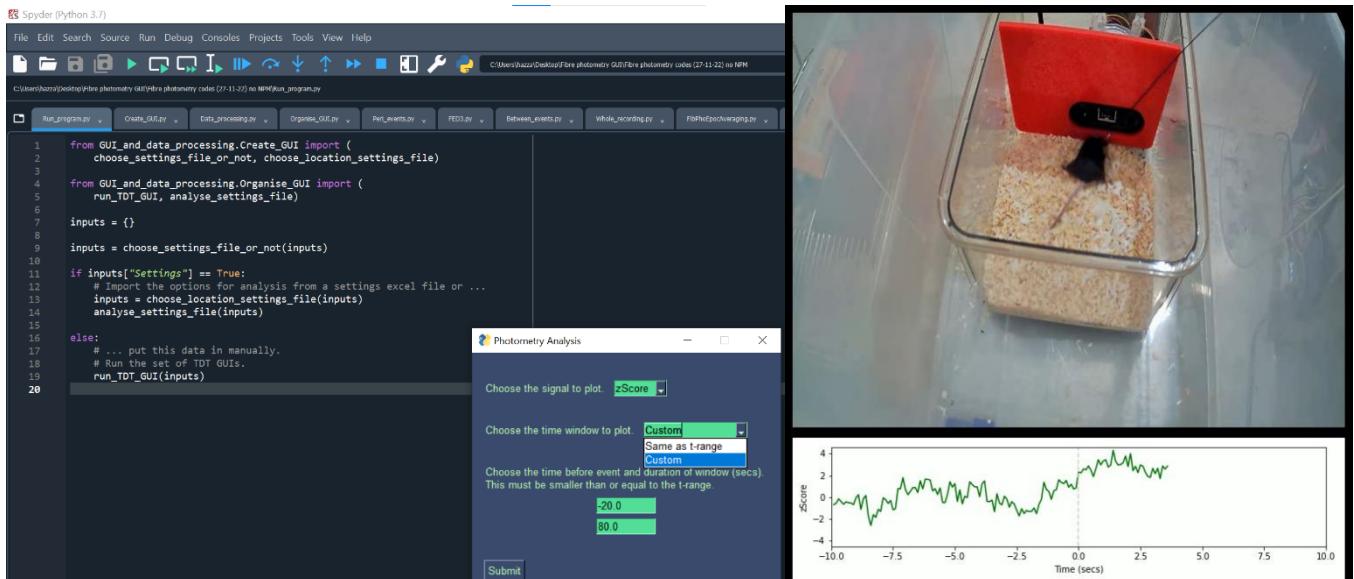
4. The type of event I selected was “Note” for the window below. If all notes should be selected, regardless of the names, select “all”. If specific sub-event(s) should count as the same event, “select” specific and choose the sub-events.



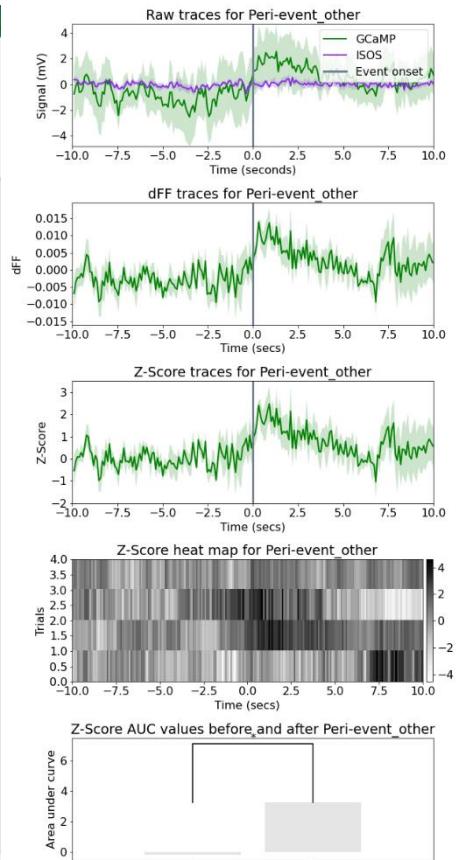
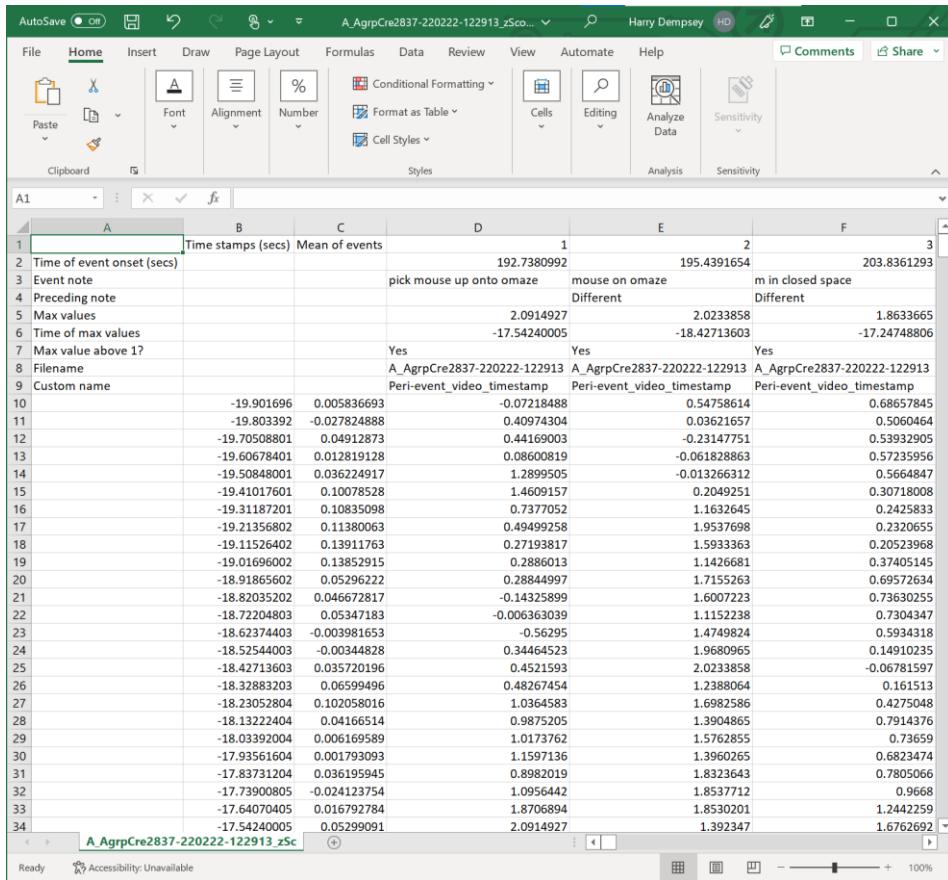
5. Choose the final options before analysis.



- **t-range:** select the peri-event window by choosing the time before event onset and total window duration. For example, if the t-range is (-20, 40), analyse the 20 secs before to 20 secs after each event.
  - **Baseline period:** choose the time period before event onset that will count as a baseline signal. This is used to calculate the Z-score values.
  - **Artifact rejection level:** this is optional. It allows signal artifacts above a given threshold to be excluded.
  - **Create video snippets of epochs:** this creates video snippets of each event and overlays the signal plots underneath. If this is set to True, you will choose the signal type and the duration to plot, as in the image 1<sup>st</sup> below. “Same as t-range” is recommended. A preview of the video snippet is in the 2<sup>nd</sup> image below.



- **Save preview image:** this plots the ISOS, GCaMP, dFF and Z-score plots with all the events overlayed on top of each other. It also includes a heatmap of the Z-scores over time for each event and the area under curve for the Z-score before 0 secs and after 0 secs. See the image below.
- **Save Z-score, dFF, ISOS or GCaMP data to CSV:** export the data type of interest. The data will be presented in the format below.



## FED3

### 6. Choose the options for FED3 (Feeding Experimentation Device) analysis.

The screenshot shows a Spyder Python 3.7 interface with a code editor and a terminal window. The code editor displays a script for FED3 analysis, and the terminal window shows the output of the script execution. A "Photometry Analysis" dialog box is open, prompting the user to choose a custom name (FED3), active poke (Left), and poke to analyse (Left). The terminal output shows the analysis settings and file paths.

```

from GUI_and_data_processing.Create_GUI import (
    choose_settings_file_or_not, choose_location_settings_file)

from GUI_and_data_processing.Organise_GUI import (
    run_TDT_GUI, analyse_settings_file)

inputs = {}

inputs = choose_settings_file_or_not(inputs)

if inputs["Settings"] == True:
    # Import the options for analysis from a settings excel
    inputs = choose_location_settings_file(inputs)
    analyse_settings_file(inputs)

else:
    # ... put this data in manually.
    # Run the set of TDT GUIs.
    run_TDT(inputs)

```

**Photometry Analysis**

Choose a custom name for this analysis: **FED3**

At the moment, "changing" just makes the active pokes the ones that preceded a pellet drop.

Choose the active poke: **Left**

Choose the poke to analyse: **Left**

Confirm the event names for the pokes and pellet events

Left poke: **Left**

Right poke: **Right**

Pellet drop: **Pelt**

Submit

**Usage**

Here you can get help of any object by pressing **Ctrl+H** in front of it, either on the Editor or the Console.

Help can also be shown automatically after writing a left parenthesis next to an object. You can activate this behavior in Preferences > Help.

New to Spyder? Read our [tutorial](#).

Source Console Object Help Variables Explorer Plot File

Enable I/A

Root\_Morales\_lab\_codes.FibPhotocAveraging\_between\_events, Analysis\_types\_FED3, Analysis\_types\_Between\_events

Use settings excel file location is C:\Users\hazza\Desktop\Fibre photometry GUI\photometry tanks\VMc\_3147K\_2614K-211118-105517

Export location is C:\Users\hazza\Desktop\Fibre photometry GUI\photometry tanks\VMc\_3147K\_2614K-211118-105517

Setup is Setup A

Camera is Cam1

Type of analysis is FED3

- **Active poke:** choose the poke that causes a pellet drop as a reward. This could be “left”, “right” or “changing”. At the moment, “changing” causes rewarded pokes to just be the ones that happen before a pellet drop.
- **Poke to analyse:** decide which pokes to include in the analysed data file. This could be “left”, “right” or “both”.
- **Confirm the event names for the left poke, right poke and pellet drop:** the event names are taken from the “epoch” section of the TDT tank. If they resemble “Left”, “Right” and “Pellet”, they are automatically filled in. Otherwise, you can manually change these.

7. Fill in the final options before analysis as in step 5

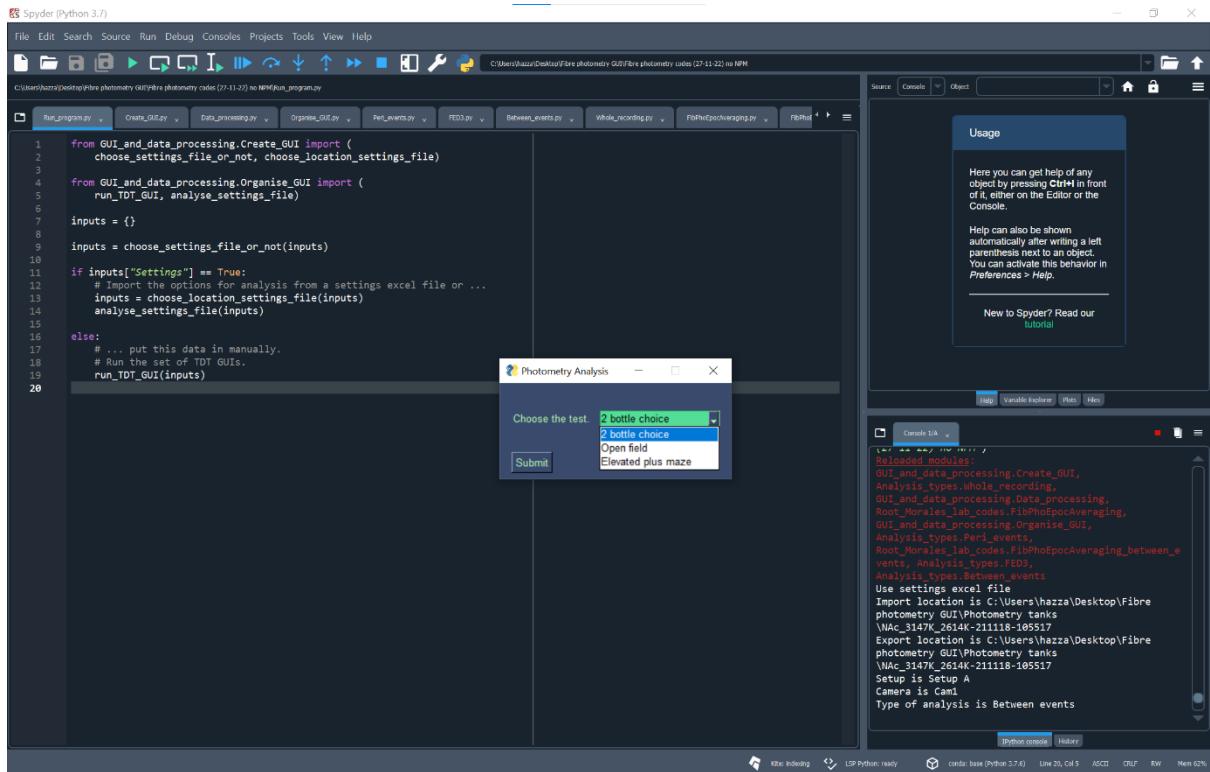
8. Here is the FED3 output excel file. This has the same features as the excel file generated in step 5. In addition, it splits columns into sheets based on nose pokes, pellet retrievals and rewarded/non-rewarded pokes.

The screenshot shows an Excel spreadsheet titled "NAc\_3147K\_2614K-211118-105517.zScore.FED3\_Setup.A". The "Overall" sheet displays a table of data with columns for Time stamps (secs), Mean of events, and various event types (Left, Right, Pellet). The "Rewarded pokes" sheet highlights rows where the event type is "Rewarded". The "Pellets" sheet shows data related to pellet retrievals. The "Non-rewarded pokes" sheet shows data for non-rewarded pokes. The "All pokes" sheet contains all the raw data. The Excel ribbon at the top includes tabs for Home, Insert, Page Layout, Formulas, Data, Review, View, Automate, and Help. The status bar at the bottom indicates "Ready" and "Accessibility: Good to go".

## Between events

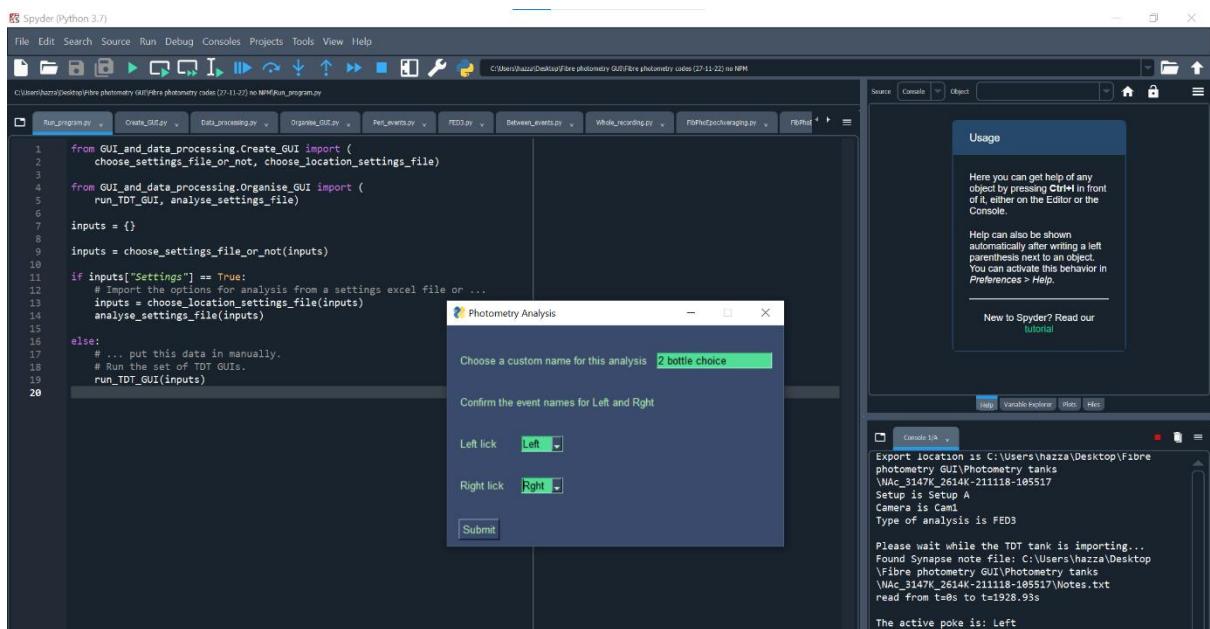
9. Choose the test to analyse. This involves finding the time between 2 types of events, as in the table below. If you want to analyse a different type of test, just select the first option and change the event names yourself.

	Event 1	Event 2
<b>2 bottle choice</b>	Left bottle lick	Right bottle lick
<b>Open field</b>	Entering outer zone	Entering inner zone
<b>Elevated plus maze</b>	Entering closed arm	Entering open arm



10. Choose the type of event for analysis. See step 3 for a description of each type.

11. Confirm the automatically suggested event names or change them.



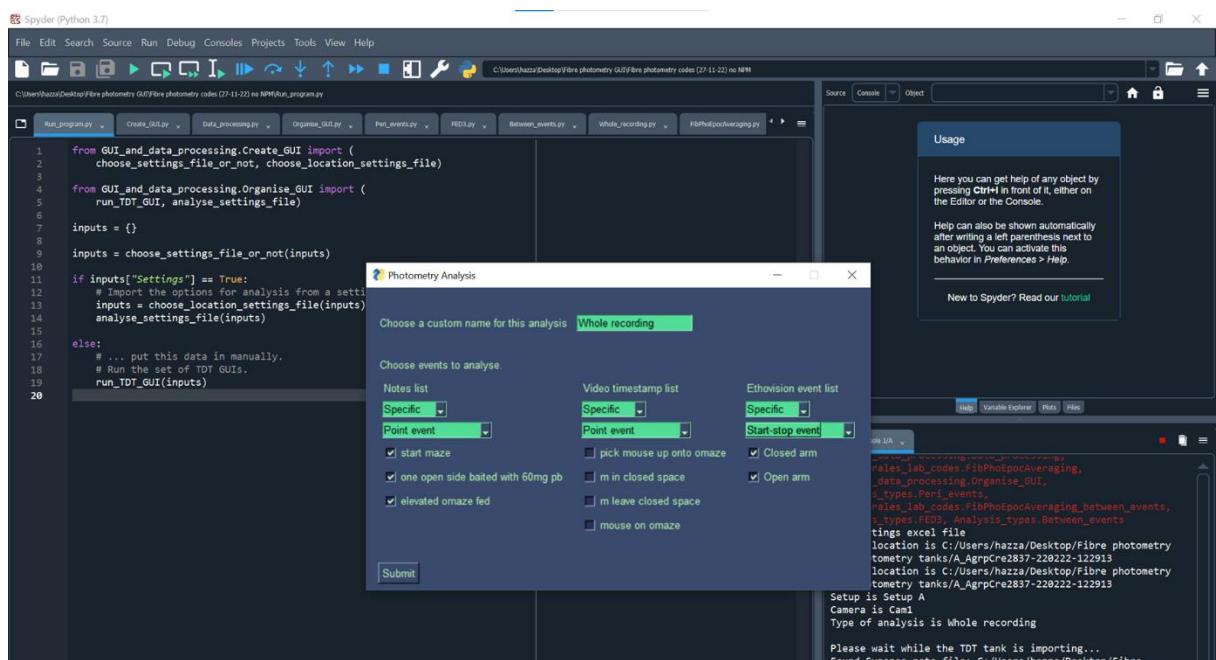
12. Fill in the final options before analysis as in step 5.

13. Here is the between events excel file. The only differences to the excel file in step 5 are that there is a “mean of columns” row and the columns for each event are split into separate sheets. The columns are also different lengths, as the interval between events changes every time.

	A	B	C	D	E	F	G	H
1	Time of event onset (secs)	Time stamps (secs)	Mean of events	Left	Right	Same	Rght	Same
2			43.63812864		69.96426752		80.7911424	173.3738496
3	Event note			Right	Different			
4	Preceding note				Right	Different		
5	Max values					Same		
6	Time of max values			1.29628706	0.615802705		2.337168932	1.670908451
7	Max value above 1?			3.73551925	4.91519902		11.30495977	23.00313554
8	Mean of column			Yes	No	Yes	Yes	Yes
9	Filename		NAc_3147K_2614K-211118-105517	NAc_3147K_2614K-211118-105517	NAc_3147K_2614K-211118-105517	NAc_3147K_2614K-211118-105517	NAc_3147K_2614K-211118-105517	NAc_3147K_2614K-211118-105517
10	Custom name		2 bottle choice					
11	0.098303998	0.040270021		0.7249040401	0.291448236		0.431824237	-0.631054223
12	0.196607996	-0.020426506		1.10021353	-0.108464986		0.348541319	-0.812222064
13	0.294911994	0.024421469		1.022125244	-0.228676051		0.655444443	-0.845661233
14	0.393215992	0.005224274		0.837642848	0.06911055		0.584679782	-0.959947526
15	0.49151999	0.050334527		1.009865403	0.141710147		0.707287908	-1.100058675
16	0.589823998	0.006496697		0.686111867	-0.049226835		0.540153444	-0.777784646
17	0.688127986	-0.046935186		0.402100205	-0.223354583		0.960816324	-0.97197479
18	0.786431984	0.030008057		0.560505748	0.125625357		1.241219521	-1.222699165
19	0.884735982	0.01671666		0.580583513	0.039128039		1.230975509	-1.012295008
20	0.98303998	-0.096899906		0.325837195	-0.200932324		1.142610908	-1.06744647
21	1.081343978	-0.083665251		0.548588395	-0.45520699		1.236590147	-0.759150982
22	1.179647976	-0.05620114		0.505128086	0.17919071		1.082881808	-0.699599504
23	1.277951974	-0.009317531		0.348502547	0.236668229		0.922017753	-0.8676579
24	1.376255972	-0.085609421		0.349093735	-0.247051284		0.874348104	-0.857358992
25	1.474559971	0.059346279		0.714983046	-0.142091528		0.803883851	-0.724713564
26	1.572863969	0.069583001		0.539927304	0.148054898		0.976917267	-0.78879106
27	1.671167967	0.112283932		0.301136583	-0.016718054		0.725963593	-0.789437175
28	1.769471965	0.051267708		0.579013467	0.415261358		0.825586557	-0.955159903
29	1.867775963	0.131610662		0.841900706	-0.013326001		1.211905718	-0.574810743
30	1.960719961	0.150717066		0.777826488	0.323265702		0.998510122	-0.776644289
31	2.064383959	0.149026068		0.769549847	-0.071711287		0.876160443	-0.644225061
32	2.162687957	0.062283579		1.25183177	-0.184379101		0.690757334	-0.595959784
33	2.260991955	0.016443068		0.951337993	-0.195851561		0.635453939	-0.58669685
34	2.359295953	0.069054116		0.760355771	0.109912083		0.547693491	-0.841144797

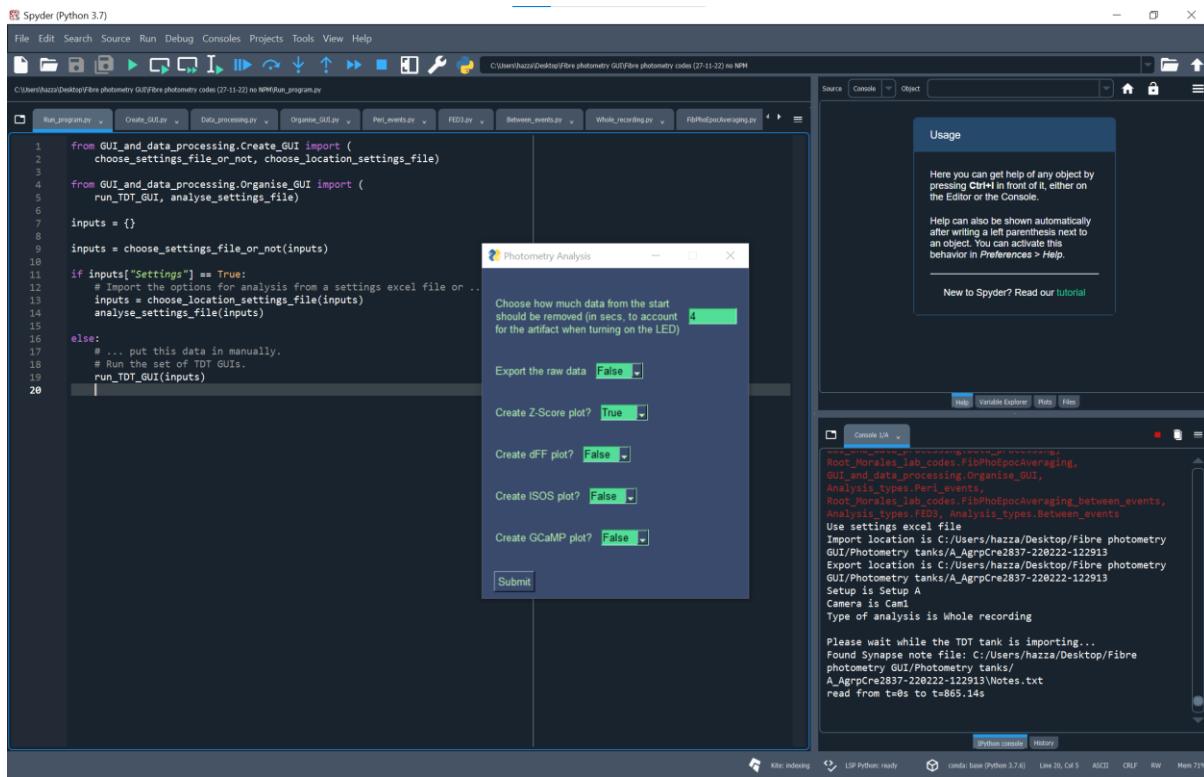
## Whole recording

14. Choose the events to annotate on a plot of the whole recording signal.



- All types of events (other, notes, video timestamps and Ethovision) are read and shown in the window above. In this example, there are no “other” events. These event types are explained in step 3.
- If all events within one event type should be plotted, select “all”. If only specific events should be plotted within one event type, select “specific”.
- If the events start and end at the same time (like dropping a peanut butter chip in a cage), select “point event”. If the events have a start and an end time (like being in the outer zone of an open field), select “start-stop event”.

## 15. Fill in the final options before analysis.

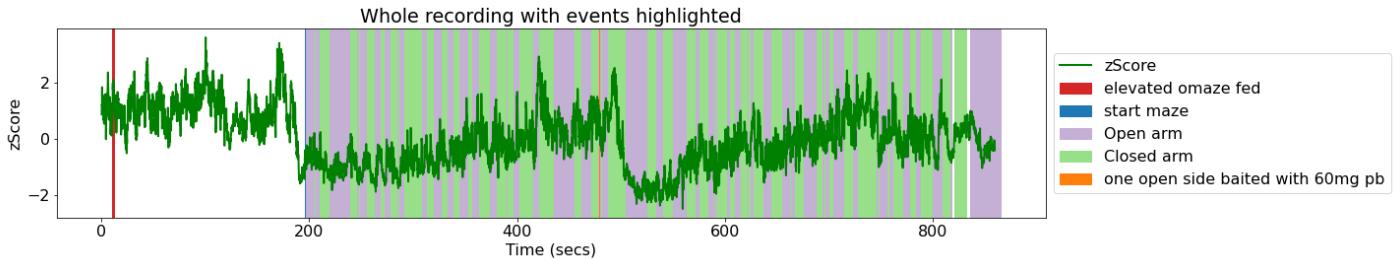


- The first line determines how much data should be excluded from the start of a recording. When the fibre photometry system is turned on, there is usually a large artifact. This should be excluded using a value such as 4 secs.
- You can export the raw data, which includes the time stamps, signal data (either Z-score, dFF, ISOS or GCaMP) and event information columns. See the image to the right.

A screenshot of Microsoft Excel showing a data table. The table has three columns: A (Timestamps (secs)), B (zScore), and C (Events). The data is as follows:

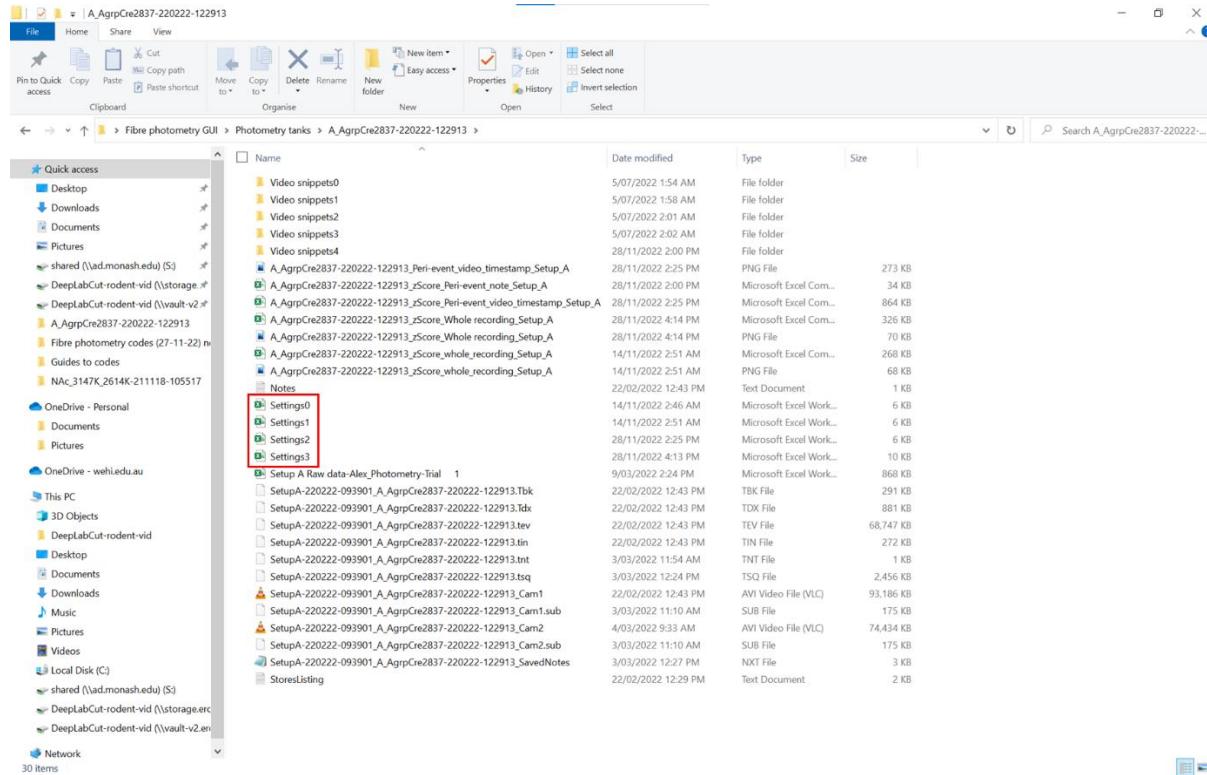
	A	B	C
1	Timestamps (secs)	zScore	Events
2	0.098303998	0.8720544	
3	0.196607996	1.035948	
4	0.294911994	1.2156827	
5	0.393215992	1.1796312	
6	0.49151999	1.839247	
7	0.589823988	1.3325518	
8	0.688127986	1.126149	

- You can create plots for each data type (Z-score, dFF, ISOS or GCaMP). Here is a Z-score plot with the events above annotated.



## Settings excel file

16. A settings excel file will be exported after every analysis run. These contain the settings needed to re-create the analysis. They will be called “Settings” with a 0, 1, 2, ... at the end depending on how many are already in the TDT tank.



17. Each file can be edited to run multiple analyses. You just need to leave a blank row between each set of options.

The screenshot shows two Microsoft Excel spreadsheets side-by-side. Both spreadsheets have the same column headers: A, B, C, D, E, F, G, H, I, J.

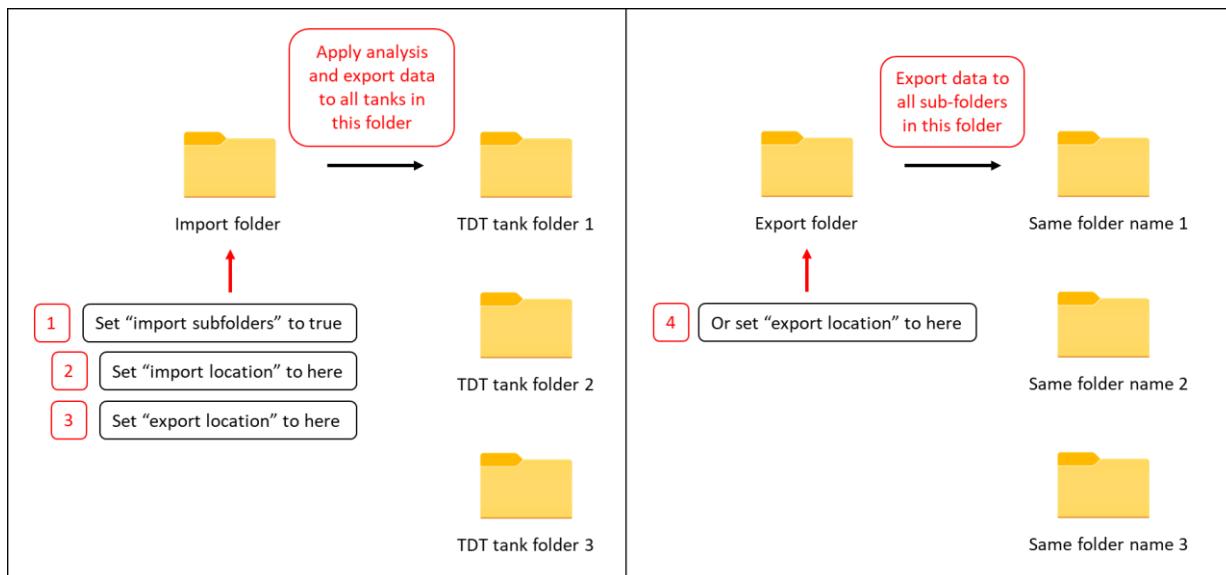
**Left Spreadsheet (Sheet1):**

	A	B	C	D	E	F	G	H	I	J
1	Import subfolders	FALSE								
2	Import location	C:\Users\hazza\Desktop\Fibre photometry GUI\Photometry tanks\NAc_3147K_2614K-21111								
3	Export location	C:\Users\hazza\Desktop\Fibre photometry GUI\Photometry tanks\NAc_3147K_2614K-21111								
4	Setup	Setup A								
5	Camera	Cam1								
6	Analysis	Peri-events								
7	Type	Other								
8	Name	Pelt								
9	Analysis name	Peri-event_other								
10	t-range	-10 20								
11	Baseline period	-10 -5								
12	Artifact RL	inf								
13	Image	TRUE								
14	Create snippets	TRUE								
15	Export ISOS	FALSE								
16	Export GCaMP	FALSE								
17	Export dFF	FALSE								
18	Export zScore	TRUE								
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										
32										
33										
34										

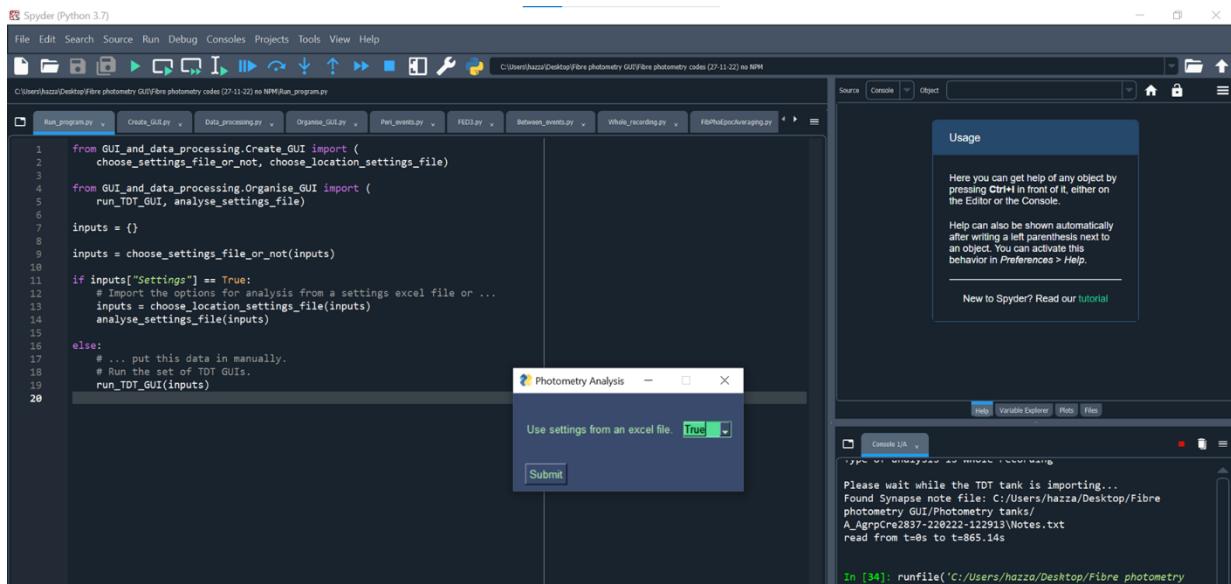
**Right Spreadsheet:**

	A	B	C	D	E	F	G	H	I	J
1	Import subfolders	FALSE								
2	Import location	C:\Users\hazza\Desktop\Fibre photometry GUI\Photometry tanks\NAc_3147K_2614K-21111								
3	Export location	C:\Users\hazza\Desktop\Fibre photometry GUI\Photometry tanks\NAc_3147K_2614K-21111								
4	Setup	Setup A								
5	Camera	Cam1								
6	Analysis	Peri-events								
7	Type	Other								
8	Name	Pelt								
9	Analysis name	Pelt_Setup_A								
10	t-range	-10 20								
11	Baseline period	-10 -5								
12	Artifact RL	inf								
13	Image	TRUE								
14	Create snippets	TRUE								
15	Export ISOS	FALSE								
16	Export GCaMP	FALSE								
17	Export dFF	FALSE								
18	Export zScore	TRUE								
19										
20										
21										
22										
23										
24										
25										
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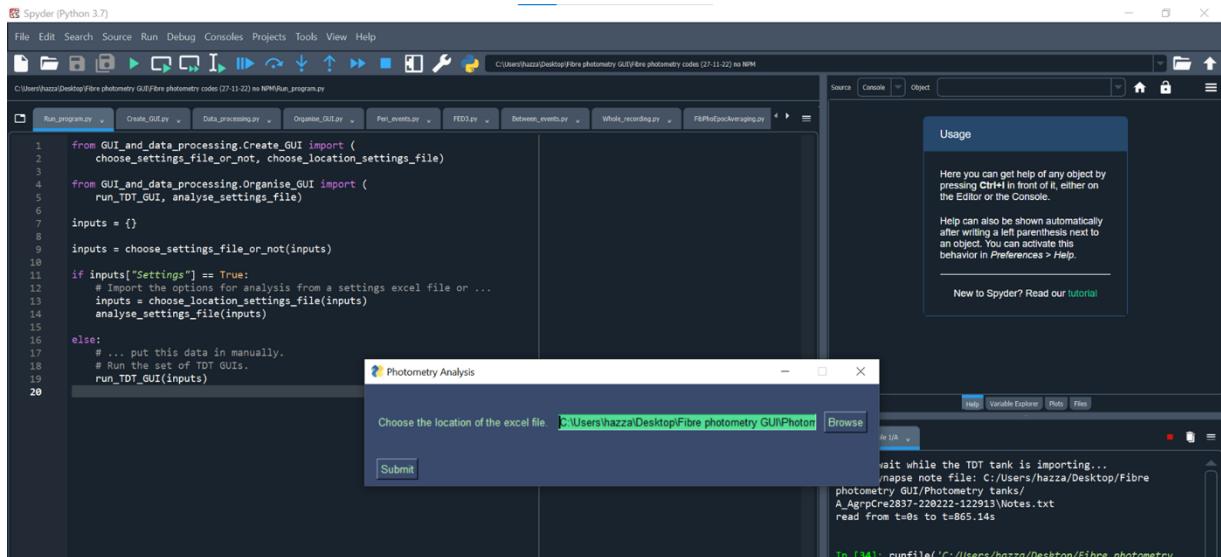
- There is an additional option called “import subfolders”.
  - Normally, the import location is the folder for a TDT tank. But this location can be changed to a folder of many tank folders.
  - If “import subfolders” is set to “TRUE”, the code will apply the same analysis settings to every tank within that folder.
  - The export location can be the same as the import location, or it can be another folder, with contains the same sub-folder names.



18. At the very start of the GUI, click true for using a settings excel file.



19. Choose the location of the settings excel file.



20. Hit submit to analyse all your data.