1. Fluctuation Profiling Around Edge Motion Events (FPAEME)
2. Spatial gradient along the distance from the cell edge

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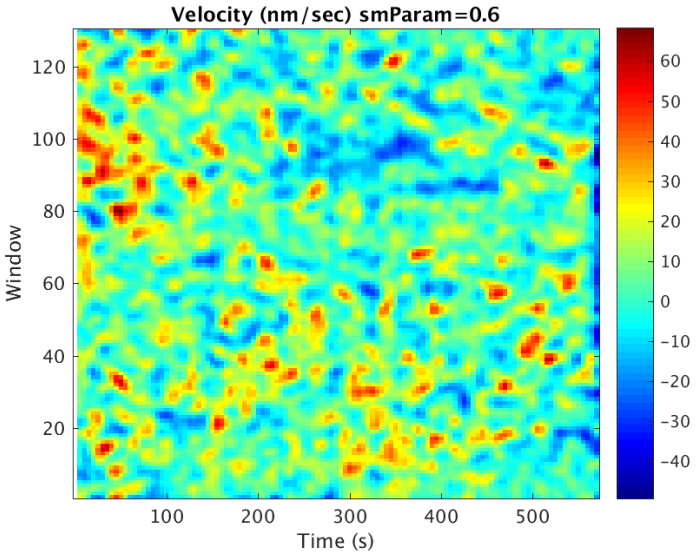
List of .m functions for FPAEME and spatial gradient analysis

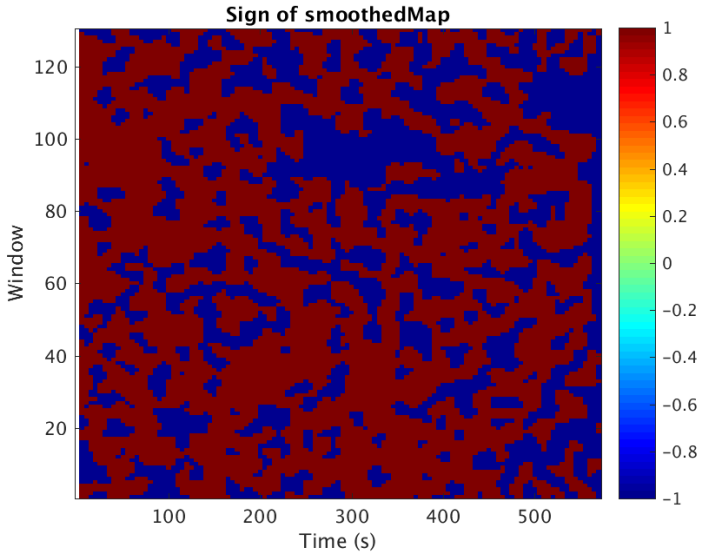
1. mapDescriptives_Vel_LB
    1. (matlab internal) lbqtest.m
2. phaseMasking
    1. (externalLibraries) irle
3. phaseDescriptives_OneChan
    1. long_run_variance
    2. shadedErrorBar**v2**
4. phaseDescriptives_MaxMinVel_OneChan
    1. phaseMaskingInternal > rle, irle
    2. (externalLibraries) rle
    3. shadedErrorBar**v2**
5. MLsummary_FluctuationProfiling

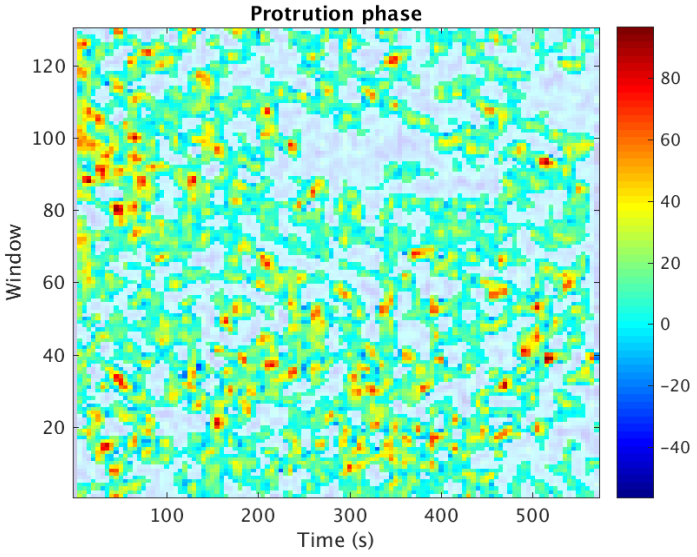
6. mapDescriptives_meanCV_alongDepth
7. MLsummary_alongDepth

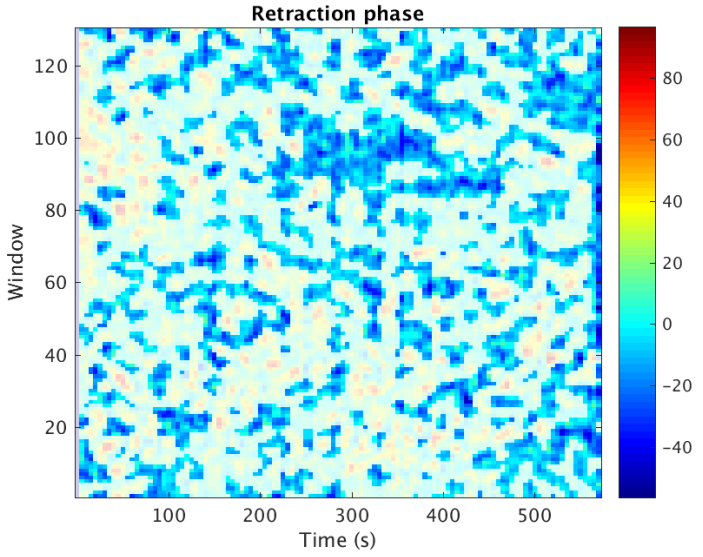
1. Fluctuation Profiling Around Edge Motion Events (FPAEME)

Protrusion/retraction phase detection



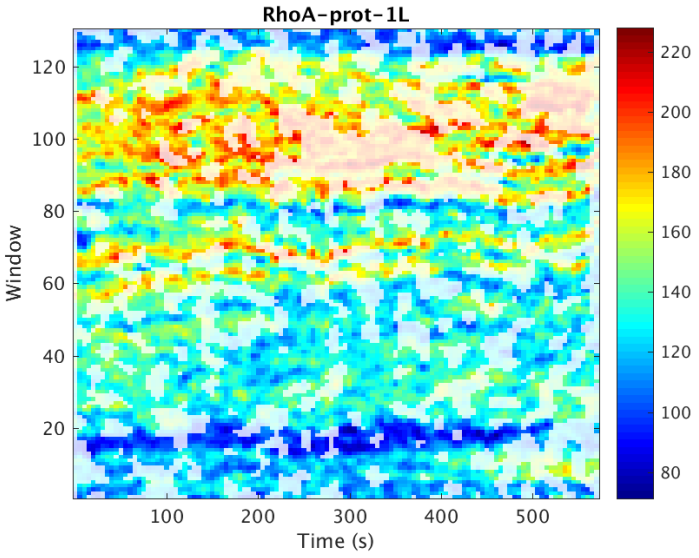


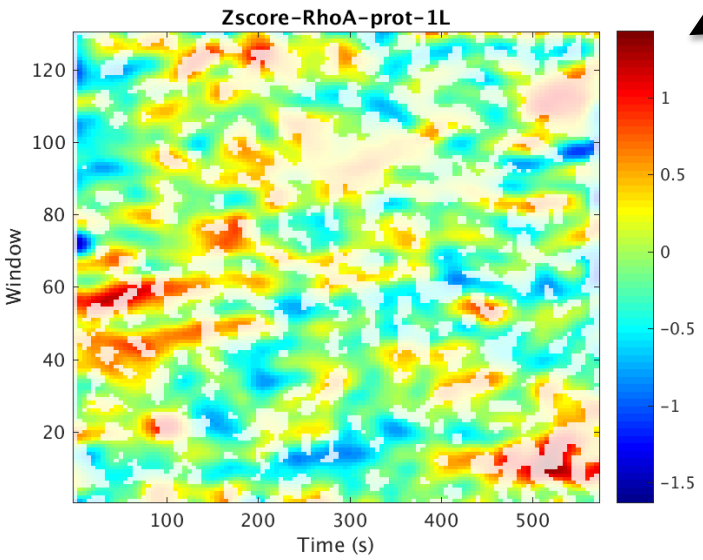




- Moderately over-smooth the velocity map
- Construct a protrusion mask map where smoothed velocities are positive.

RhoA activity in phases





Protrusion

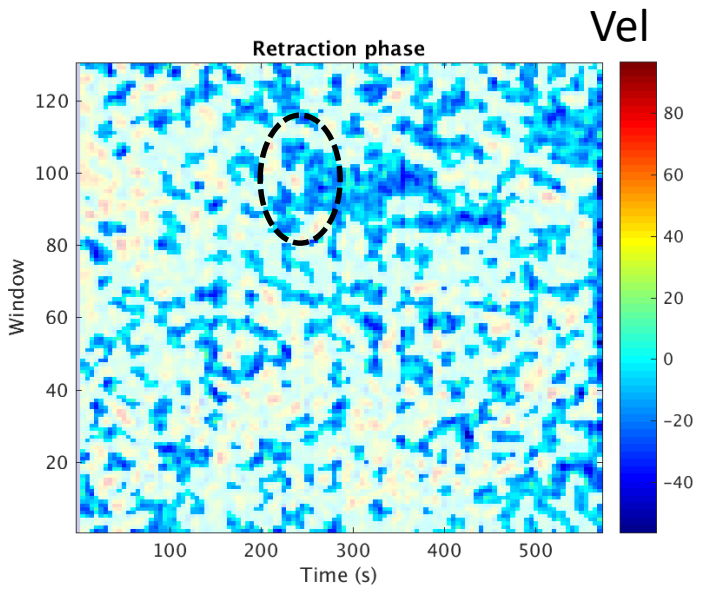
- Smoothed activity map
  - To focus on the temporal fluctuation only (not the spatial distribution), for each window, the activity time series was normalized (standardized) so that the normalized activities in a window have mean 0 and variance 1.

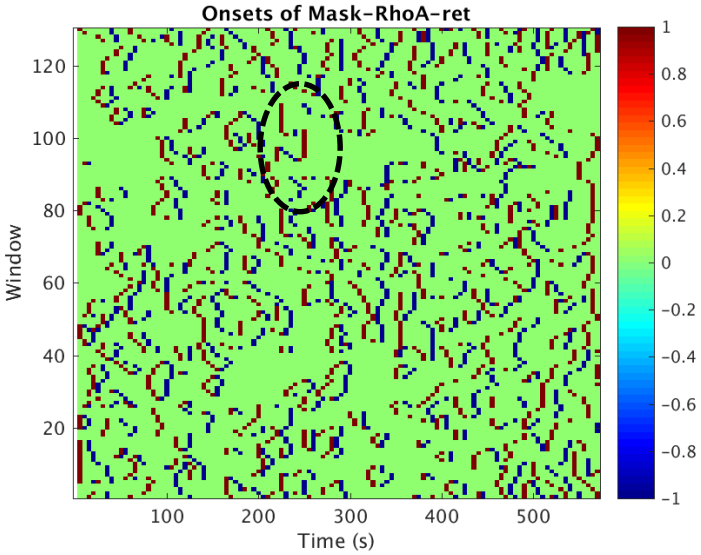
$$Z(w,t) = \frac{A(w,t) - 1/T \sum_{t=1}^{T} A(w,t)}{SD(A(w,1), \ldots, A(w,T))}$$

w: window index, t: time frame index

- Window-wise variation (heterogeneity) was normalized to focus on temporal fluctuation
- Smoothed map

Detecting onsets



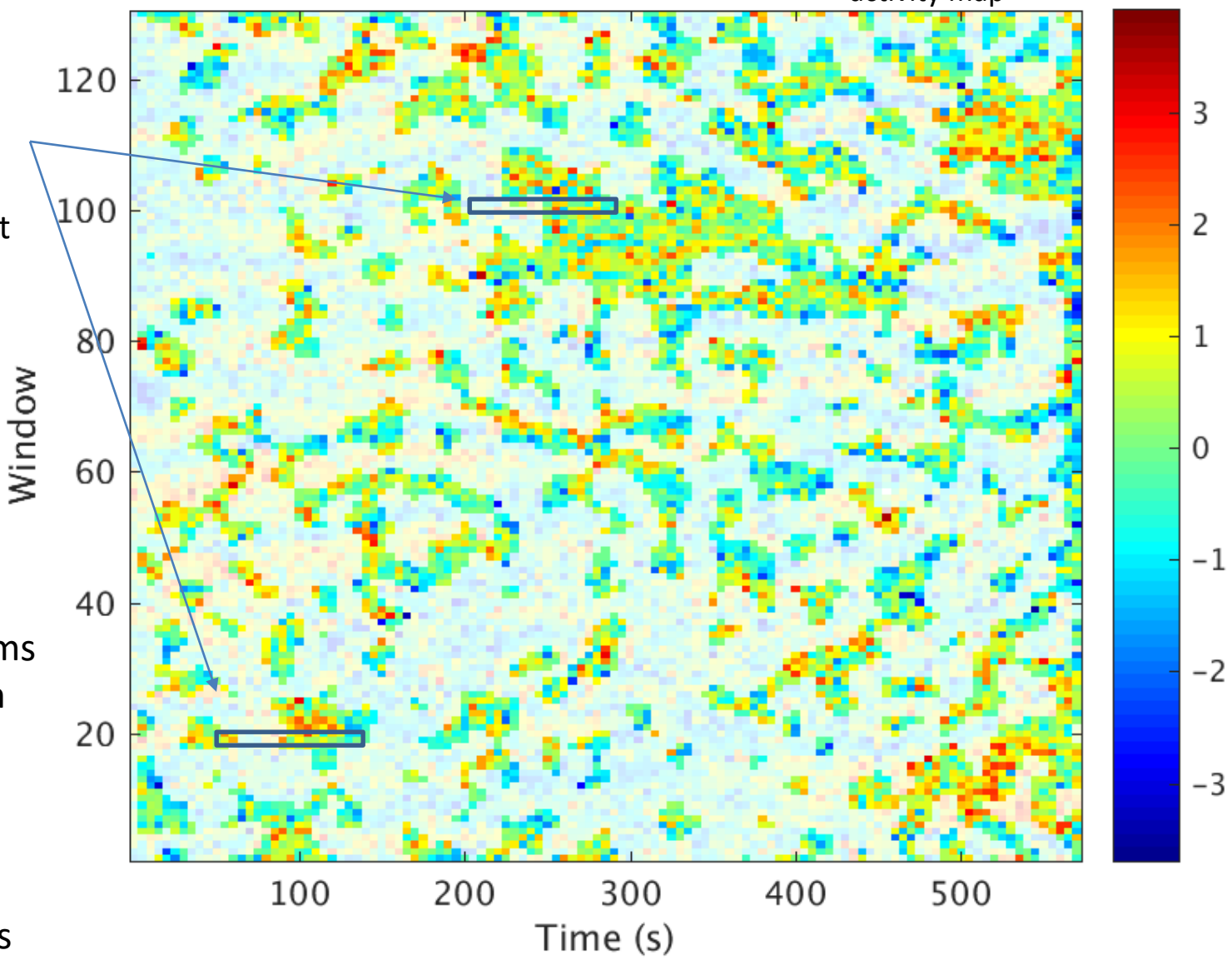


Local sampling normalized RhoA signals around ret-onsets

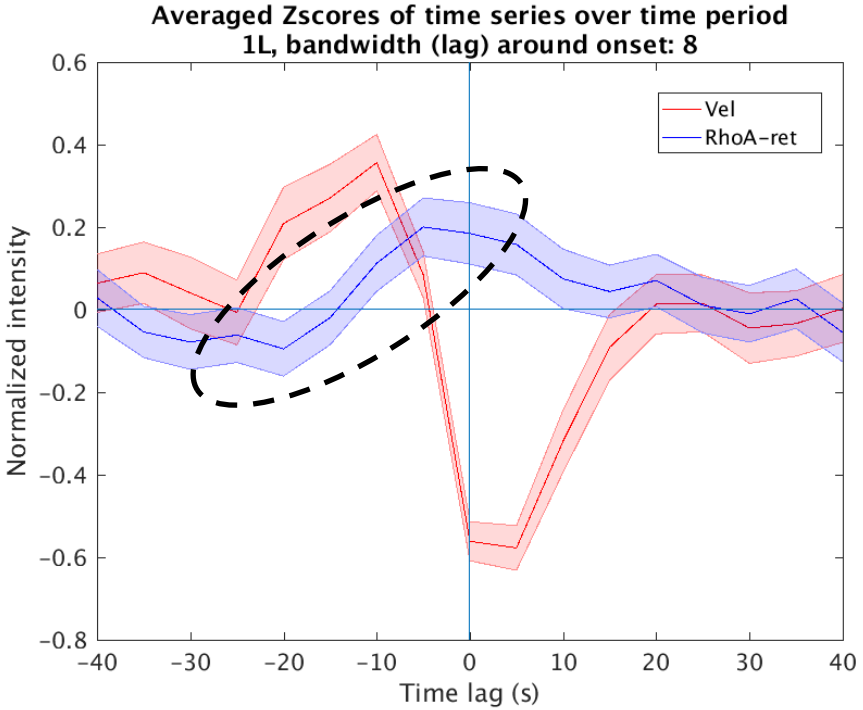
Local sampling window with +/- 8 frames around the retraction onset

Based on the idea of Lee et al. (Cell systems 2016), we can profile typical pattern of activities (normalized) around onsets

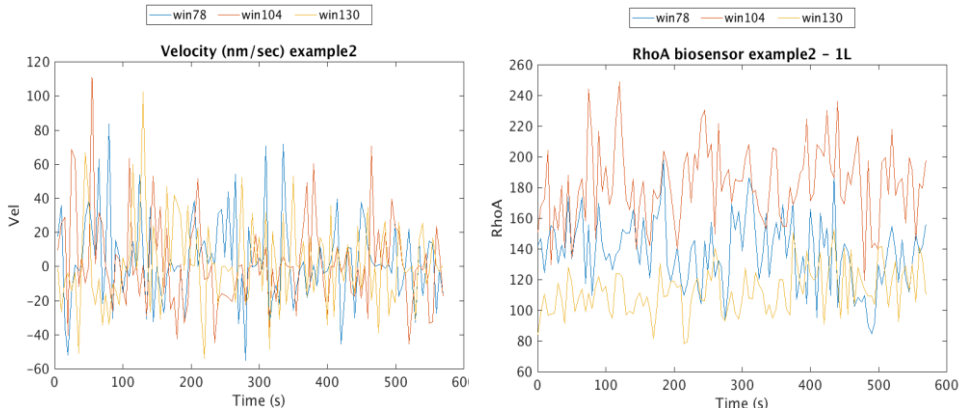
Non-smoothed normalized activity map



Kinetics profiling around onsets



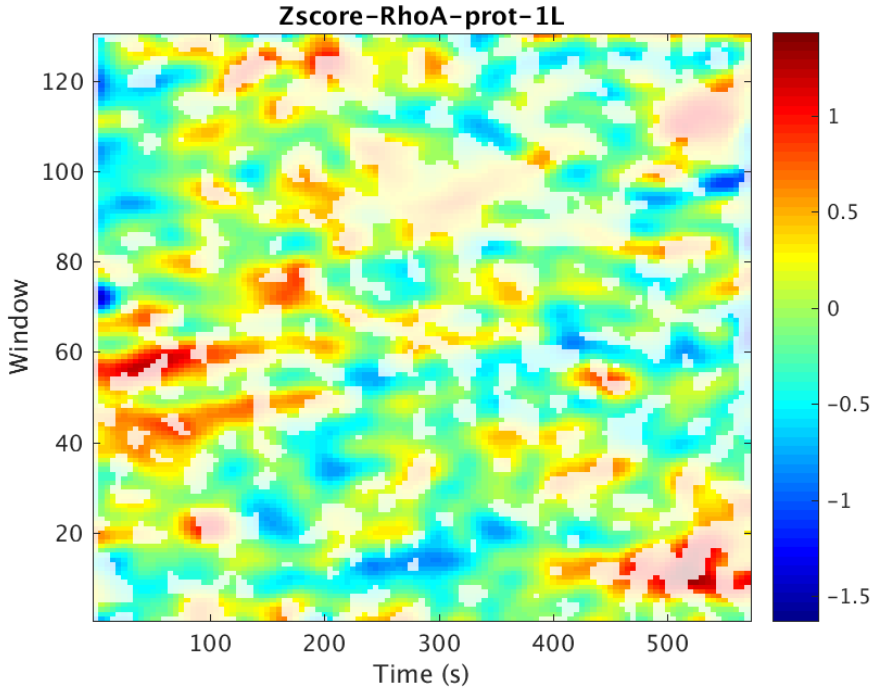
Protrusion-phase Ret-onset Retraction-phase

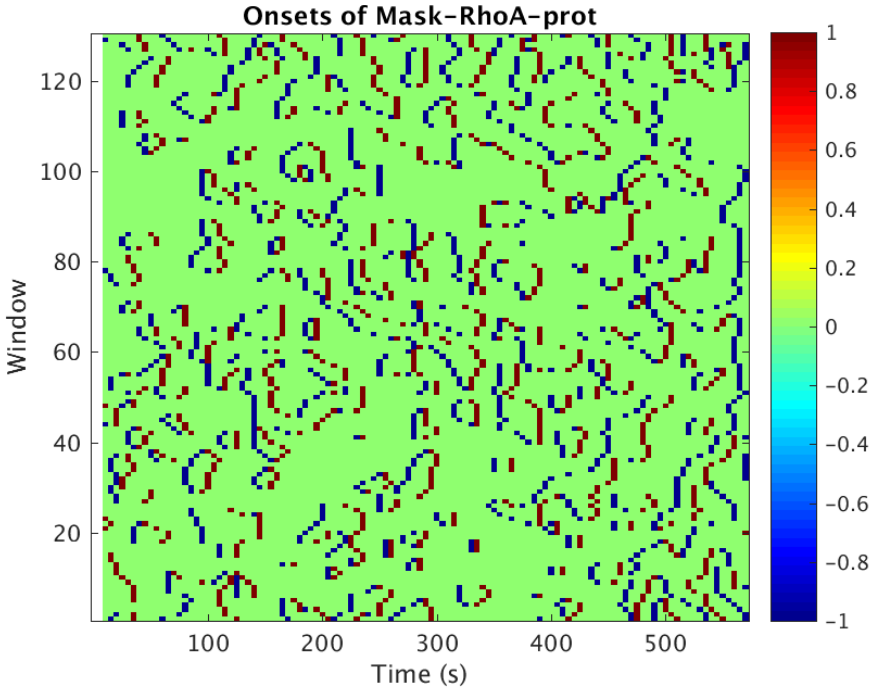


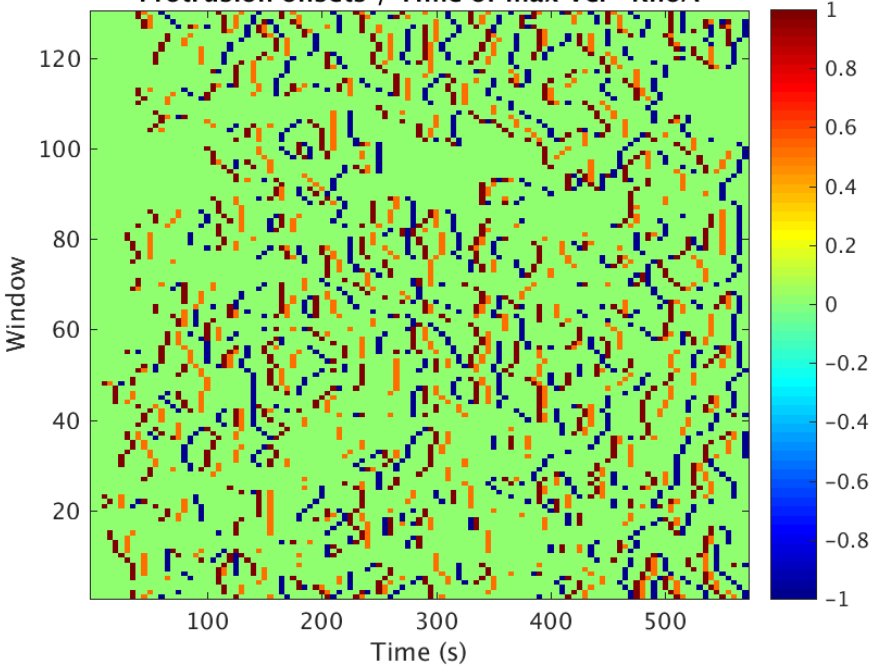
1. Actual fluctuations (time series) have higher frequency than the averaged standardized TS

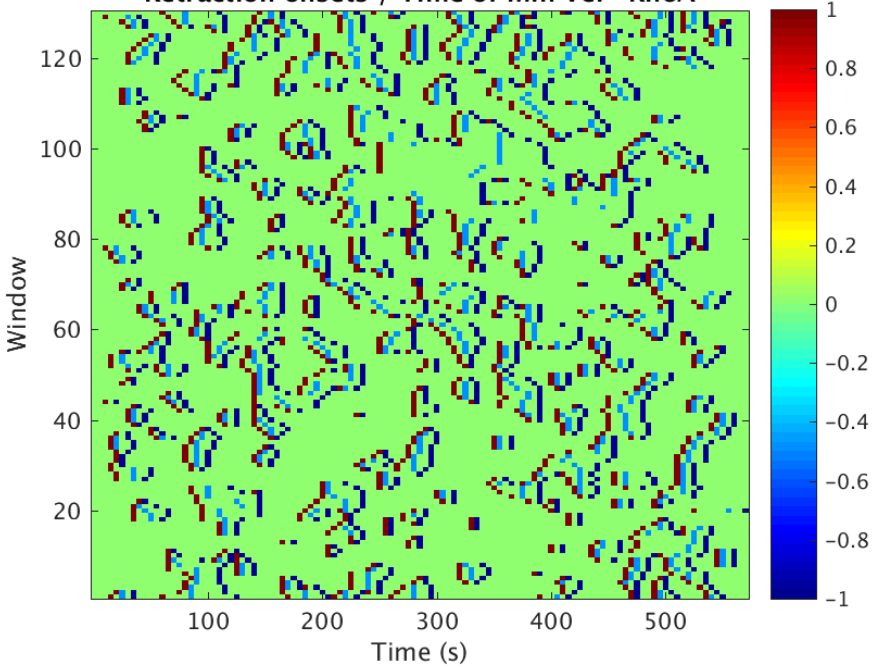
2. This kinetics profiling for activity maps is semi-automatic, data-driven.
3. Narrow confidence interval of velocity between -10 sec and 10 sec indicates that onset-computation is reasonable (Lag 0 belongs to retraction mask)

4. The pattern shows (RhoA initiated retraction):
    1. The increase of RhoA proceeds the decrease of velocity before retraction onset.


Zscore-RhoA-prot-1L
Window
Time (s)

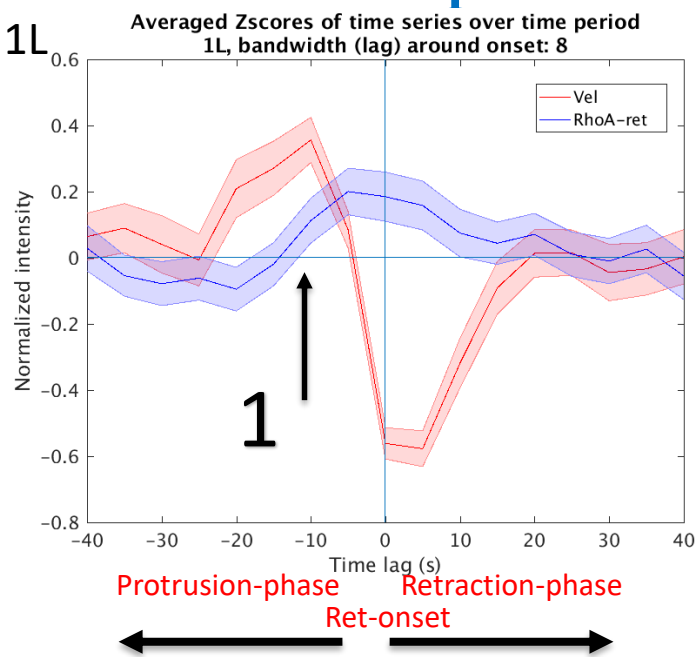

Onsets of Mask-RhoA-prot
Window
Time (s)


Protrusion onsets / Time of max Vel -RhoA
Window
Time (s)


Retraction onsets / Time of min Vel -RhoA
Window
Time (s)

Kinetics profiling around onsets

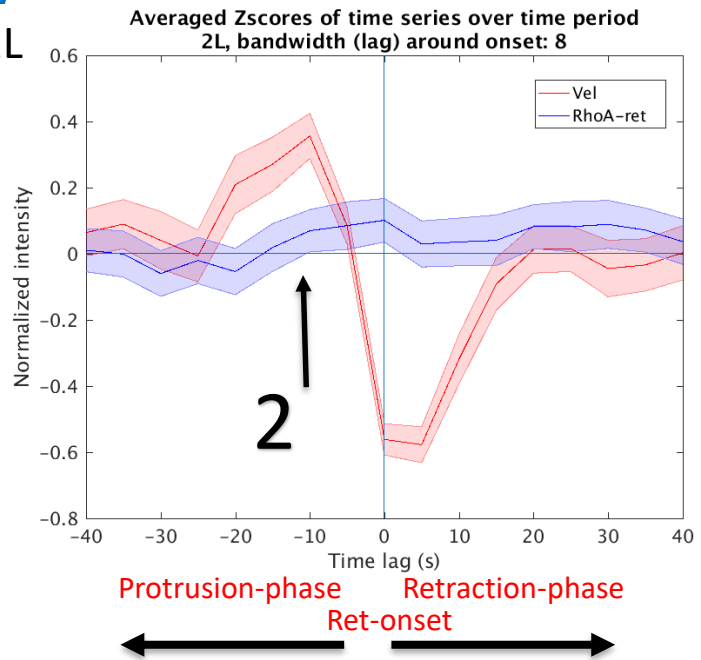
1L



Protrusion-phase Retraction-phase

Ret-onset

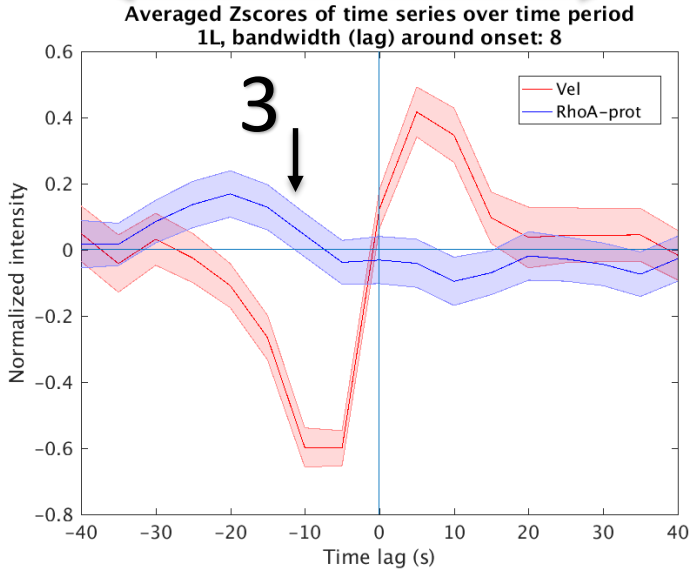
2L



Protrusion-phase Retraction-phase

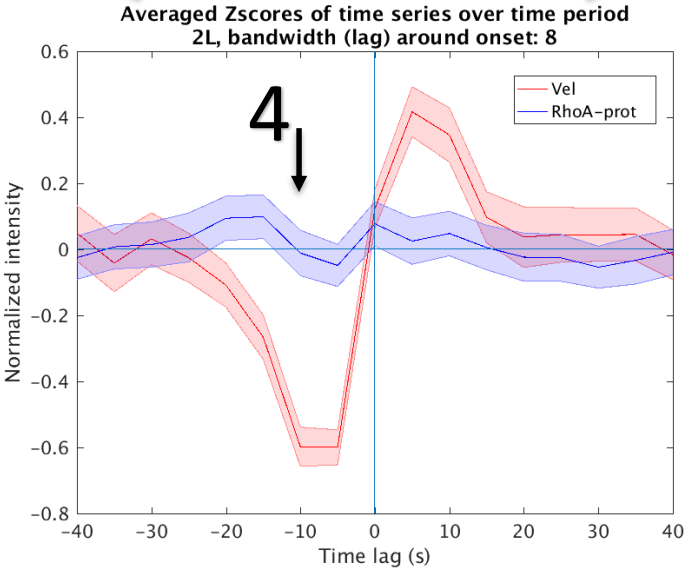
Ret-onset

Ret-onset



Retraction-phase Protrusion-phase

Prot-onset

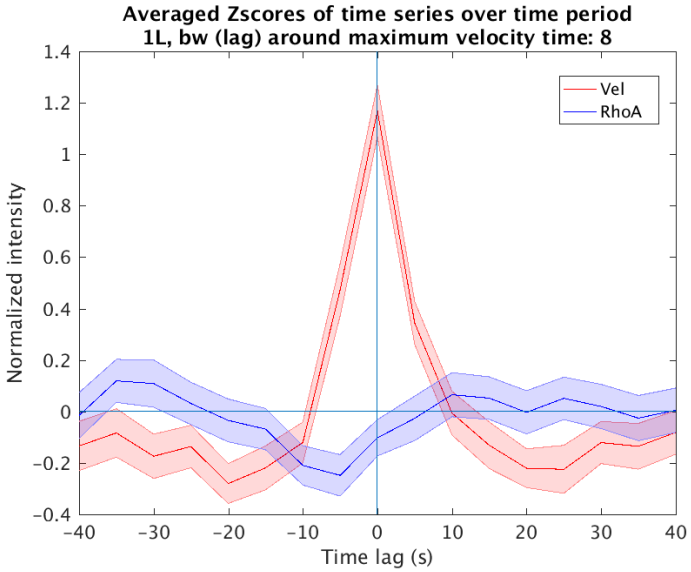


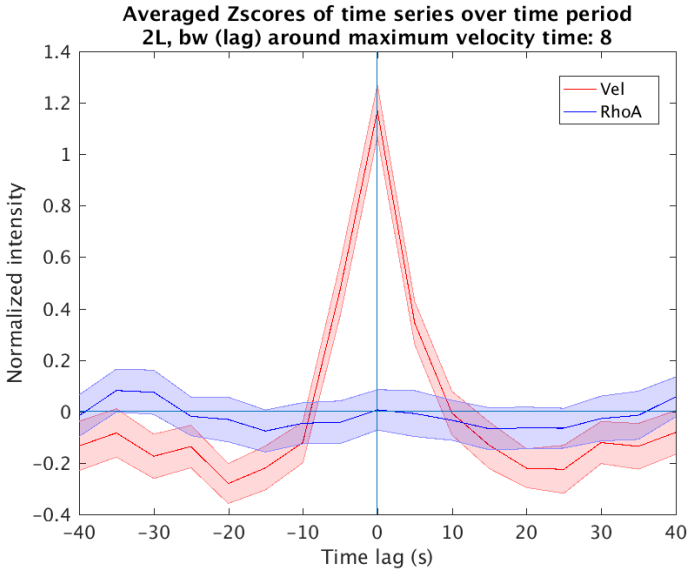
Retraction-phase Protrusion-phase

Prot-onset

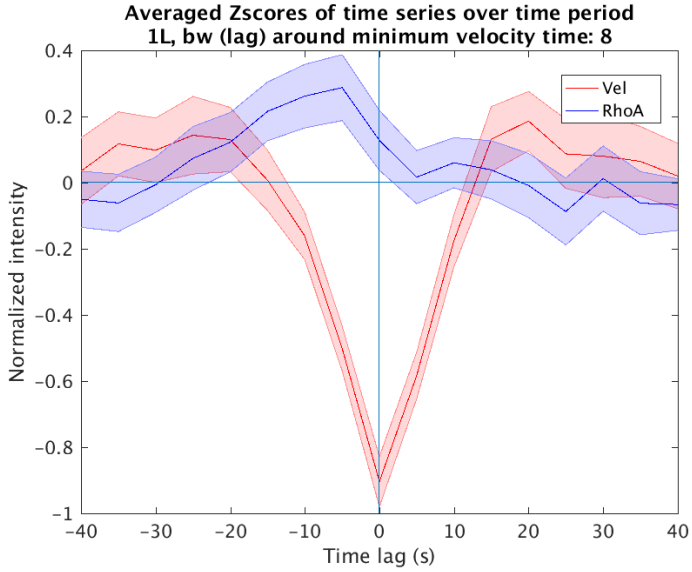
Prot-onset

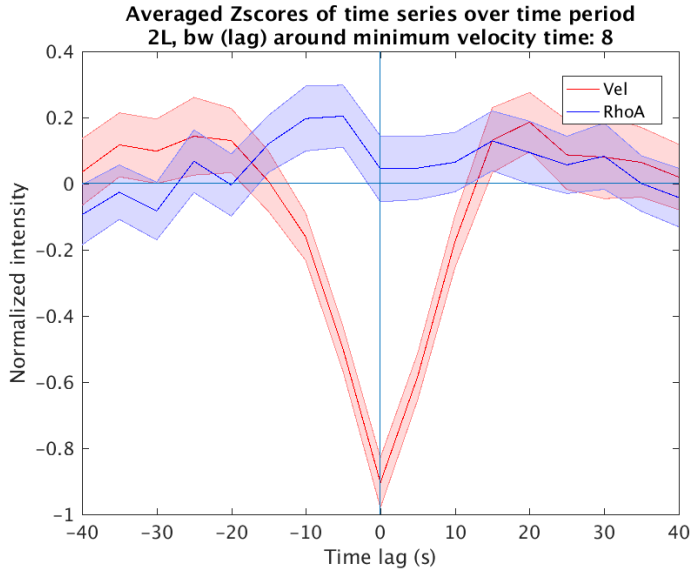
Among the kinetics of RhoA activity, the increase of RhoA before retraction onset (#1) is stronger than others.





Maximum Vel





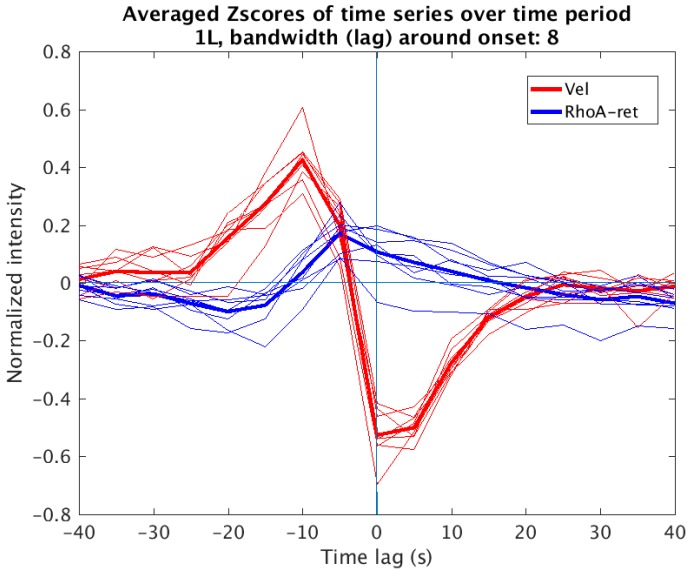
Minimum Vel

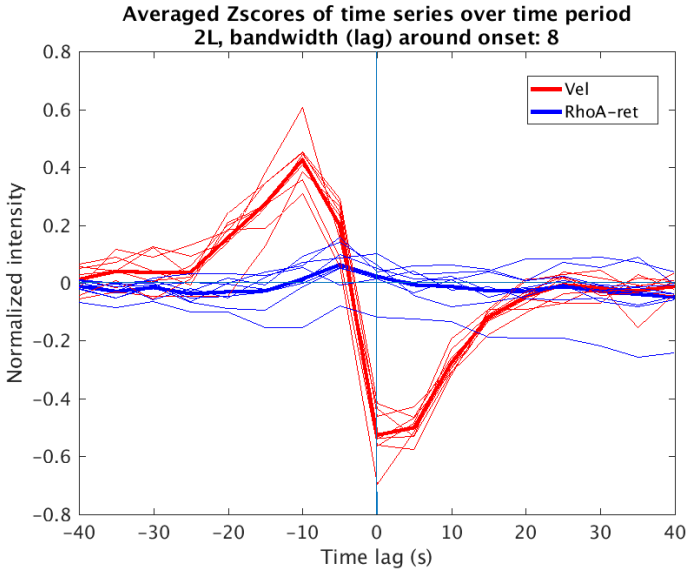
Maximum or minimum peaks of RhoA activity proceed the peaks of velocity.

1Layer

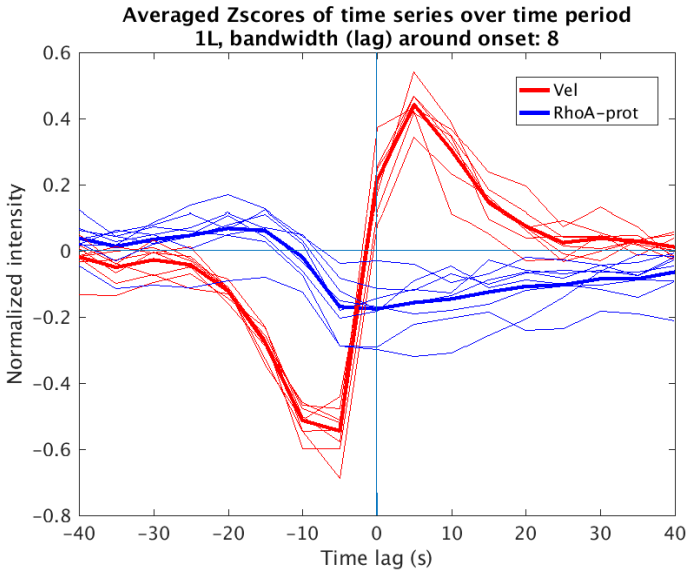
2Layer

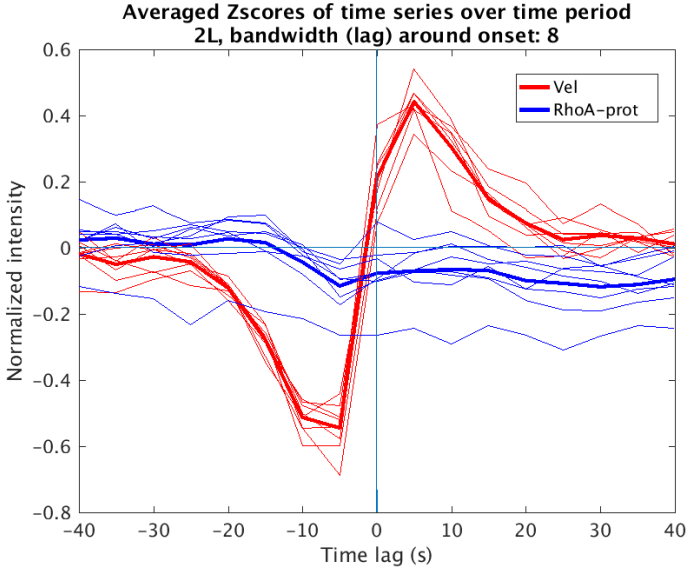
Summary for 8 movies (considering cell-to-cell variation)





Ret-onset



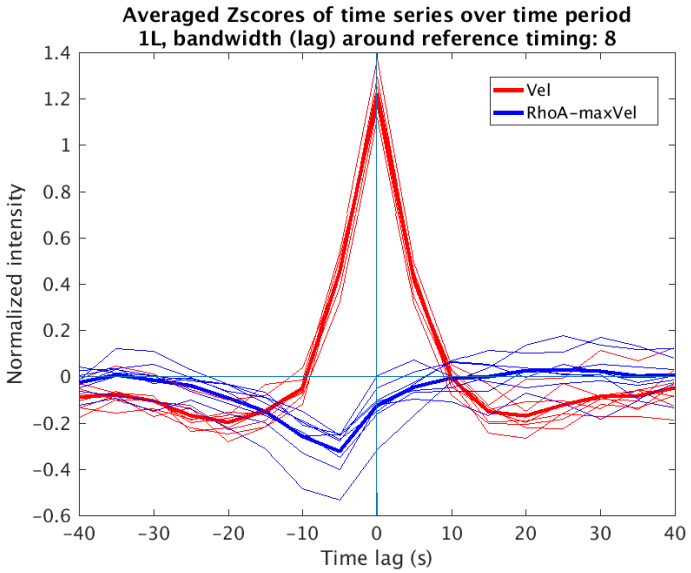


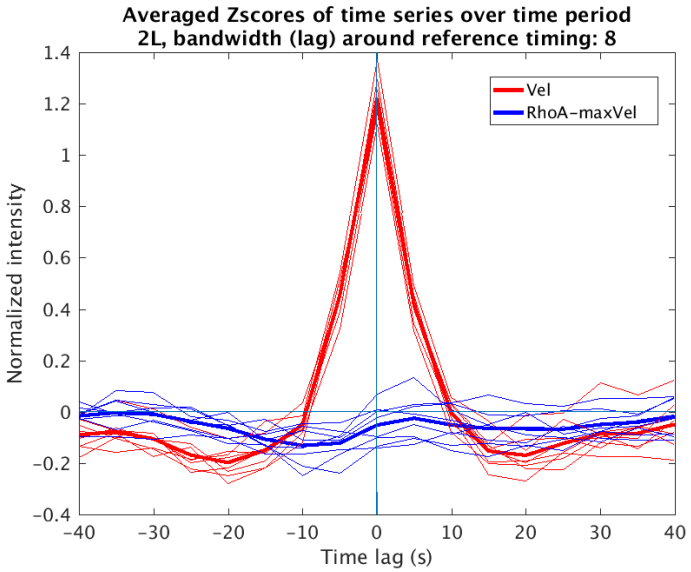
Prot-onset

1Layer

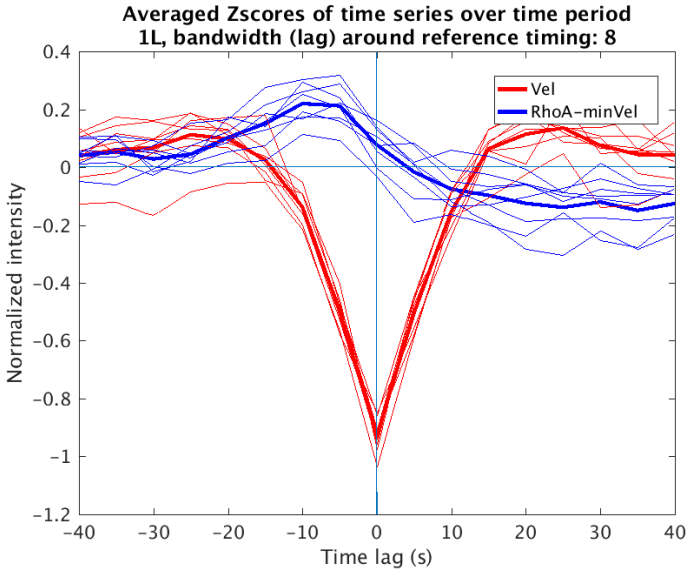
2Layer

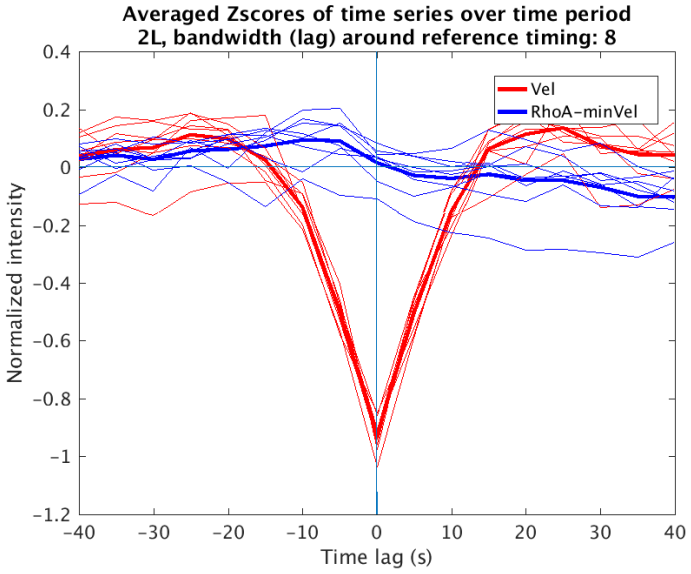
Summary for 8 movies (considering cell-to-cell variation)





Maximum Vel



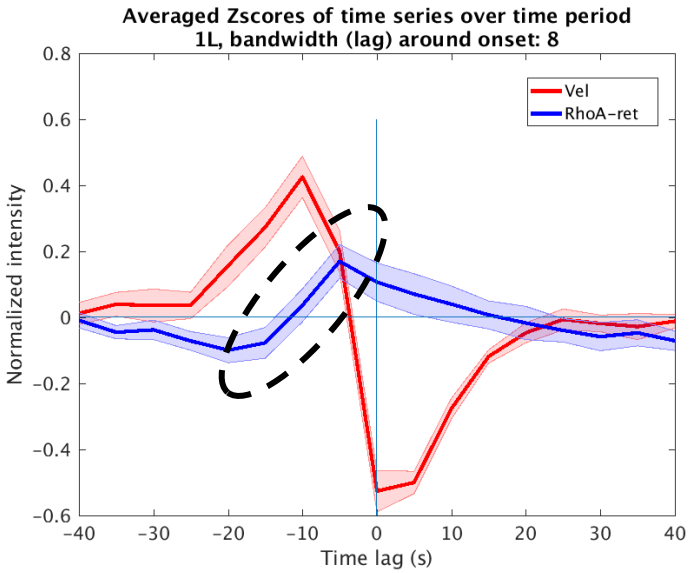


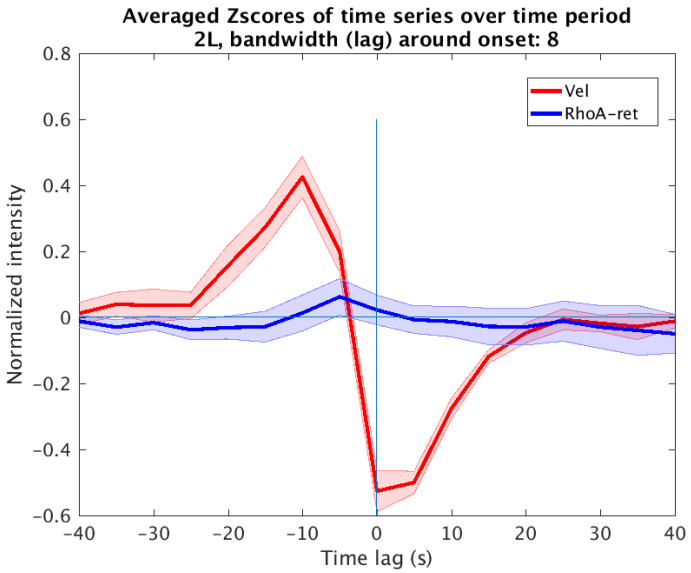
Minimum Vel

1Layer

2Layer

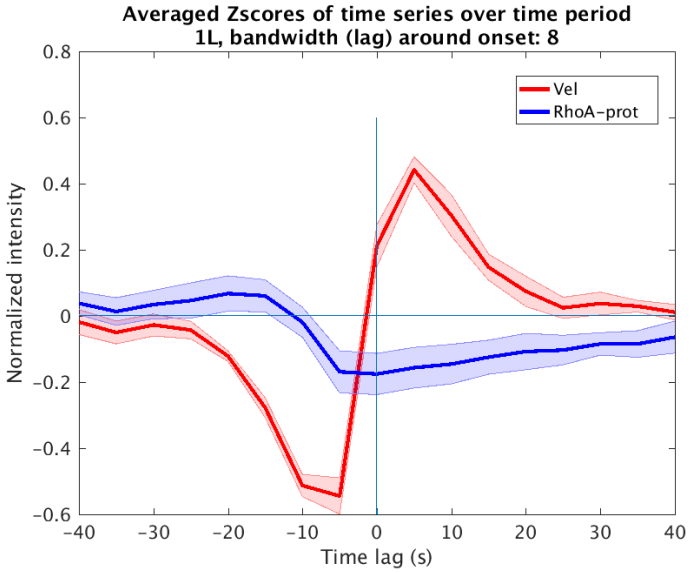
Summary for 8 movies (considering cell-to-cell variation)

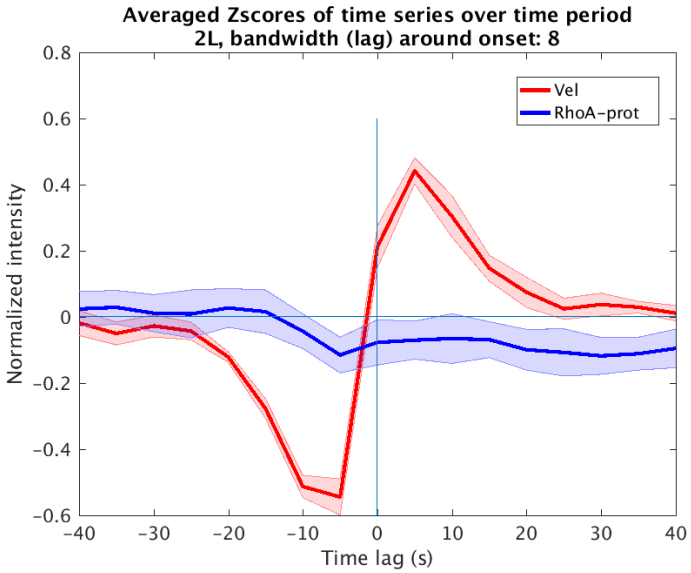




Ret-onset

1. The increase of RhoA proceeds the decrease of velocity before retraction onset.

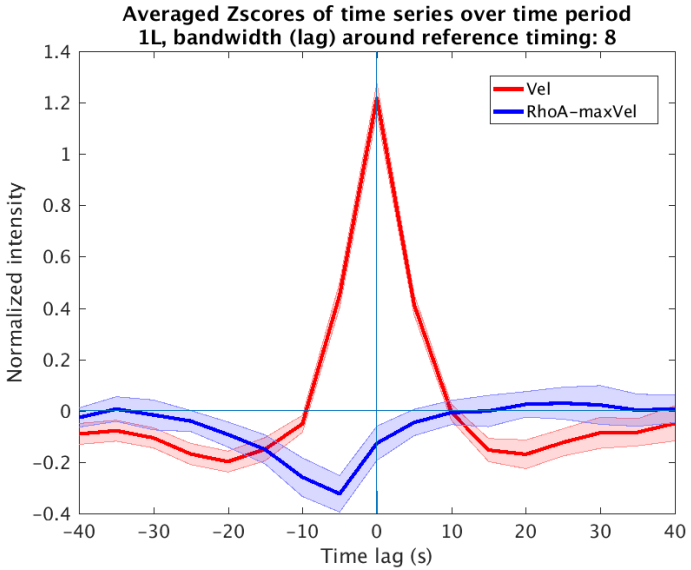


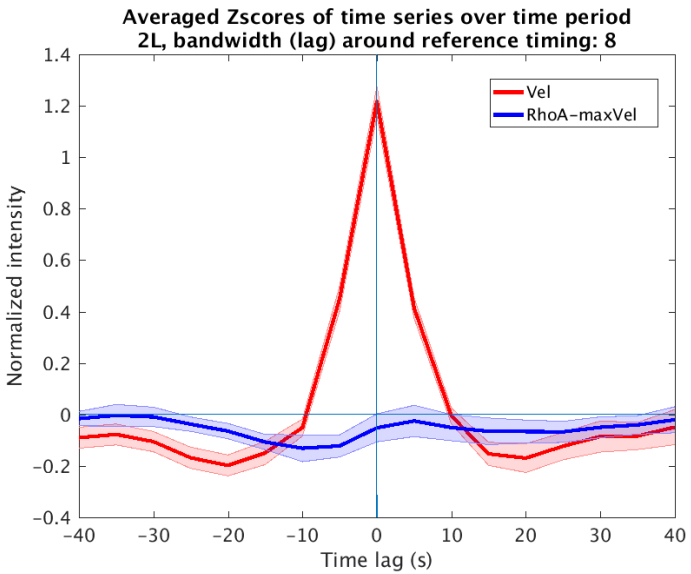


Prot-onset

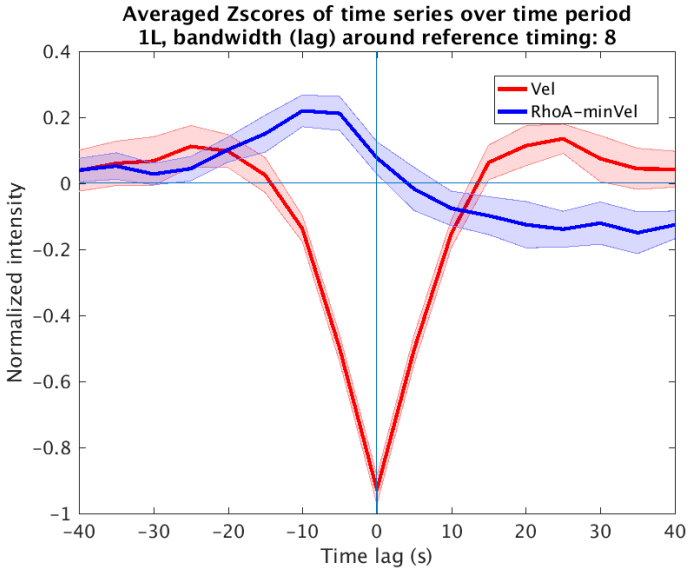
Retraction-phase Protrusion-phase

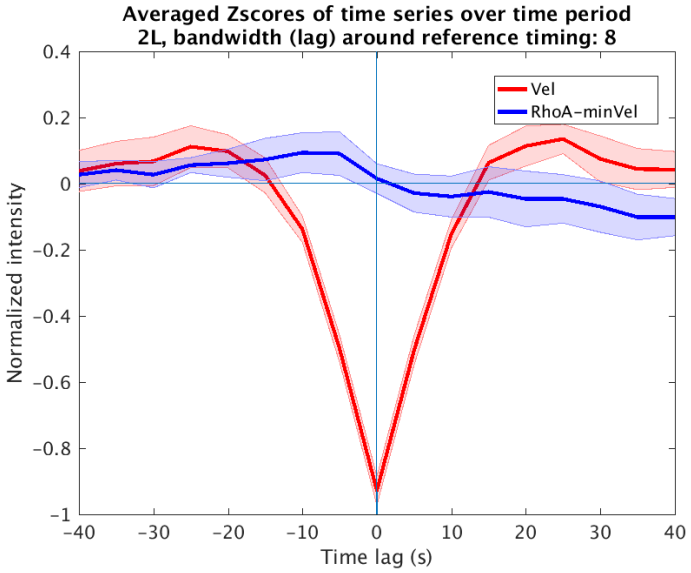
Prot-onset





Maximum Vel





Minimum Vel

Maximum or minimum peaks of RhoA activity proceed the peaks of velocity in a reversed fashion.

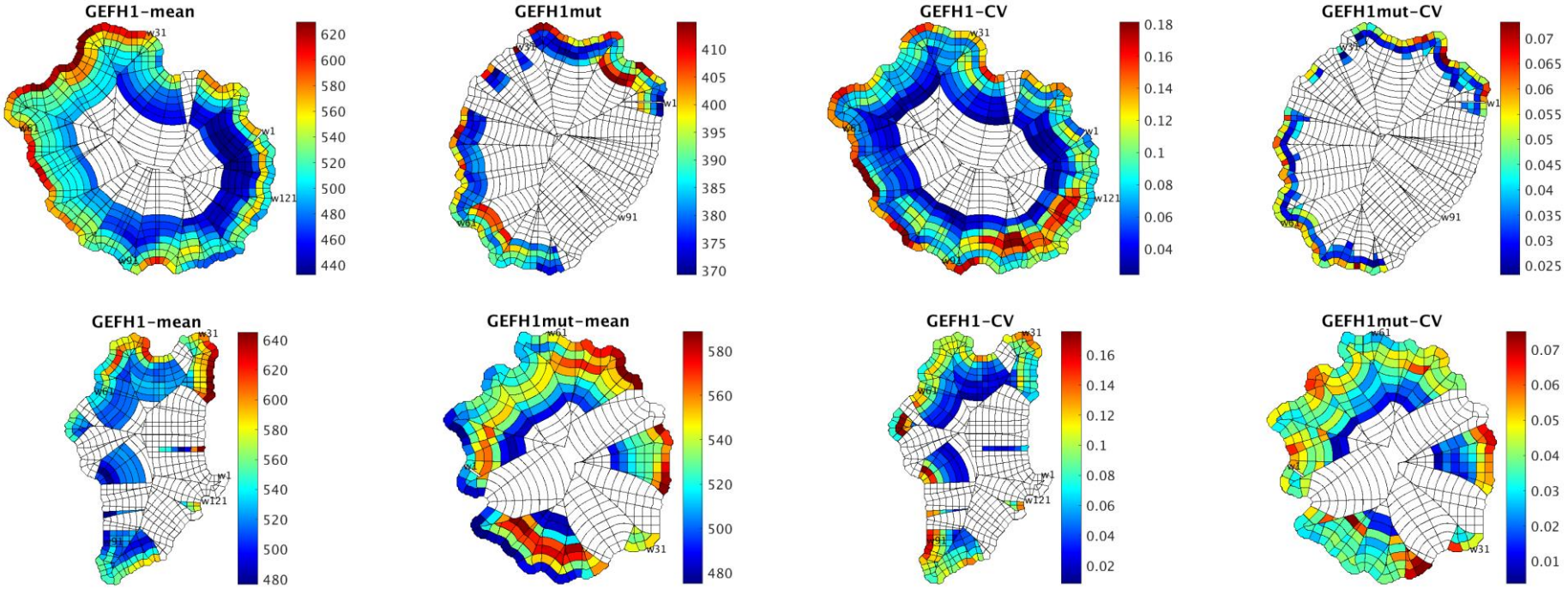
1Layer

2Layer

2. Spatial gradient along the distance from cell edge

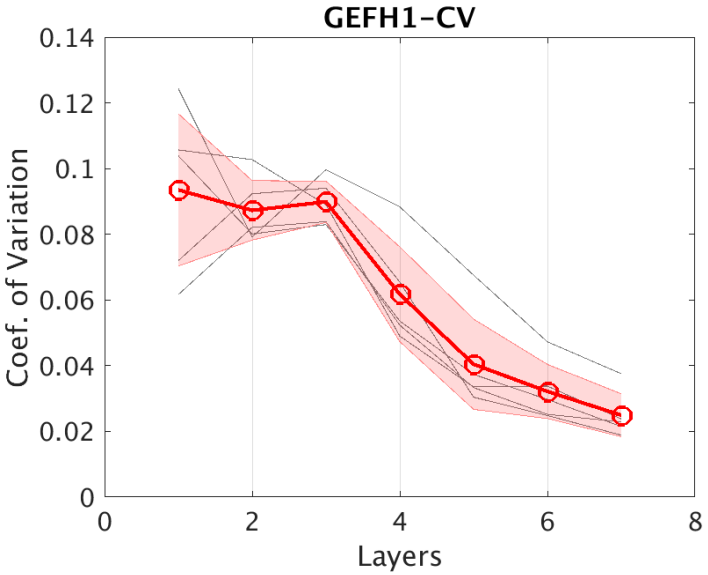
Average activity vs. Temporal variability

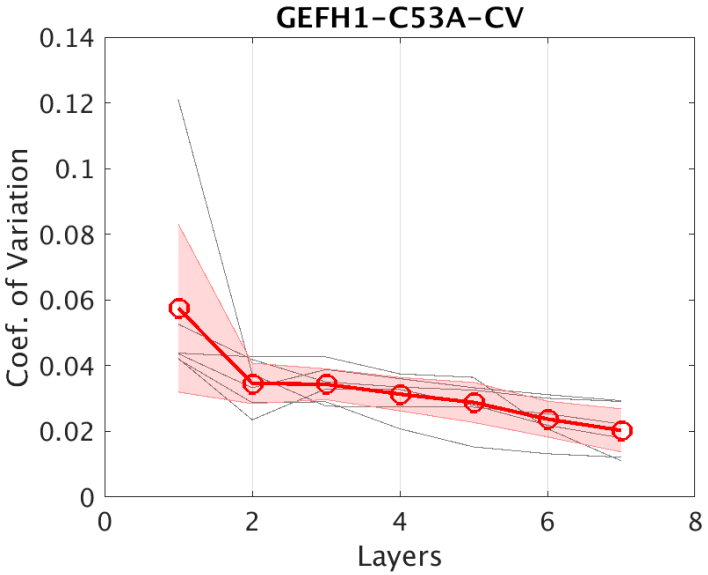
WT C53A WT C53A

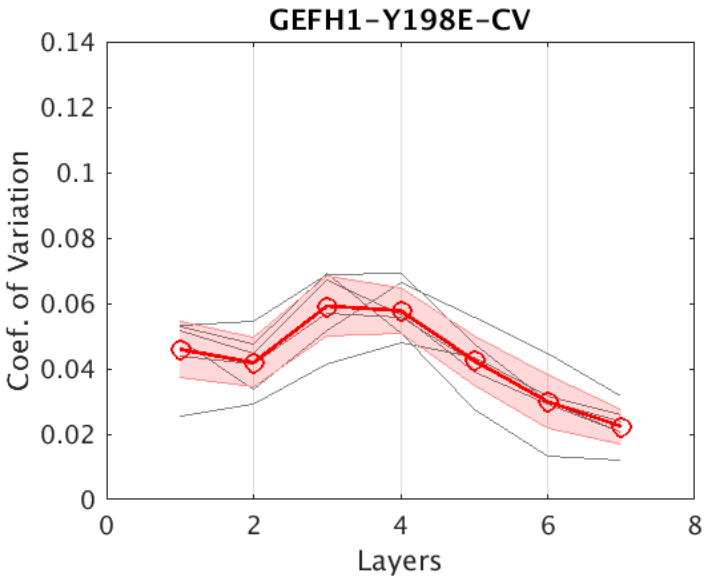


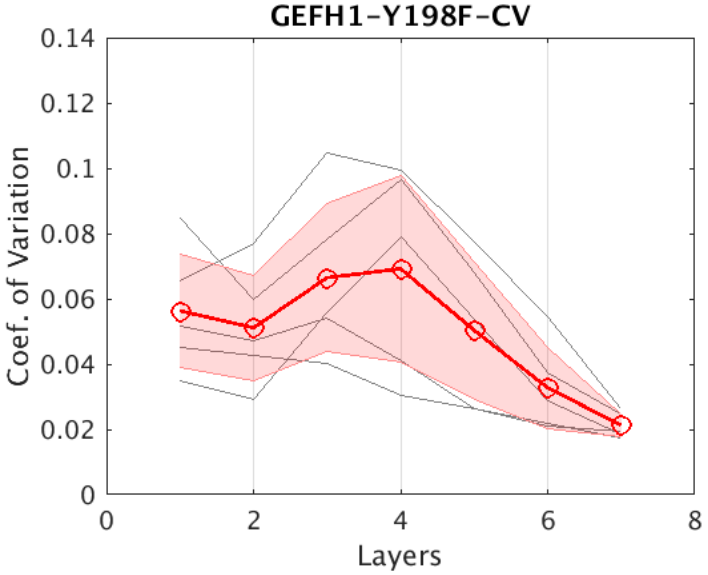
- Coefficient of variation (CV) = $\frac{\sigma}{\mu}$ = SD/mean
- It shows normalized variation (over time, in this case).

Temporal variability



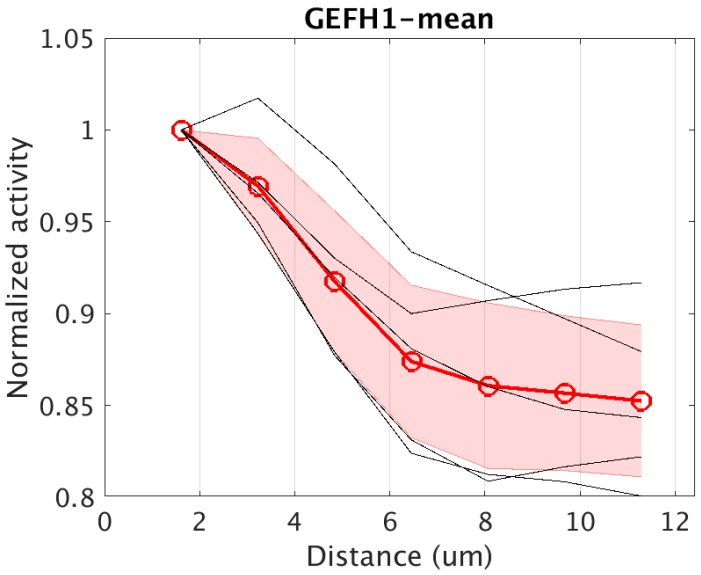




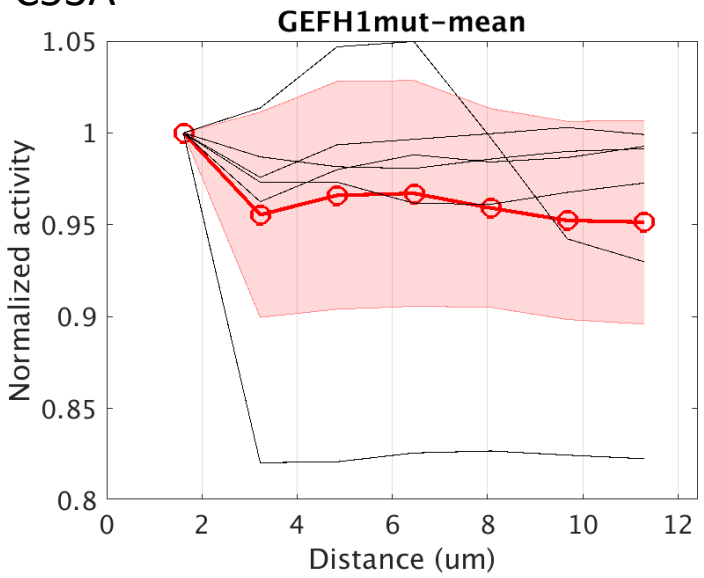


Normalized avg activity

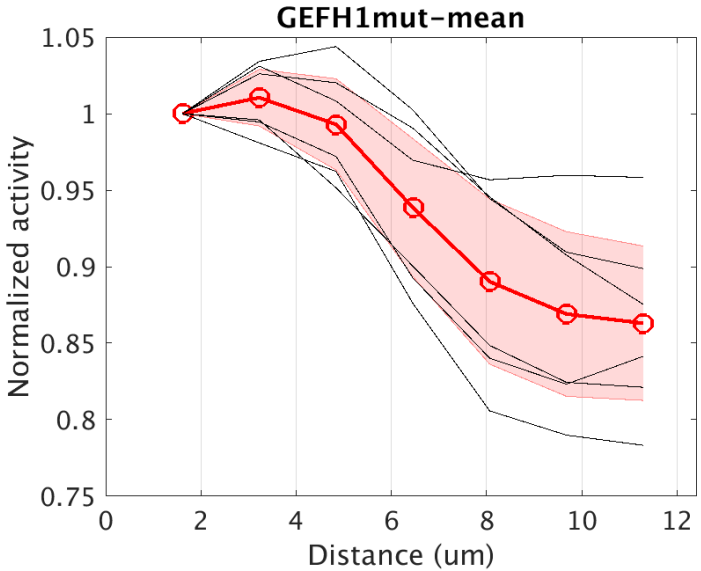
WT



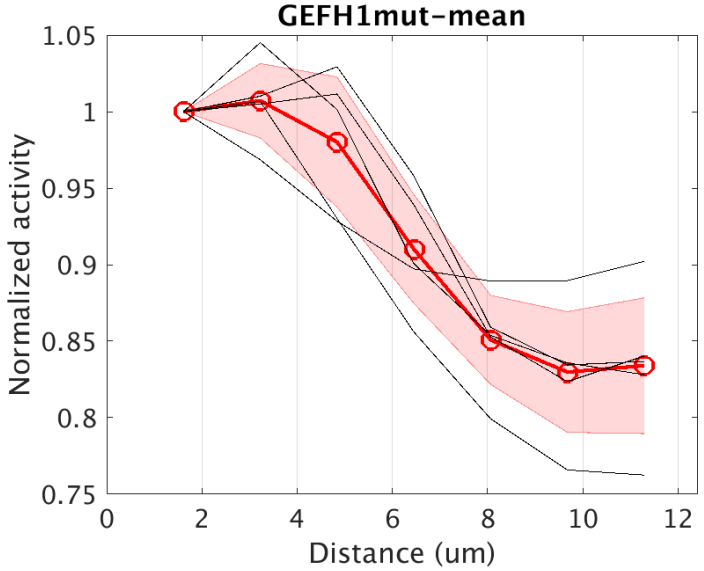
C53A



Y198E



Y198F



See also the following to measure the coupling between molecular dynamics and edge motion as shown in the figure

1. Readme_mapDDX.pdf (map Descriptives, Diagnostics and Xcorrelation analysis)
2. 6 examples of pipeline codes (after movieData are processed by windowing.)
    1. example_pipeline_DX_FPAEME_1chan_actin.m
    2. example_pipeline_DX_FPAEME_2chan.m
    3. example_pipeline_DX_FPAEME_1chan_biosensor.m
    4. example_pipeline_DX_FPAEME_1chan_LB_actin.m
    5. example_pipeline_DX_FPAEME_2chan_LB.m
    6. example_pipeline_DX_FPAEME_1chan_LB_biosensor.m