Transcription site classifying analysis for in vivo imaging

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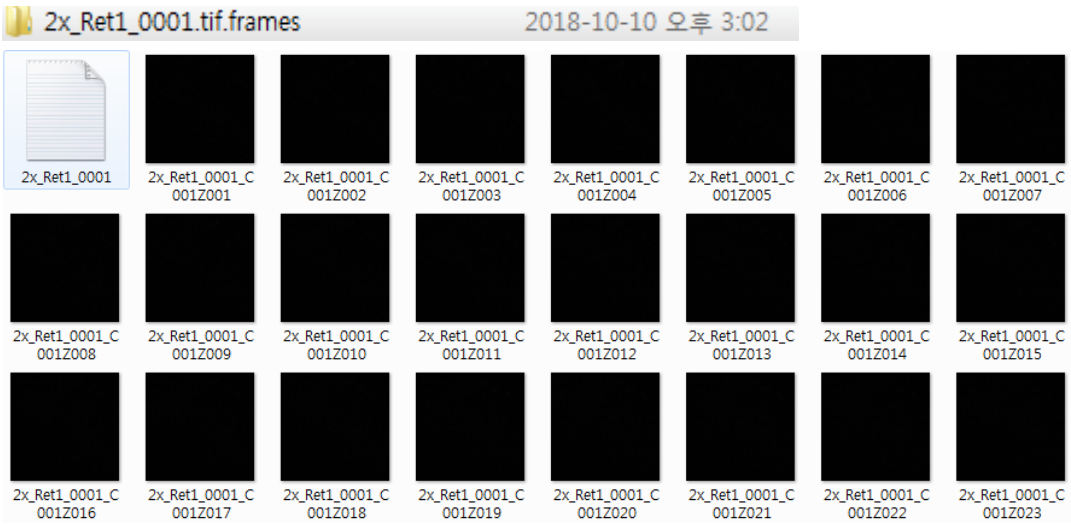
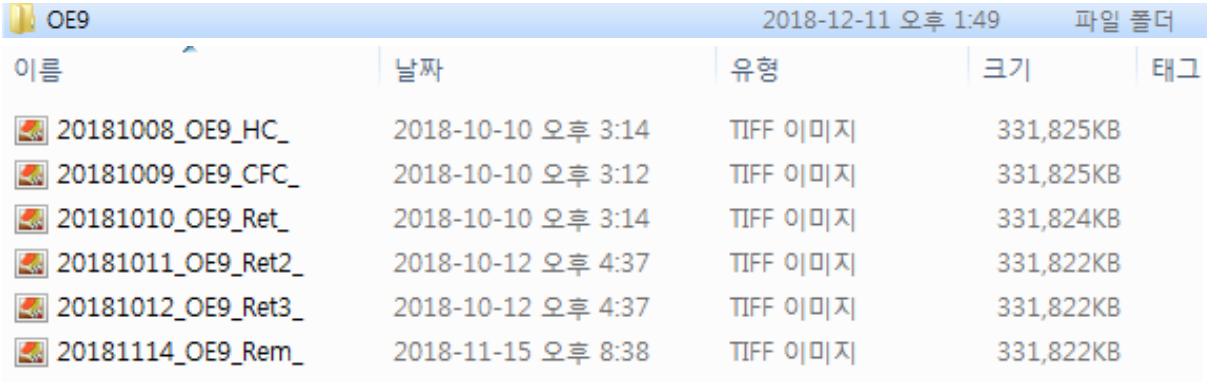
This document is written on the purpose of explaining the analysis method of transcription population. The analysis process is consist of (1) XY translation of image based on lipofuscin tracking, (2) Image registeration with different time point, (3) cell centroid detection, (4) classification, (5) measuring freezing behavior, (6) combining TXN data of several time point and freezing into one file and (7) statistics.

● XY translation of image based on lipofuscin

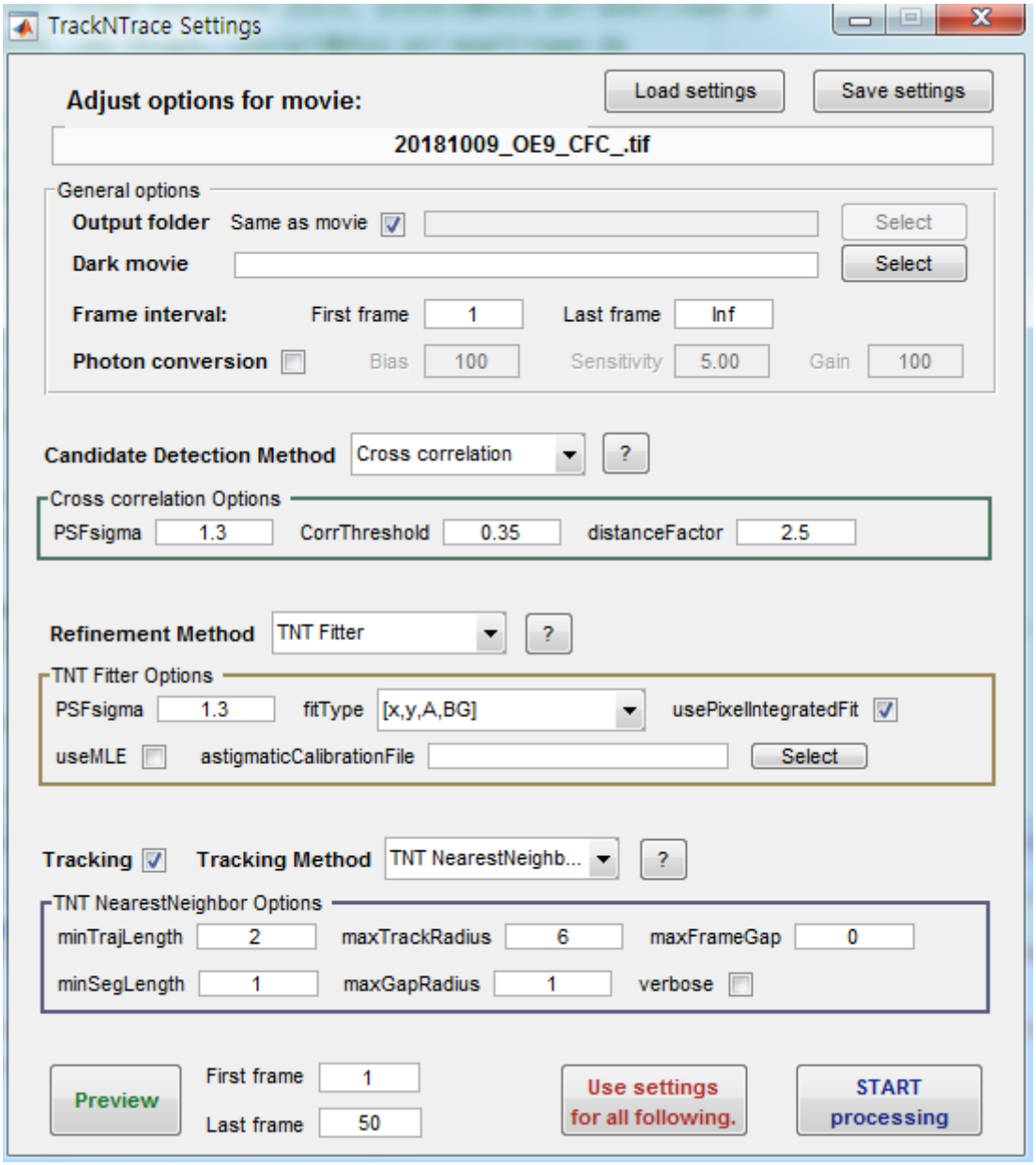
1. After imaging, you might have image files like this.



2. Prepare image files in stack file like this. ***Name sequence is very important. Don't forget the last under bar ('_'). ***
   Red z1, z2, z3, z4 ... Green z1, z2, z3, z4 … sequence.

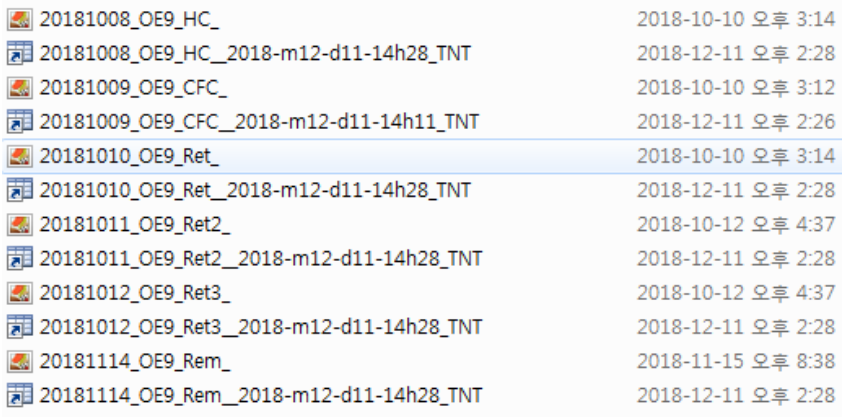


3. Run TrackNTrace. Run ***RunTrackNTrace.mat*** file.
   The tracking condtion is shown below.

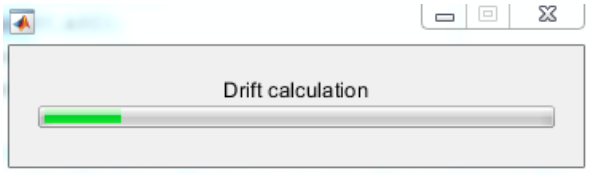


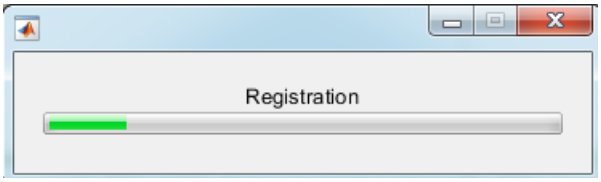
Parameters are not important to do the analysis except ***maxGapRadius***. It should be 1.

4. Now you will see the Image files and tracking files like this.



5. Then, run ***One_lipofuscin_tracking_registration.mat*** file.
6. Select the all raw images and all tracking data.





After two pop-up window, you will see the new files are appeared in the image file folder.

● Image registeration with different time point

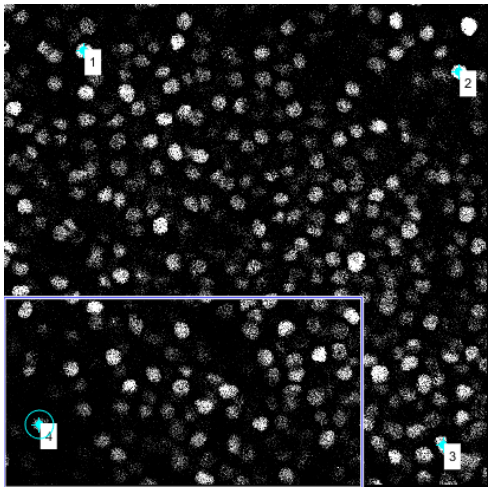
7. Open the new subtracted registrated files with Fiji.
Select one bright cell and crop the cell and do the reslice.
Match the z slice and set the range of each session.
ex) HC : 10~67 stack,
CFC : 22~79 stack,
Ret : 14~71 stack,
Ret2 : 14~71 stack,
Ret3 : 10~67 stack,
Rem. : 22~79 stack.

8. Open ***Two_image_registration,mat'*** file.

In the 28th line, you should enter the range of zstack which you got in 7. And set the output folder.

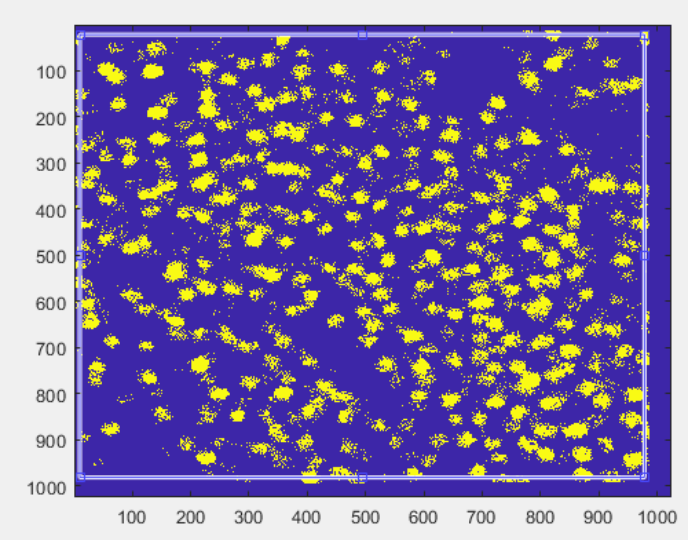
9. Run the code. Select the subtracted registrated image.

Choose 4 matching points. It is recommended to select the points in the corner. Export two variables, *'MovingPoints'* and *'FixedPoints'*.



● Cell centroid detection

10. Run ***Three_crop_cell_segment.mat***.



11. Define the crop position. After set the rectangle and double click. Then, close the window. The cell detection will start automatically after denoting crop position.

12. When the cell detection is over, you will see the Cell list file in the transformed folder.

● Classification

13. Open ***Four_cell_classify.mat***.

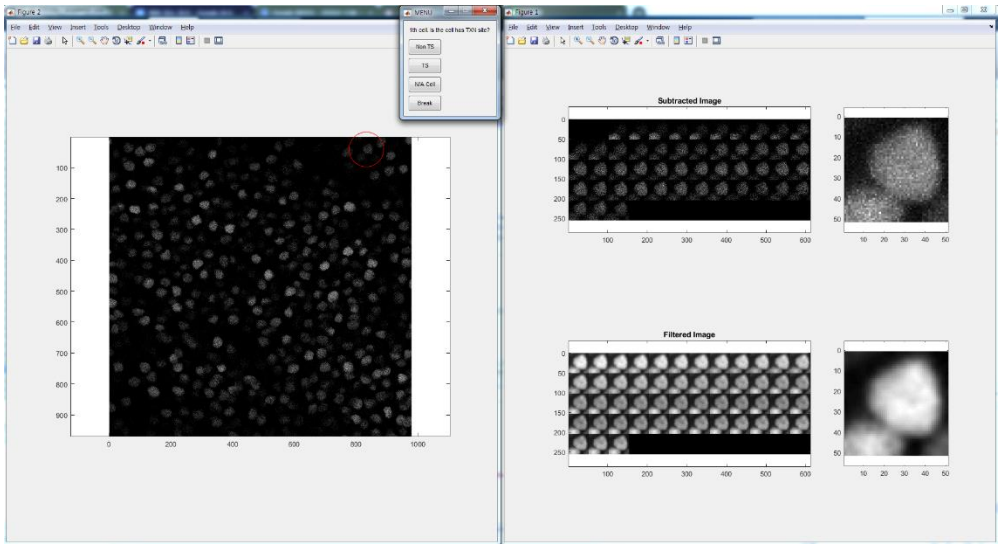
14. Set classifying folder in 4th line and set the cell size which indicates size to be cropped in 8th line.

15. Run ***Four_cell_class.mat***. Select the cropped subtracted registered image and cell list file.

16. If error comes from variable '*sp_name'*, check the *sp_name{2,1}* indicates the session of behavior.

17. You will see the window like below.

18. If you want to rest, you can break. And you can resume by restarting the code or by running from 18th line.

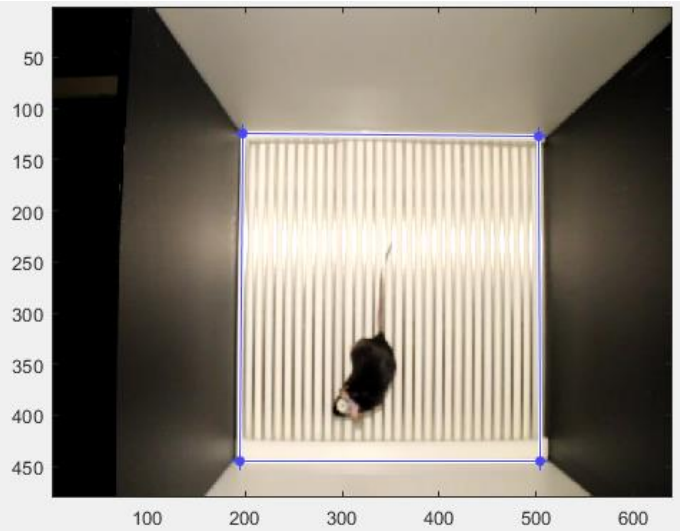


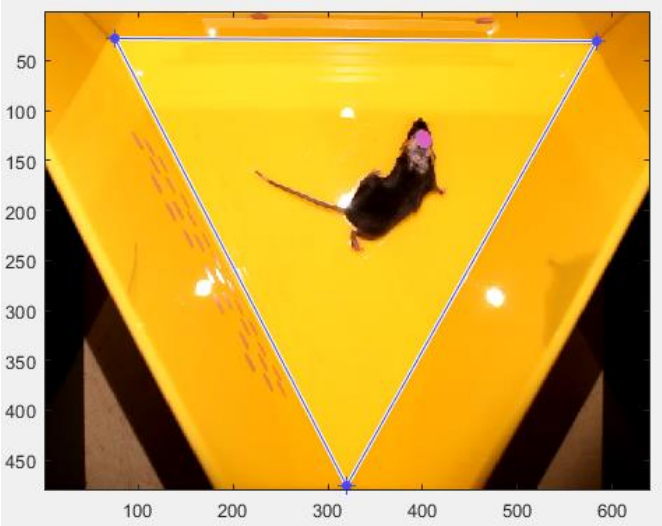
19. Once you finish the classification, you will see the NF_map file in the classification path.

● Measuring freezing behavior and freezing into one file and (7) statistics

20. Open ***Video_tracking.mat.*** If you are analyzing the trigonal prism shape context, open ***Video_tracking_triangle.mat***. The name of the video file (behavior video) should be format in 'mouse name_behavior session'. (ex, OE9_CFC)

21. In 3rd line and 4th line, change video path and video file name to your condition.
22. If you are analyzing CFC, in 7th line, the variable ‘maxtime’ should be 240. Otherwise, ‘maxtime’ is 180.
23. If the window pops up, create the ROI. Don’t make dark side inside of the ROI !





24. Repeat to other behavior session.

25. By seeing retrieval session, measure the freezing rate by looking every 3 second.

sec	3	6	9	12	15	18	21	24	Rate
Moving	O	X	O	O	X	X	O	X	
Valid Freezing	X	X	O	O	X	X	X	X	25 %

26. Run ***Freeze_tracking.mat.*** In 10th line, change the threshold until the variable Freez_rate_Ret is same with the rate you measure in 24.
27. Now you will see a file with mouse name in the behavior video folder (ex, ‘OE9.mat’)

● Combining TXN data of several time point

28. Open ***Combining_Data.mat***. In 6th line set your output path and in 7th line define the behavior session. In 8th line, set the data is overlap or not.
29. Run the code and select all NF_map files and behavior file (result if 27).
30. You will see the final result file which have all of the information in variable Final

Final

PLOTS | VARIABLE | VIEW

No Variable Selected

plot | Plot as m

SELECTION

1x1 struct with 7 fields

Field	Value
Data	*463x9 double*
Distance	*1x1 struct*
Freeze	*1x1 struct*
Mouse	'OE9'
Mouse_info	*2x13 cell*
behavior	*1x6 cell*
overlap	1

Final.Freeze

Field	Value
Baseline	0.0167
CFC	0.1905
Retrieval	0.4000
Retrieval2	0.6000
Retrieval3	0.6167
Retrieval4	0.4667

Final.Data

	1	2	3	4	5	6	7	8	9
1	120.0081	751.7190	6.7216	1	1	2	2	2	1
2	959.6892	293.7318	9.1169	1	1	2	2	2	1
3	354.9217	671.5190	2.6072	1	1	1	1	2	1
4	955.5030	108.8256	11.7704	1	1	1	1	2	1
5	966.7885	414.1132	9.0984	2	1	2	2	2	1
6	702.3047	751.0083	10.5075	1	1	1	1	1	1
7	405.0225	595.7030	9.8345	1	1	2	2	2	2
8	58.9713	861.5566	4.5457	2	1	2	1	2	1
9	651.6299	711.3837	10.8306	1	1	1	2	1	1
10	33.3302	750.8693	11.5106	1	2	1	2	2	3
11	942.0029	527.5320	12.6750	1	1	1	1	1	1
12	310.0201	452.7037	2.8661	1	1	1	1	2	1