Granger-Causality Analysis (GCA) pipeline manual:
from fluorescence video input to GC pathway diagram output

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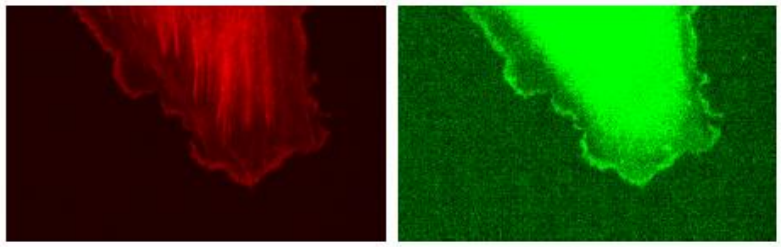
Intro

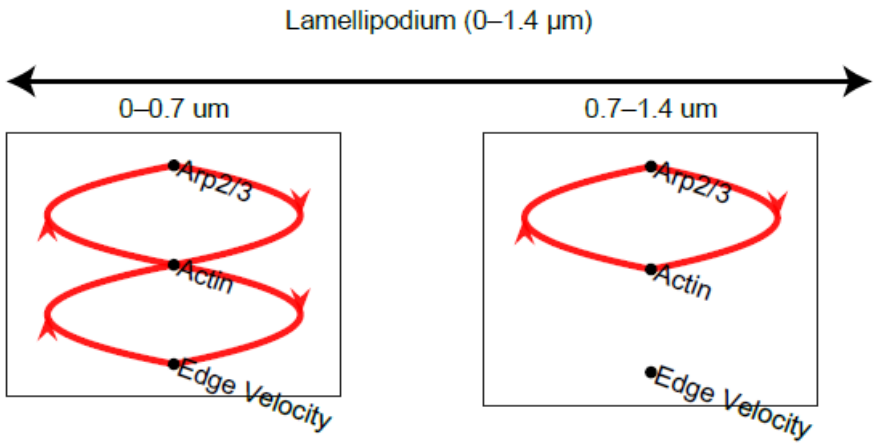
- This doc describes detailed steps of the GCA pipeline proposed in: "**Inference of Granger-causal relations in molecular systems — a case study of the functional hierarchy among actin regulators in lamellipodia**" https://www.biorxiv.org/content/10.1101/2021.05.21.445144v2
- Pipeline built on MATLAB R2020a version

- Input:
  1. well-cropped 2ch fluorescence movies to study Lamellipodia dynamics
- Output:
  9. GC pathway diagram

(i) Live cell fluorescence movies



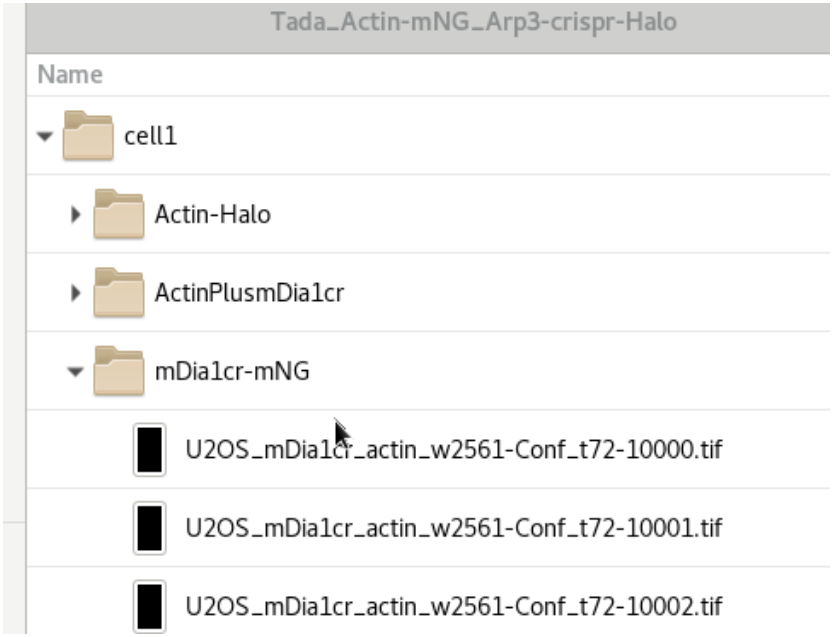
(vii) Granger-causal pathway diagram



2. Summation images (ch1+ch2) for segmentation

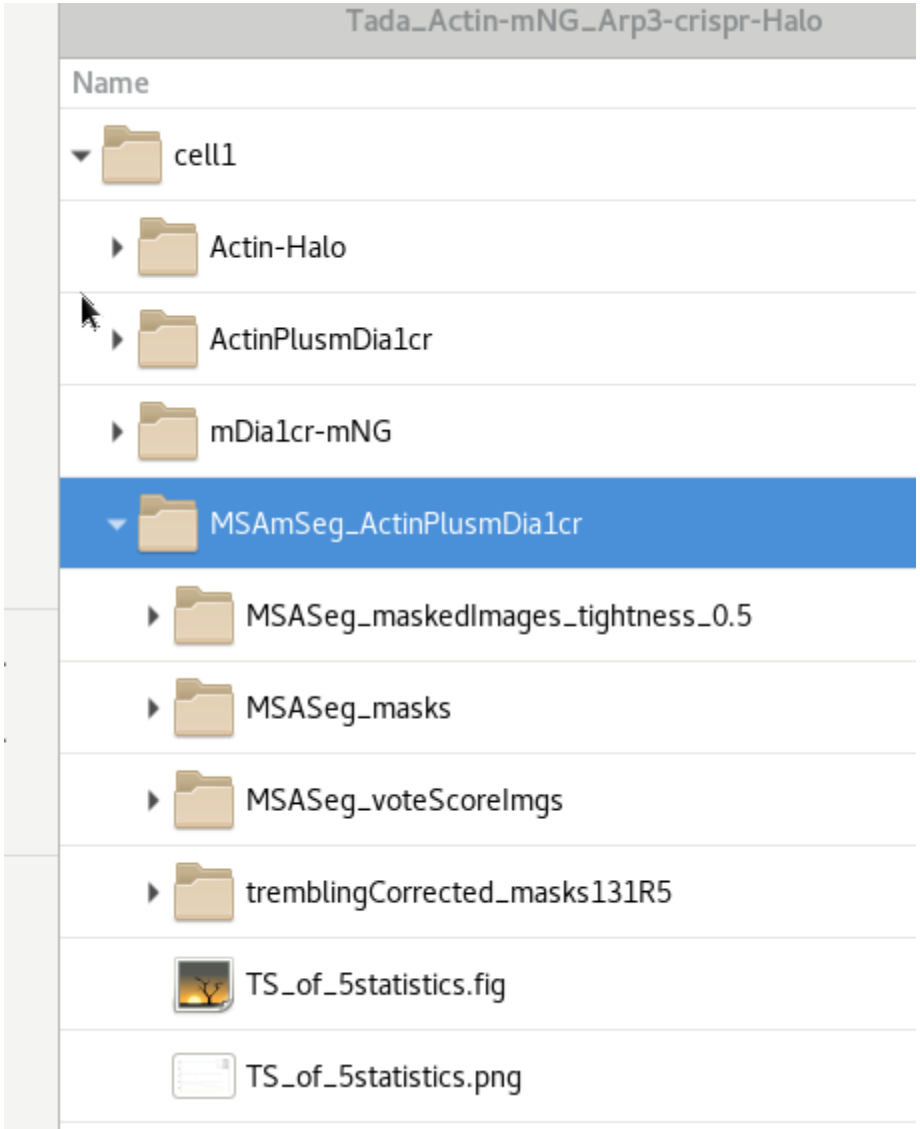
- Exercise with Actin/mDia1 movies at the example data folder: `/project/bioinformatics/Danuser_lab/ActinGrangerCausality/raw/Jungsik/Data_for_Manual_GCA/cropped-Tada200526_mDia1cr-mNG_Actin-Halo_downSampBilinear`

- Input need to be tif files per frame in two folders with channel names
- Script: `additionOf2chanImTiffs.m`
- E.g. of input/output



3. MSA segmentation of the summation images

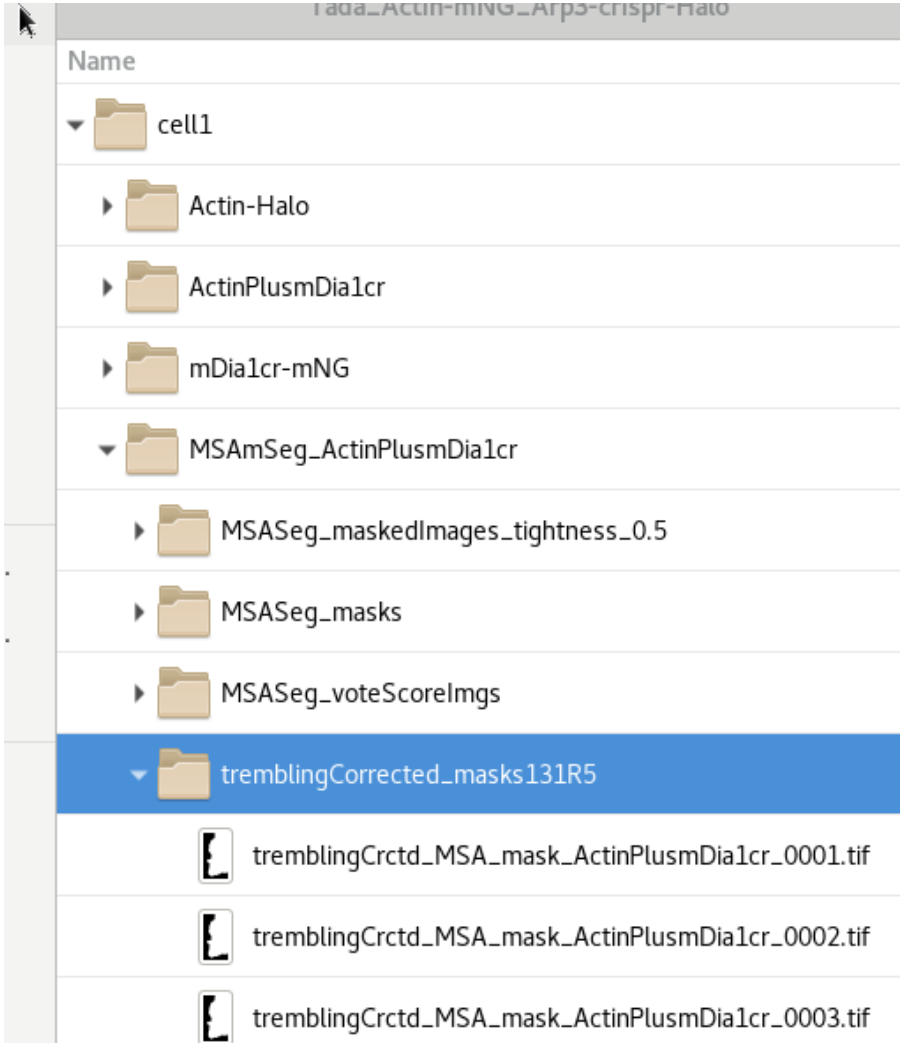
- **Ftn:** `wrapper2_MSA_multi_Seg_imDir.m`
- Eg of output



4. Edge trembling correction

- Ftn: `wrapper2_MSAmultiSeg_imDir_tremblingMaskCorrection.m`
- Eg of output

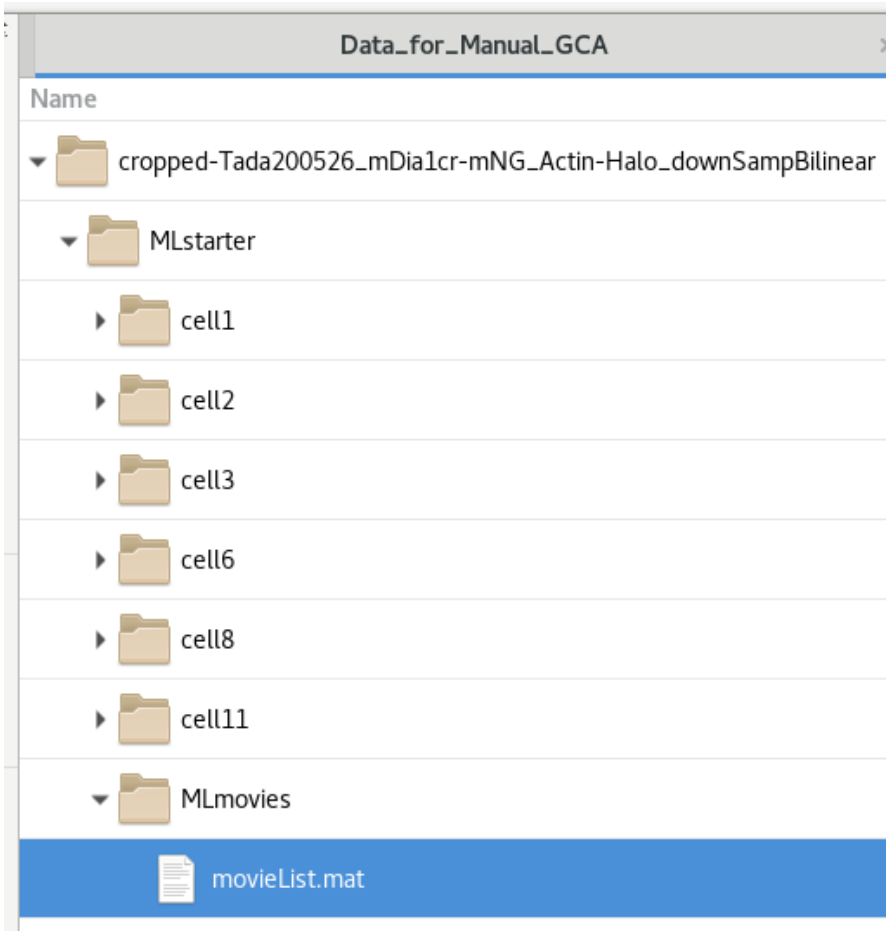
- After running the function, you need to check if the masks are correct at every frame. If it is incorrect, go back to MSA segmentation and adjust ‘tightness’ or ‘numVotes’ parameters.



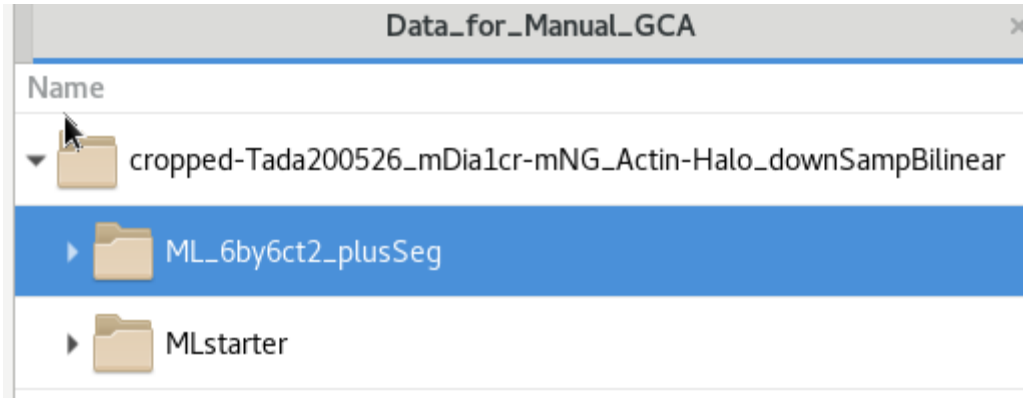
5. Make movieData & movieList with the image directories

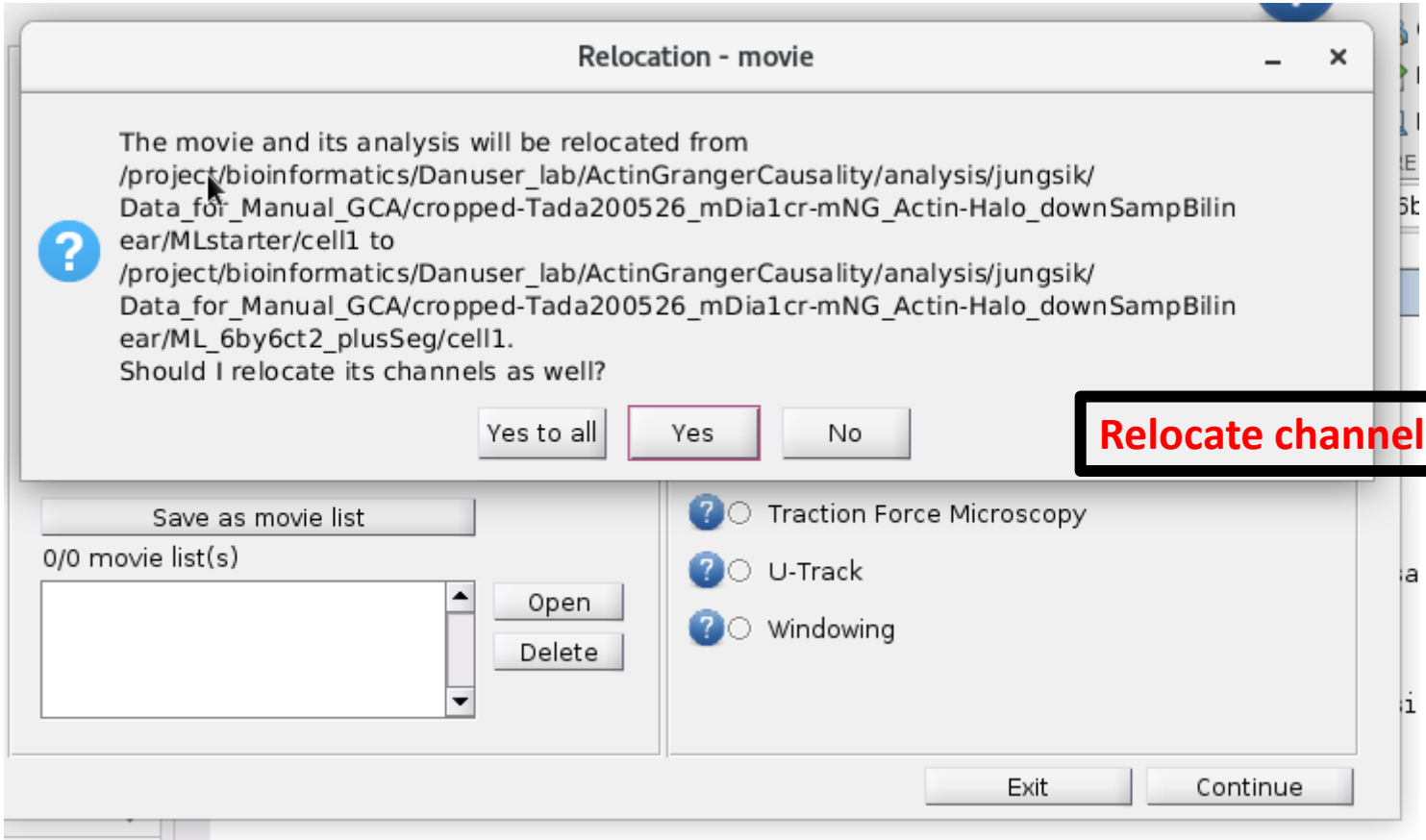
- Script:
  `Make_movieData_mat_for_folders_v2_2ch_Make_movieList.m`
- Recommended directory structure
  - Project/xxxx/raw/yymmdd_mDia1cr-mNG_Actin-Halo/cellname/Actin-Halo
  - Project/xxxx/raw/yymmdd _mDia1cr-mNG_Actin-Halo/cellname/mDia1cr-mNG
  - Project/xxxx/analysis/yymmdd _ mDia1cr-mNG_Actin-Halo/**MLstarter**/cellname/movieData.mat
  - Project/xxxx/analysis/yymmdd _ mDia1cr-mNG_Actin-Halo/**MLstarter**/MLmovies/movieList.mat

  (You might need to use the initial movieList object later multiple times, because it is necessary to try multiple analysis parameters such as window sizes, GCA smoothing parameters, etc. Thus, it is convenient to make the initial movieList object intact in a 'MLstarter' folder (say), and to copy it to a specific analysis folder.)



6. Copy/Relocate the initial MDs





7. Run externalSegmentation/windowing and make a movieList object with a script

- **Ftn:** `ML_DoExtSegRefProtWindWithN_GCA.m`

8. Run GCA pipeline to obtain final GC diagrams

- Script: `Pipeline_GCA_2chMovies_ch1ch2.m`
- (See also `Pipeline_GCA_3chMovies_2chAnalysis_ch1ch2ch3.m`)
- Input: `movieList.mat`

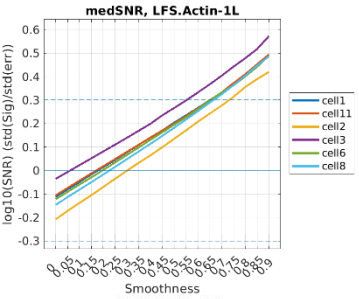
- The first section contains all the required user input for the script.

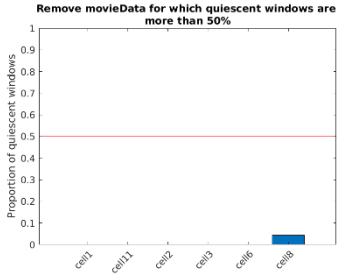
- Input parameters:
  - Half cycle of protrusion/retraction (frame) for Low-Frequency-Normalization (LFN)
  - EWMA smoothing parameter
  - Etc

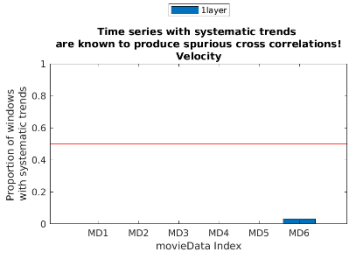
```
8
9
10 -  GCparam = struct();
11
12 -  halfOfPRCycle = 10
13 -  GCparam.name1 = 'Actin';
14 -  GCparam.name2 = 'mDialcr';
15 -  GCparam.maxLayer = 4;
16
17    % 2*half cycle of protrusion/retraction is fed into movMedian period length
18    % for low frequency normalization.
19 -  GCparam.LFNfr = 2*halfOfPRCycle;
20 -  prfxNorm = ['LF', num2str(GCparam.LFNfr), 'fr_PL_GCA_2chMov_ewma0p5_', ...
21        GCparam.name1, '_', GCparam.name2]
22
23 -  GCparam.lagMax = 2*halfOfPRCycle;
24 -  GCparam.movMedFrameSize = 2*halfOfPRCycle;
25 -  GCparam.smParamTh = 0.5;
26 -  GCparam.factoranMethod = 0000;
27 -  GCparam.highDimRegMethod = 'IC';
28 -  GCparam.infoCriterion = 'AIC';
29 -  GCparam.tLagfr = halfOfPRCycle;
30 -  GCparam.movingAvgSmoothing = false;
31 -  GCparam.EWMA = 0.5;       % lambda = 1 (no smoothing, default)
32 -  GCparam.analysisName = 'pipeline_GCA_2chMov_ewma0p5';
33 -  GCparam.prfxNorm = prfxNorm;
34
35
36 -  GCparam
37 -  save(fullfile(ML.outputDirectory_, 'GCparam.mat'), 'GCparam')
38    %  end: parameter setup
```

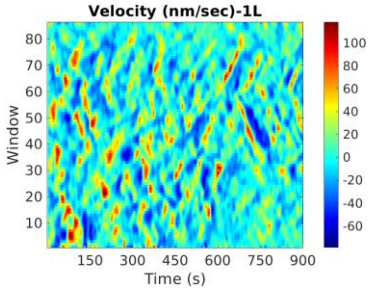
9. Output of the GCA pipeline

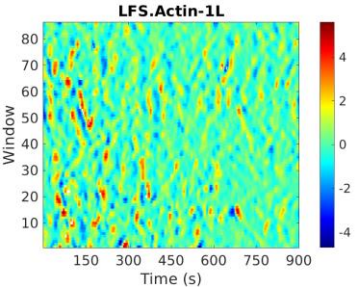
- Diagnostics
  - Signal-to-Noise-Ratio (SNR), ratio of quiescent windows, ratio of windows with non-stationary edge motion
- Cross correlations, Fluctuation Profiles Around Motion Events (FPAME)
- Intra-cellular information flows in one channel
- Cross correlations between layers for one channel
- GCA with three (two) variables, etc.

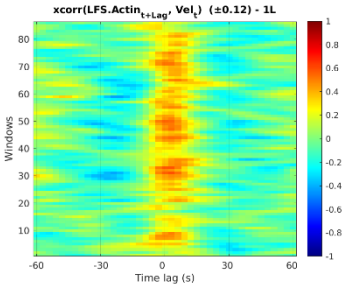


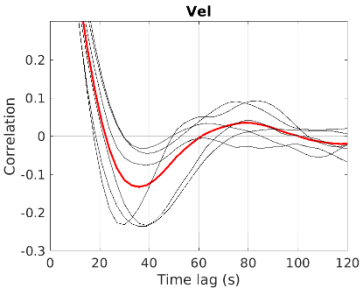


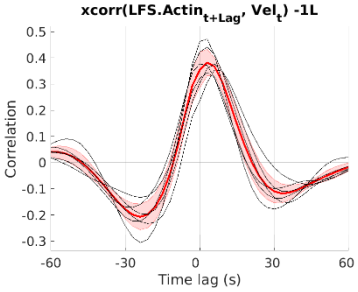


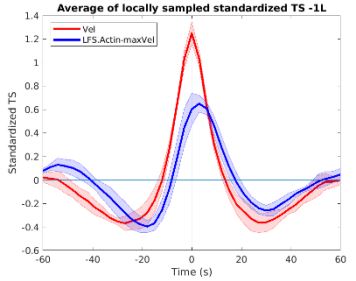


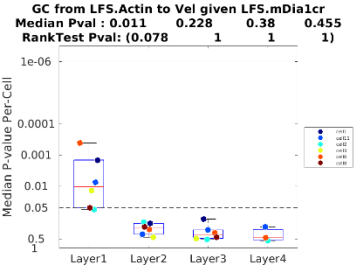


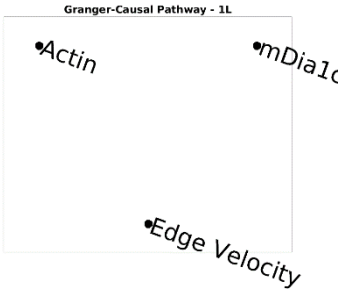












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cell1	0.00147	0.15753	0.11627	NaN
cell11	0.00773	0.35105	0.25683	0.20276
cell2	0.05643	0.14335	0.52087	0.57556
cell3	0.01407	0.44807	0.48029	NaN
cell6	0.00042	0.24919	0.32135	0.45465
cell8	0.05109	0.20676	0.43956	NaN

and much more.