

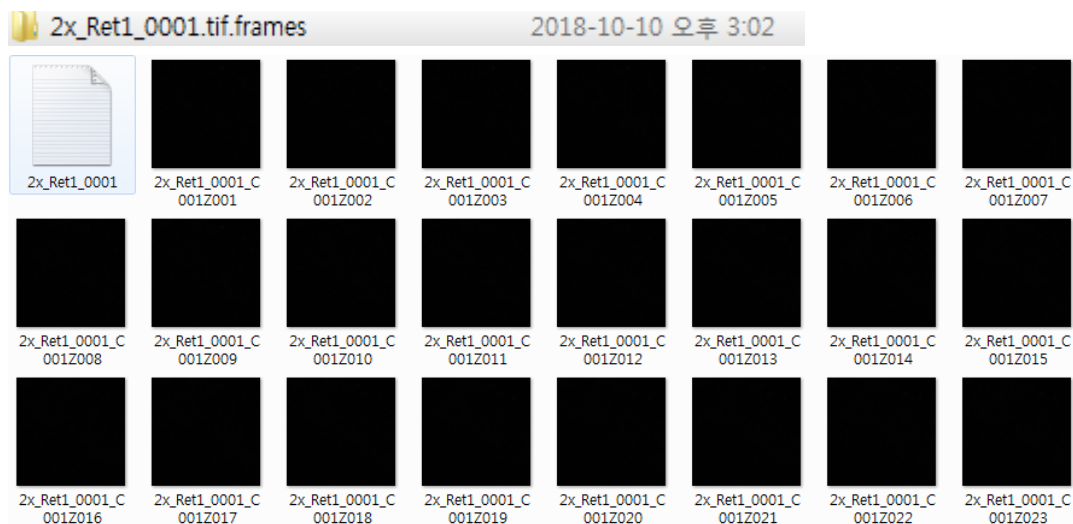
Transcription site classifying analysis for in vivo imaging

2018/12/10 BH Lee

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 registration 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.

| OE9 | | 2018-12-11 오후 1:49 | | 파일 폴더 |
|--------------------|--------------------|--------------------|-----------|-------|
| 이름 | 날짜 | 유형 | 크기 | 태그 |
| 20181008_OE9_HC_ | 2018-10-10 오후 3:14 | TIFF 이미지 | 331,825KB | |
| 20181009_OE9_CFC_ | 2018-10-10 오후 3:12 | TIFF 이미지 | 331,825KB | |
| 20181010_OE9_Ret_ | 2018-10-10 오후 3:14 | TIFF 이미지 | 331,824KB | |
| 20181011_OE9_Ret2_ | 2018-10-12 오후 4:37 | TIFF 이미지 | 331,822KB | |
| 20181012_OE9_Ret3_ | 2018-10-12 오후 4:37 | TIFF 이미지 | 331,822KB | |
| 20181114_OE9_Rem_ | 2018-11-15 오후 8:38 | TIFF 이미지 | 331,822KB | |

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

TrackNTrace Settings

Adjust options for movie: Load settings Save settings

20181009_OE9_CFC_.tif

General options

Output folder Same as movie ☒ Select

Dark movie Select

Frame interval: First frame 1 Last frame Inf

Photon conversion ☒ Bias 100 Sensitivity 5.00 Gain 100

Candidate Detection Method Cross correlation ?

Cross correlation Options

PSFsigma 1.3 CorrThreshold 0.35 distanceFactor 2.5

Refinement Method TNT Fitter ?

TNT Fitter Options

PSFsigma 1.3 fitType [x,y,A,BG] usePixelIntegratedFit ☒

useMLE ☐ astigmaticCalibrationFile Select

Tracking ☒ Tracking Method TNT NearestNeighbor... ?

TNT NearestNeighbor Options

minTrajLength 2 maxTrackRadius 6 maxFrameGap 0

minSegLength 1 maxGapRadius 1 verbose ☐

Preview First frame 1 Last frame 50 Use settings for all following. START processing

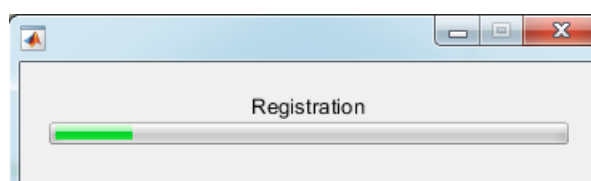
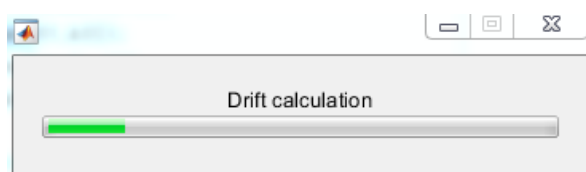
Parameters are not important to do the analysis except ***maxGapRadius***. It should be 1.

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

| | |
|--|--------------------|
| 20181008_OE9_HC_ | 2018-10-10 오후 3:14 |
| 20181008_OE9_HC_2018-m12-d11-14h28_TNT | 2018-12-11 오후 2:28 |
| 20181009_OE9_CFC_ | 2018-10-10 오후 3:12 |
| 20181009_OE9_CFC_2018-m12-d11-14h11_TNT | 2018-12-11 오후 2:26 |
| 20181010_OE9_Ret_ | 2018-10-10 오후 3:14 |
| 20181010_OE9_Ret_2018-m12-d11-14h28_TNT | 2018-12-11 오후 2:28 |
| 20181011_OE9_Ret2_ | 2018-10-12 오후 4:37 |
| 20181011_OE9_Ret2_2018-m12-d11-14h28_TNT | 2018-12-11 오후 2:28 |
| 20181012_OE9_Ret3_ | 2018-10-12 오후 4:37 |
| 20181012_OE9_Ret3_2018-m12-d11-14h28_TNT | 2018-12-11 오후 2:28 |
| 20181114_OE9_Rem_ | 2018-11-15 오후 8:38 |
| 20181114_OE9_Rem_2018-m12-d11-14h28_TNT | 2018-12-11 오후 2:28 |

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 registration with different time point

7. Open the new subtracted registered 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.

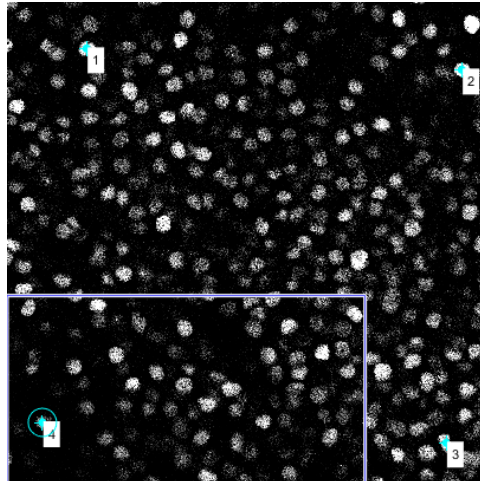


8. Open ***Two_image_registration.mat*** file.

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

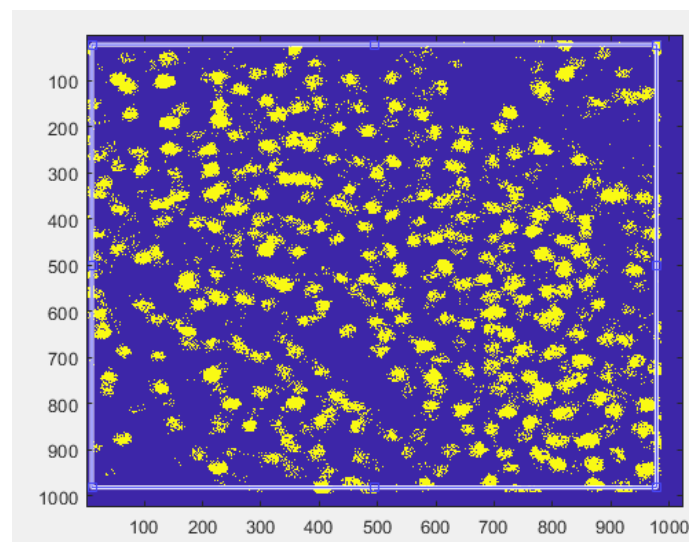
9. Run the code. Select the subtracted registered image.

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***.

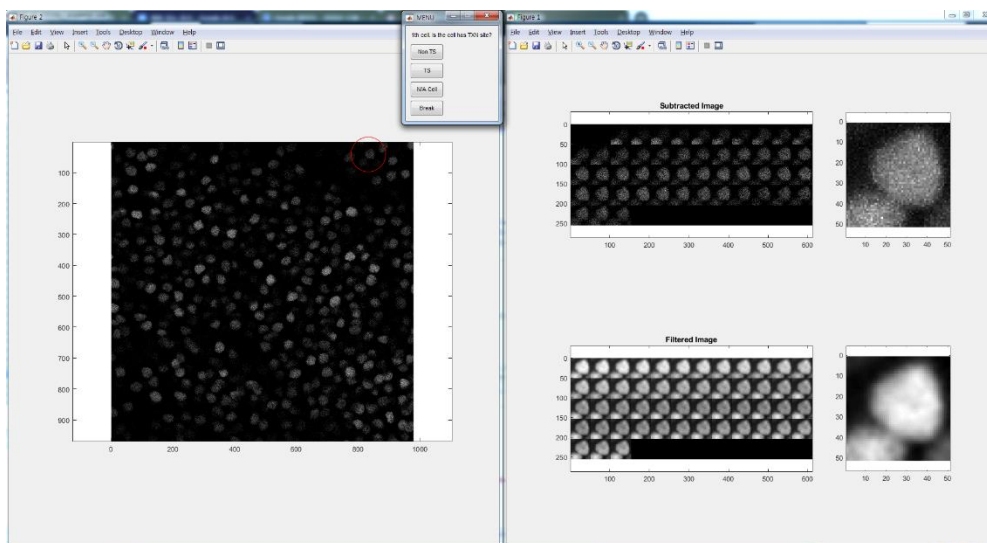
14. Set classifying folder in 4th line and set the cell size which indicates size to be cropped in 8th line.

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.

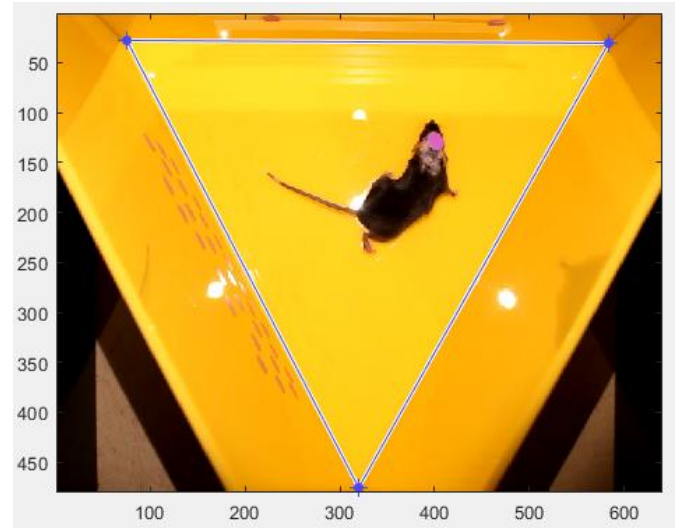
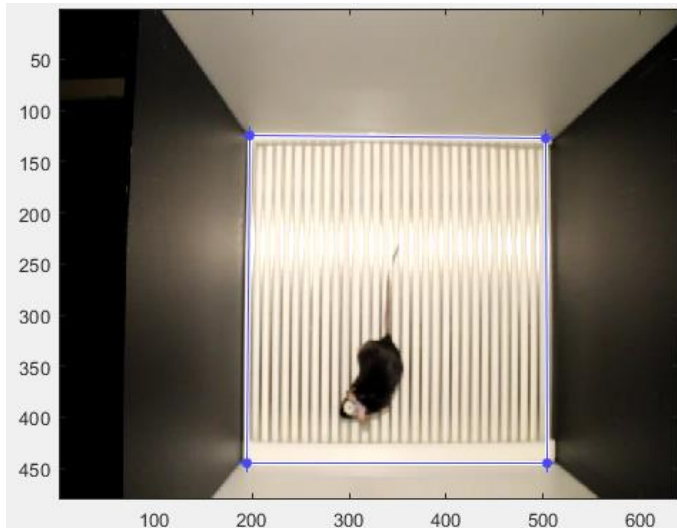


19. Once you finish the classification, you will see the NF_map file in the classification path.

● 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 |