

Quantifying monolayer cell migration

Assaf Zaritsky
UTSW, WIS

January 29th, 2018

Training school for
Bioimage Analysts
Szeged, Hungary

Today's goals

- Tools:
 - Identifying clusters of coordinated motion in flow-fields
 - Quantifying spatiotemporal dynamics of wound healing (“scratch” assay)
 - Using these tools to learn new biology
- Ideas on:
 - Phenotypic screening
 - Extracting new information from “old” data

Resources

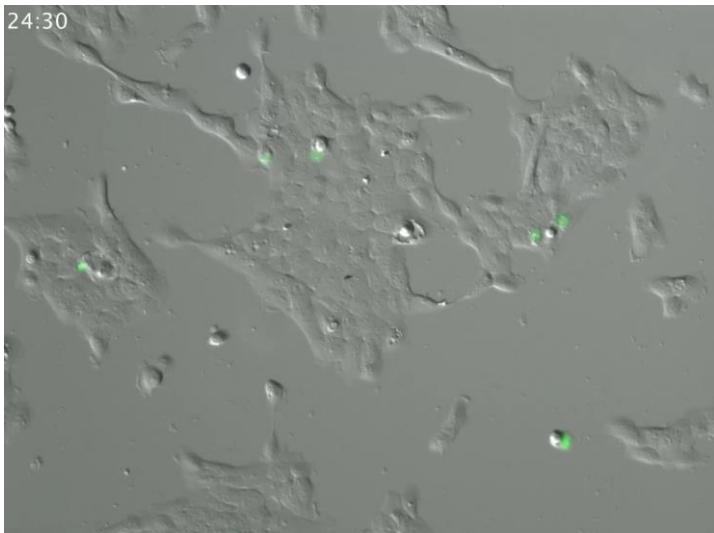
- Slides, description, relevant papers
https://github.com/miura/NEUBIAS_AnalystSchool2018/tree/master/Assaf
- Source code: <https://github.com/assafzar/MonolayerKymographs>
- Data: <https://cloud.biohpc.swmed.edu/index.php/s/R8e7zes51ZMC00f>

Agenda

1. **Collective cell migration**
2. Detection of coordinated clusters (+ exercise)
3. Example (data reuse)
4. GEF screen (+ exercise)
5. DeBias – if times allow (co-localization)

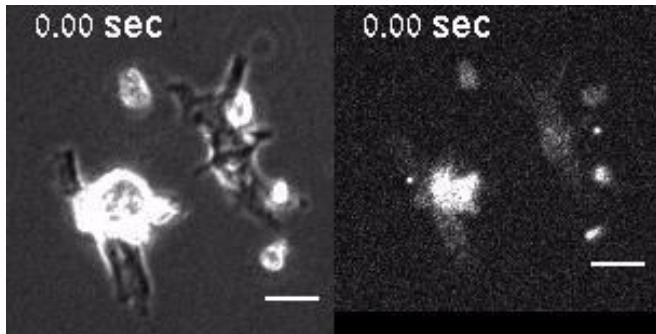
Emergence of collective cell behavior from single cell action and cell-cell communication

Collective cell death



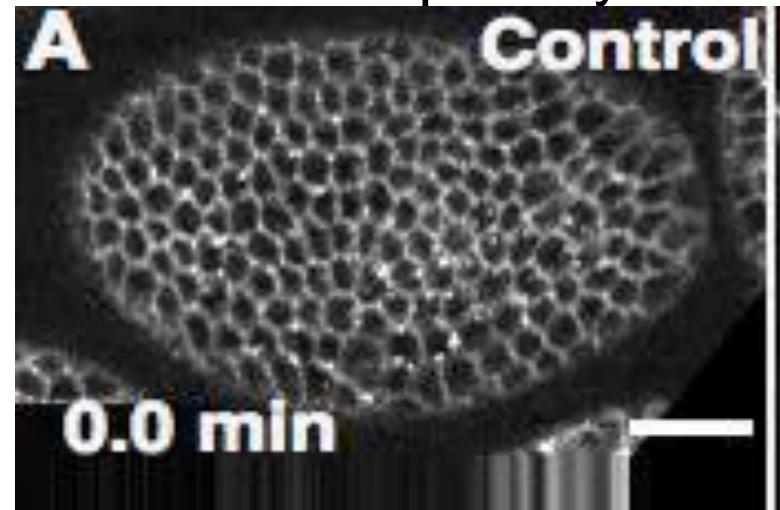
Overholtzer lab

Synchronized cardiac cells



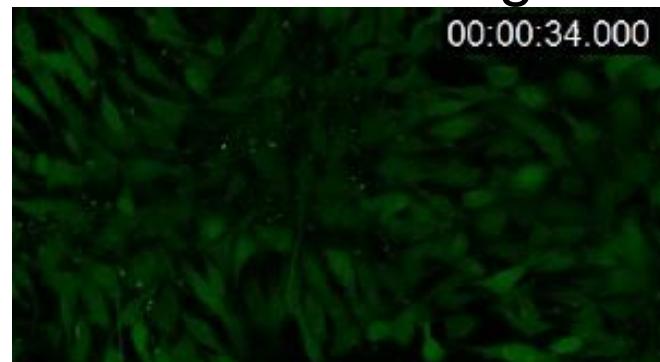
Nitsan et al. (2016)

Planar cell polarity



Barlan et al. (2017)

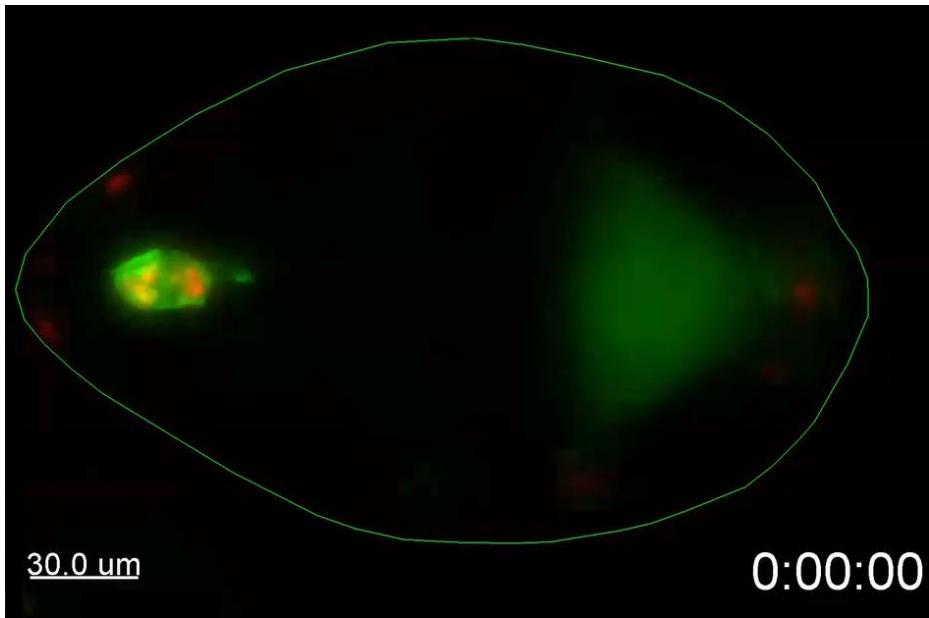
Collective calcium signaling



Sun et al. (2012)

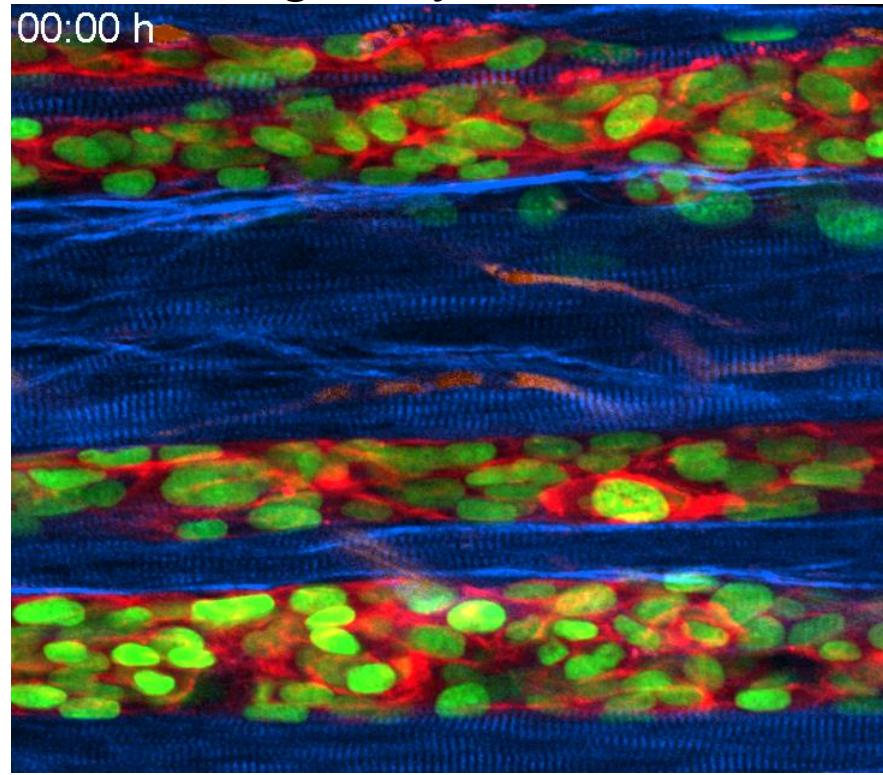
Collective cell migration

Border cells migration,
Drosophila Oogenesis



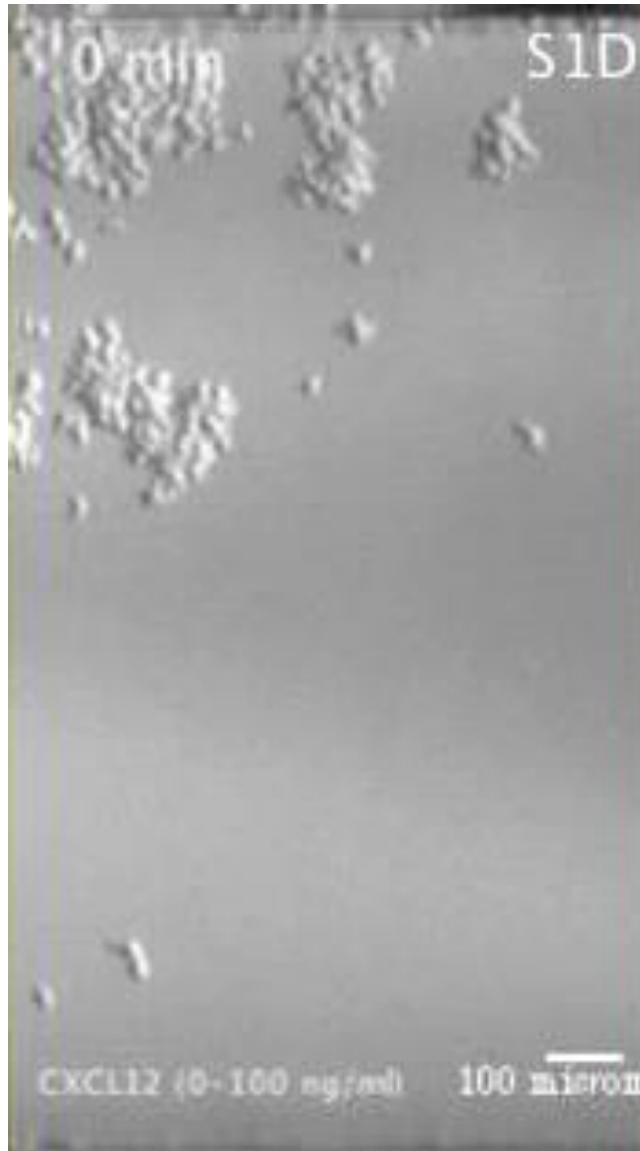
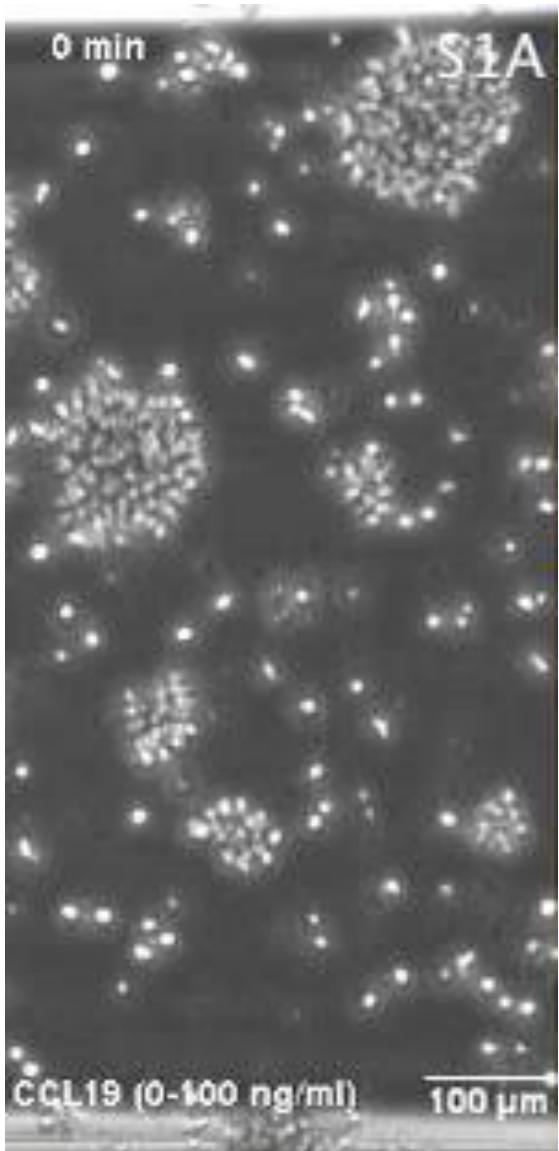
Cai et al. (2014)

Collective tumor migration
on “highways” in vivo



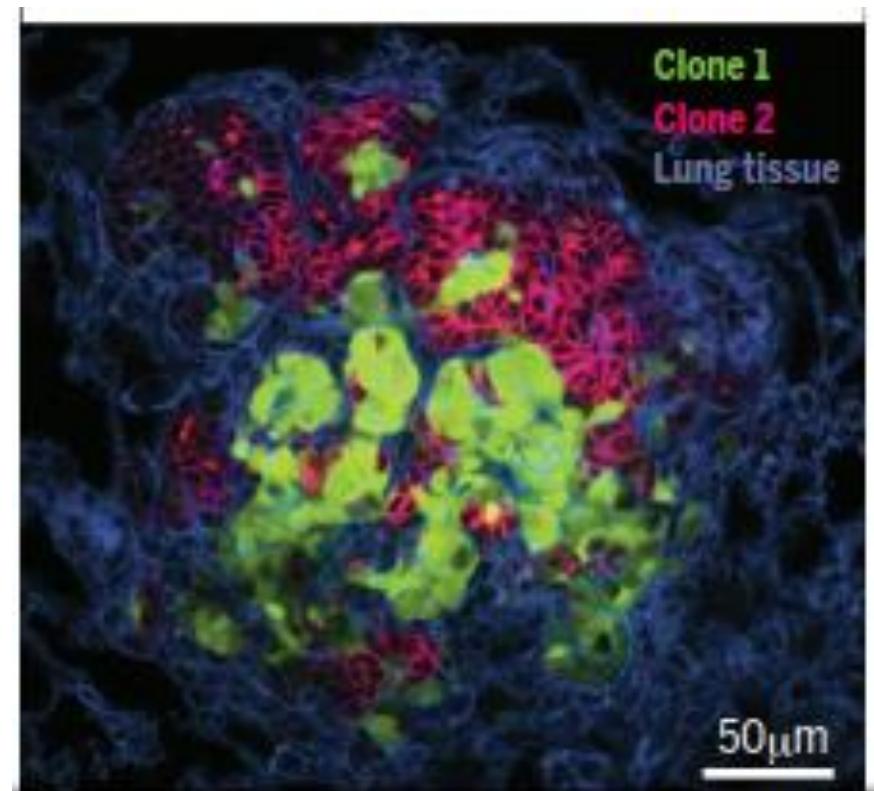
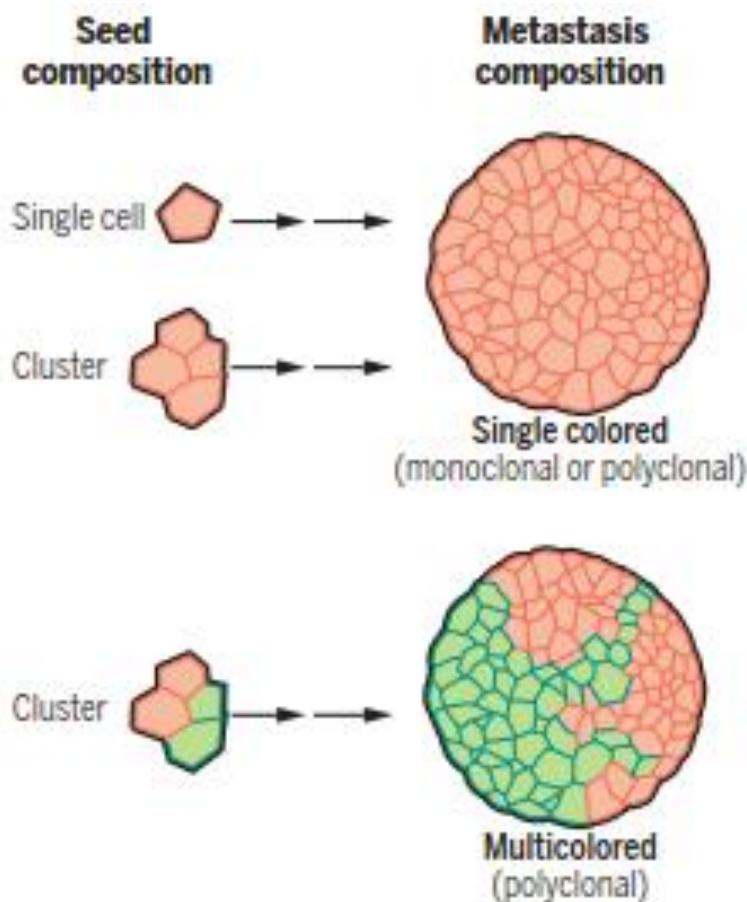
Bettina Weigelin, Peter Friedl

Cells migrate more efficiently in groups than individually



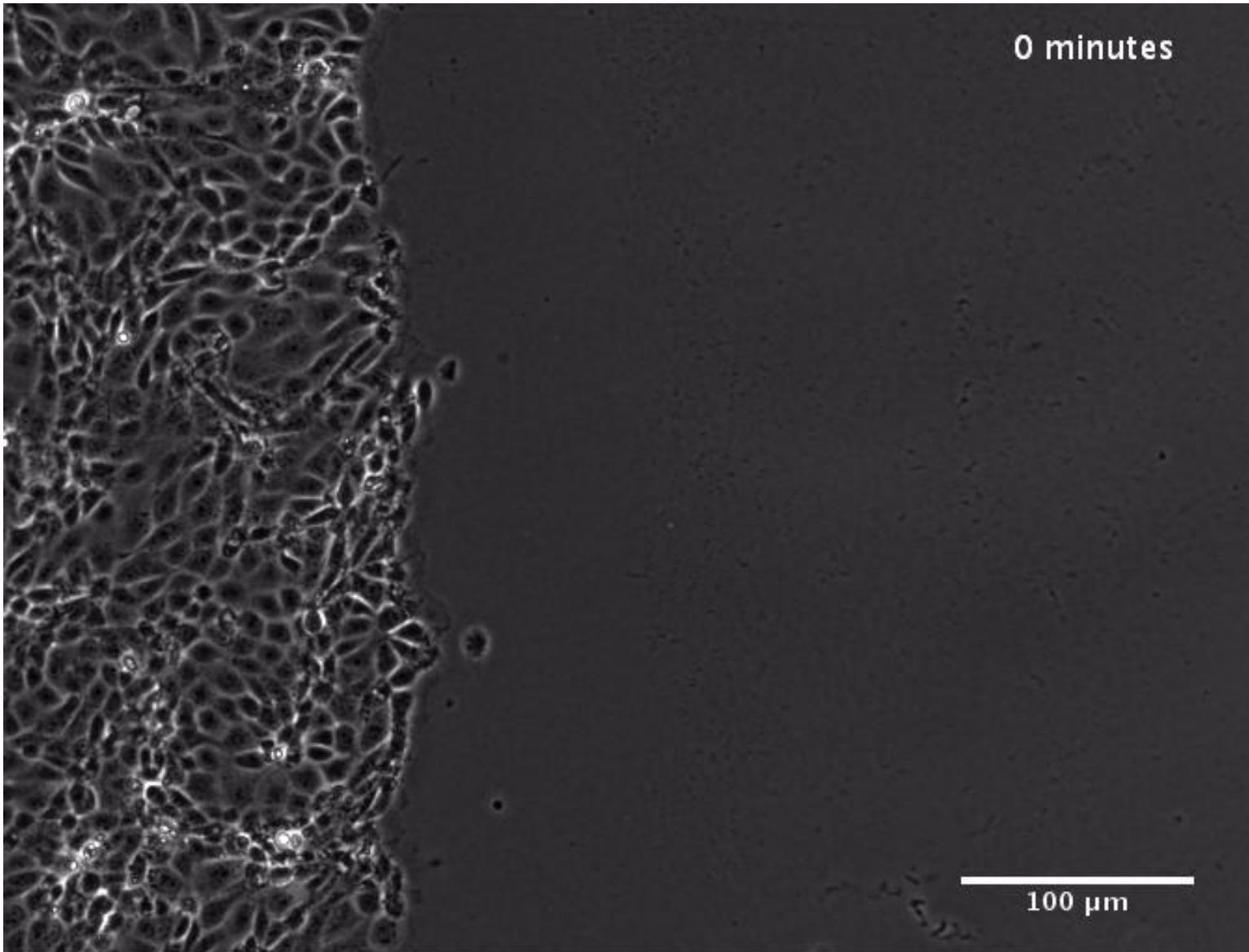
Malet-Engra
et al. (2015)

Tumor cell clusters as precursors of metastasis

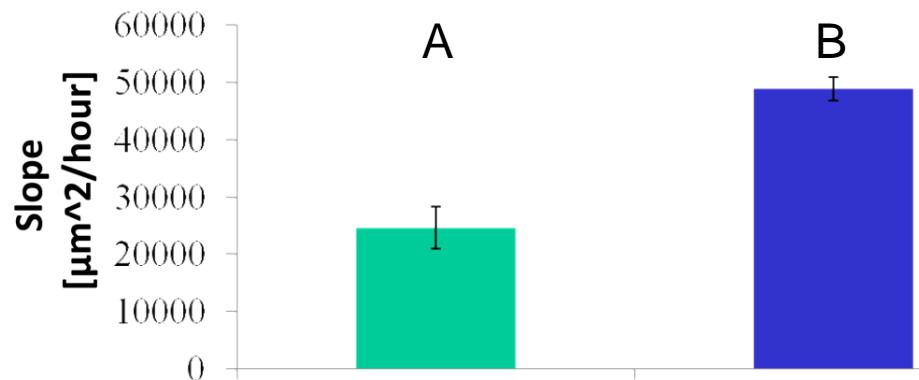
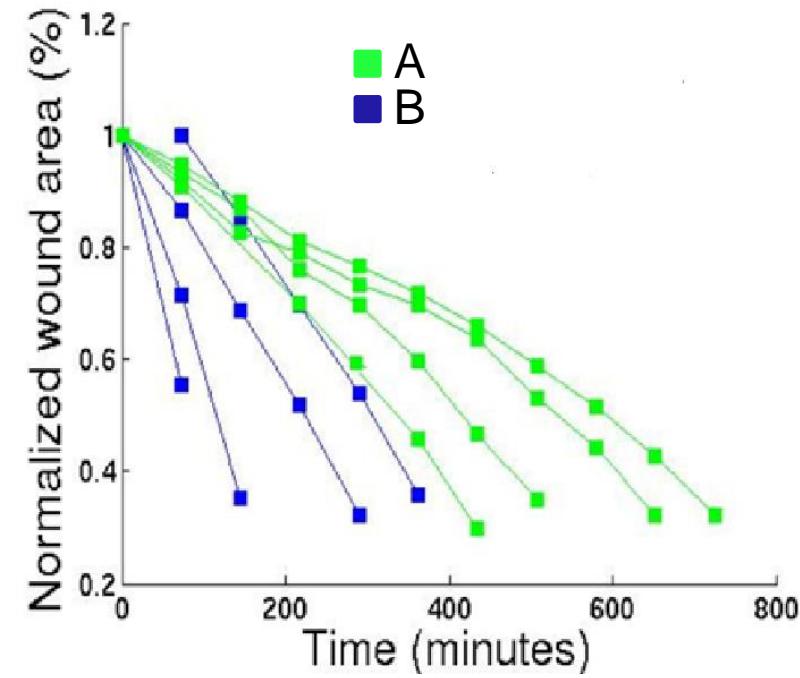
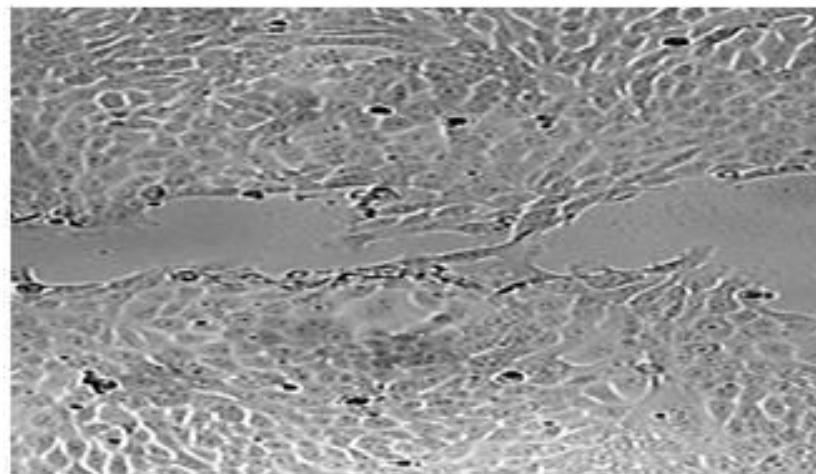
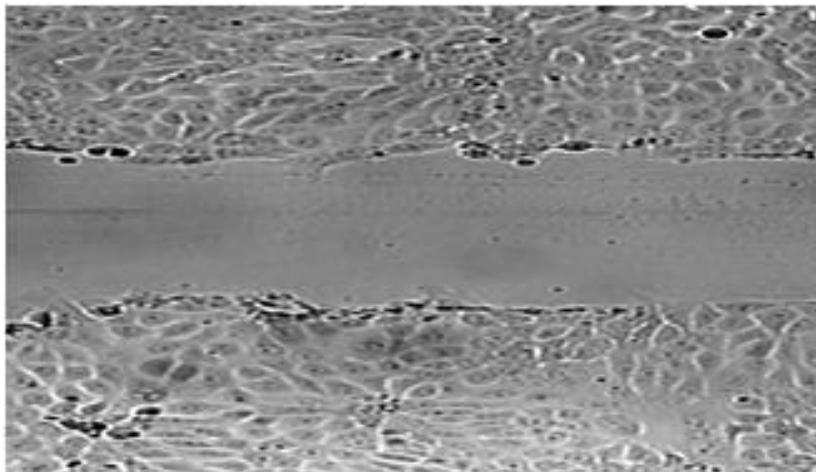


Cheung et al. (2016)
Aceto et al. (2014)

A simple model system to study collective cell migration



Standard quantification: monolayer migration rate



Tools available

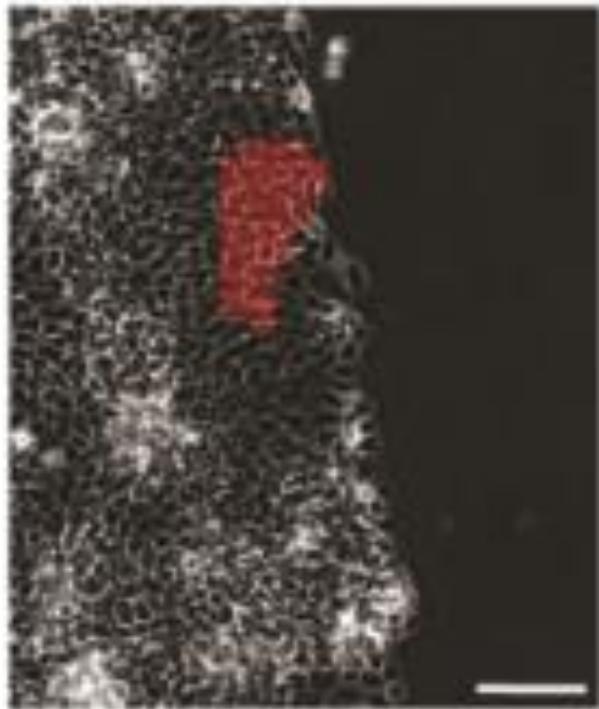
OpenLab	PerkinElmer [®]	Mac/A	2D/3D FL	MSExcel, comma-separated values	Wound-healing measurements, percentage of wound closure
AveMap	[102] ⁸	MATLAB; Windows, Mac/A	2D PC	Tab-delimited text	Wound-healing measurements: local velocities, monolayer edges, wound area, wound shape, productive velocities
Cell Image Velocimetry	[89] ⁹	MATLAB; Windows/A	2D PC, FL	MATLAB mat	Wound-healing measurements, velocity fields, angular velocity distributions
MultiCellSeg	[58] ¹⁸	MATLAB; Mac/A	2D PC	MATLAB mat	Wound-healing measurements: multicellular segmentation features
TScratch	[59] ²¹	MATLAB; Windows, Mac/A	2D PC	MSExcel	Wound-healing measurements: open wound area

Agenda

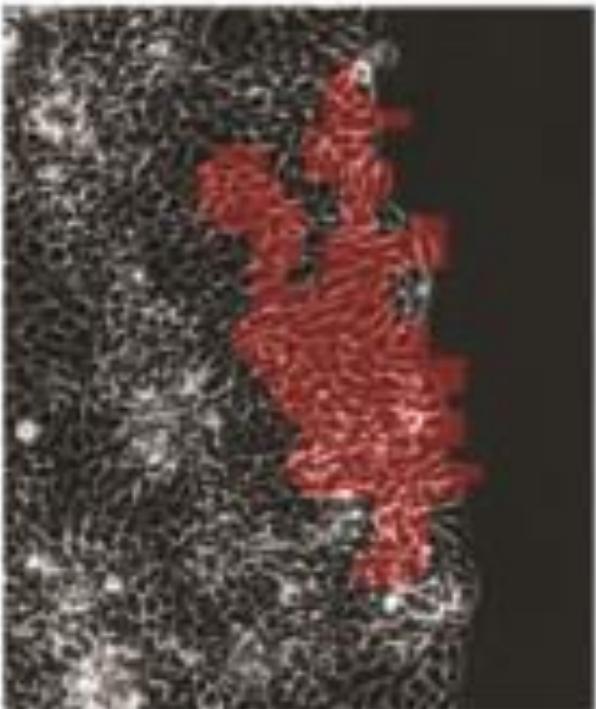
1. Collective cell migration
2. **Detection of coordinated clusters (+ exercise)**
3. Example (data reuse)
4. GEF screen (+ exercise)
5. DeBias – if times allow (co-localization)

Explicit detection of coordinated cells

0 min



60 min



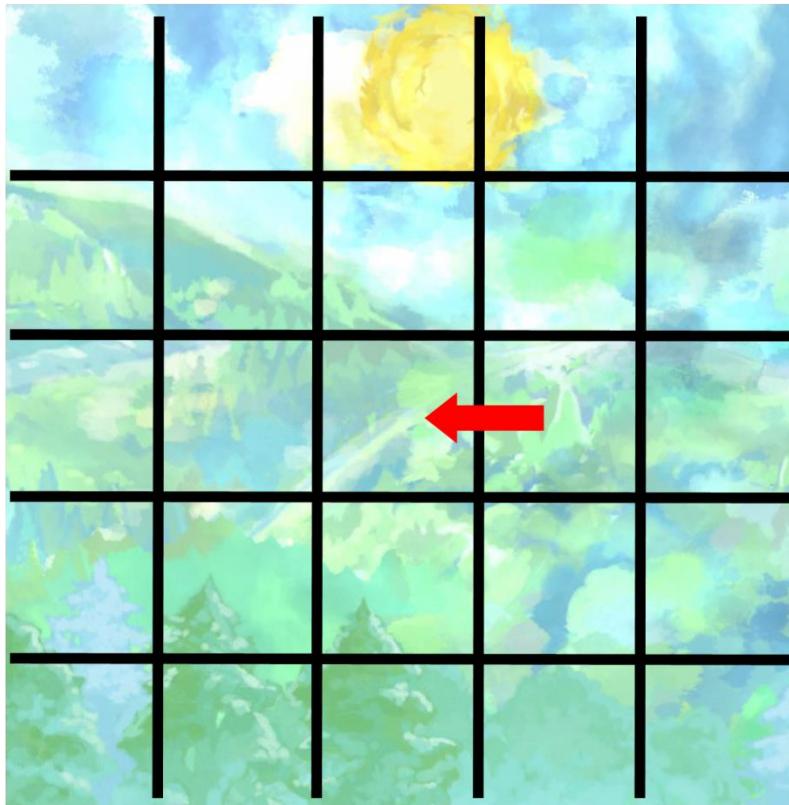
120 min



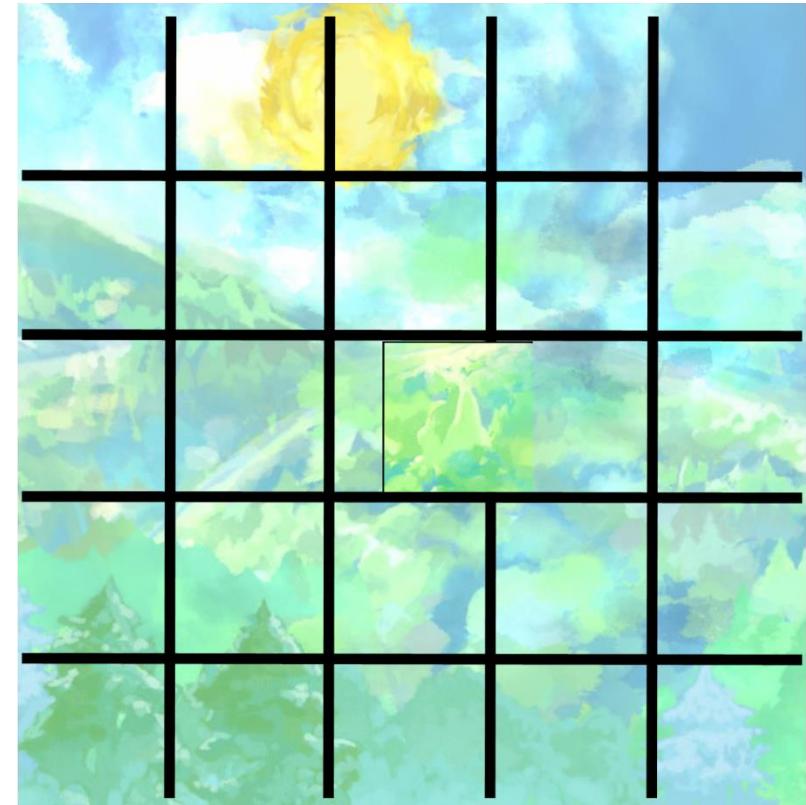
Motivation

Exploiting spatial heterogeneity
to assess mechanisms of
coordinated migration

Determining flow fields: Particle Image Velocimetry (PIV)

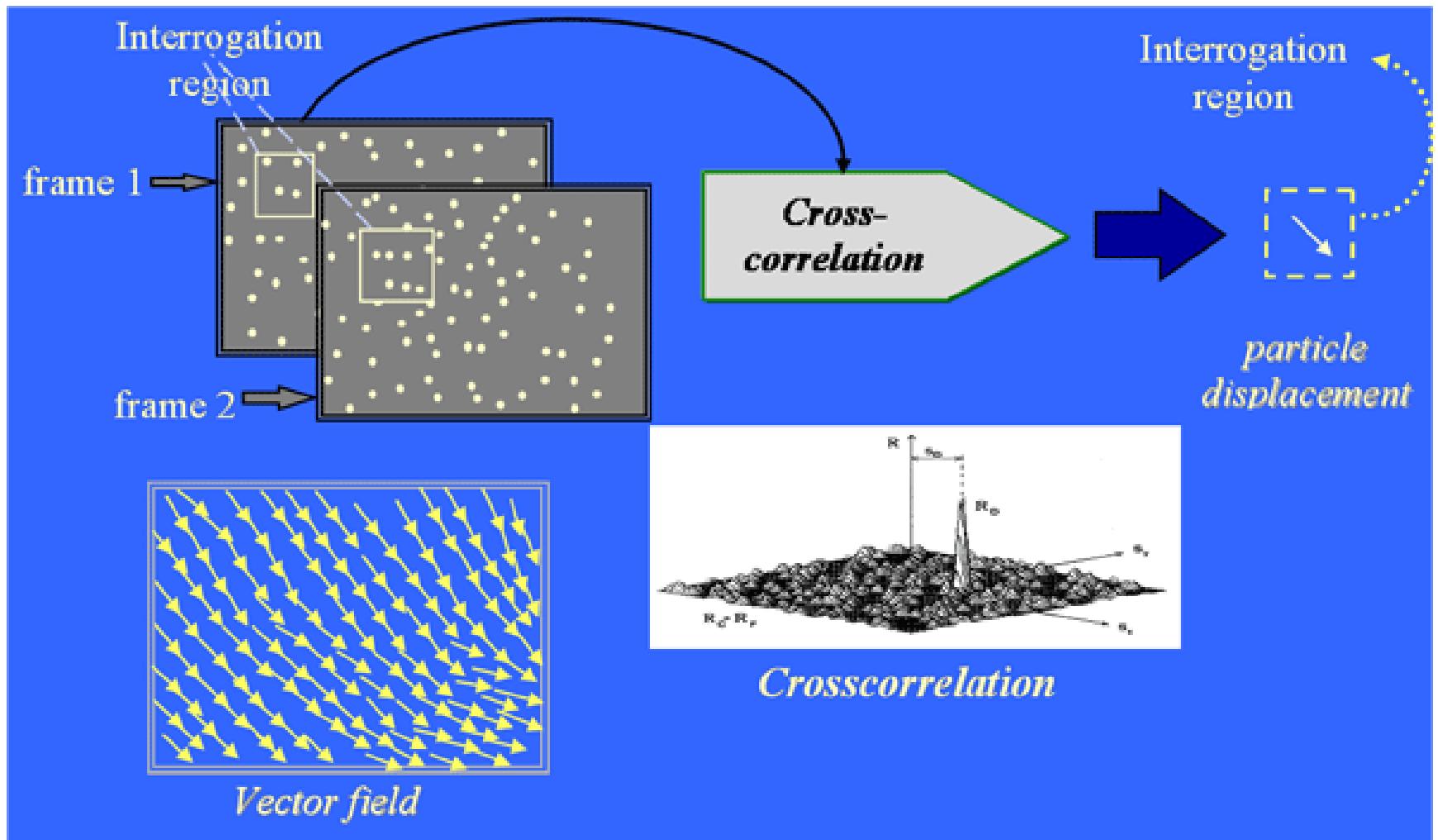


Time 1



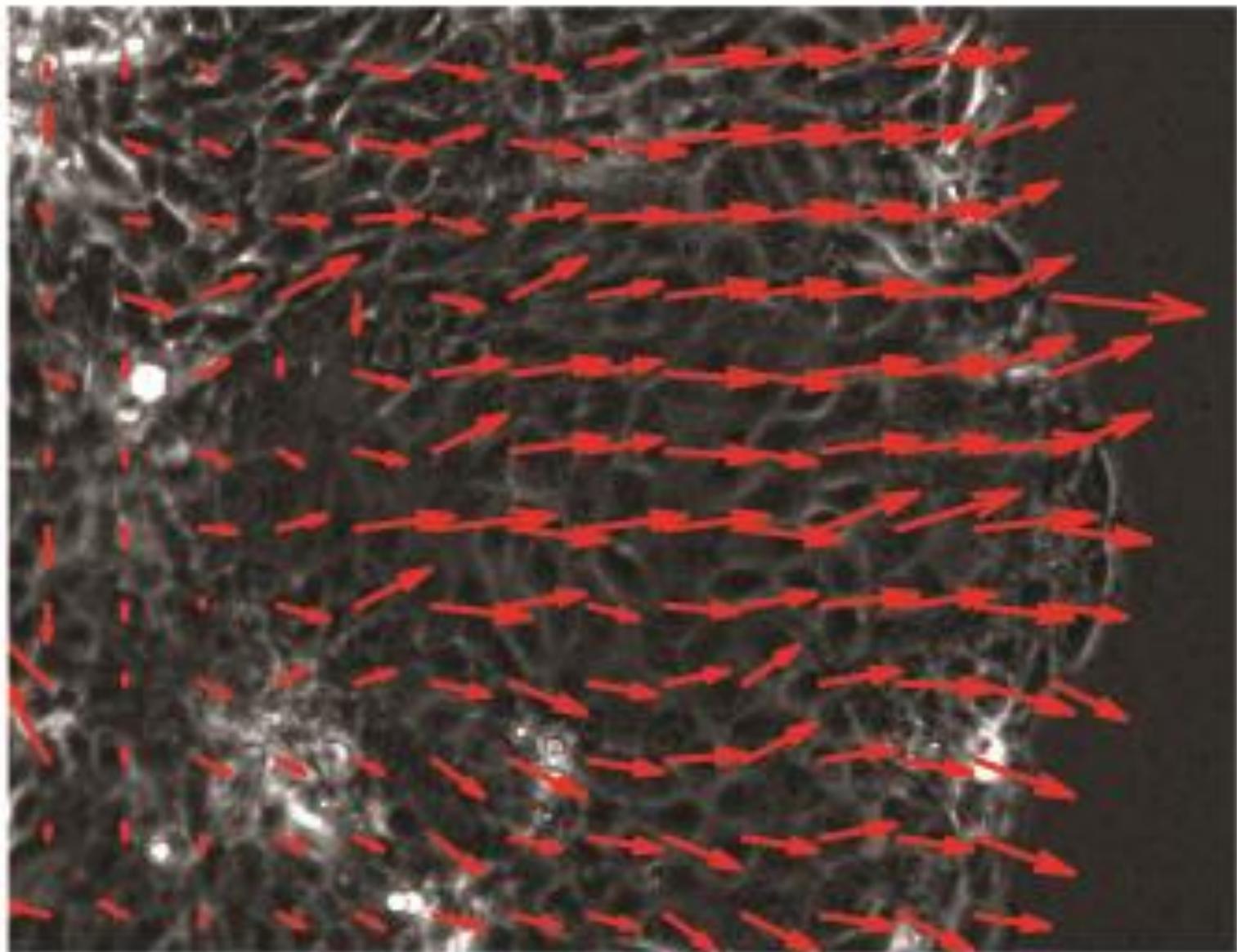
Time 2

Particle Image Velocimetry (PIV)



Source: <https://www.erc.wisc.edu/piv.php>

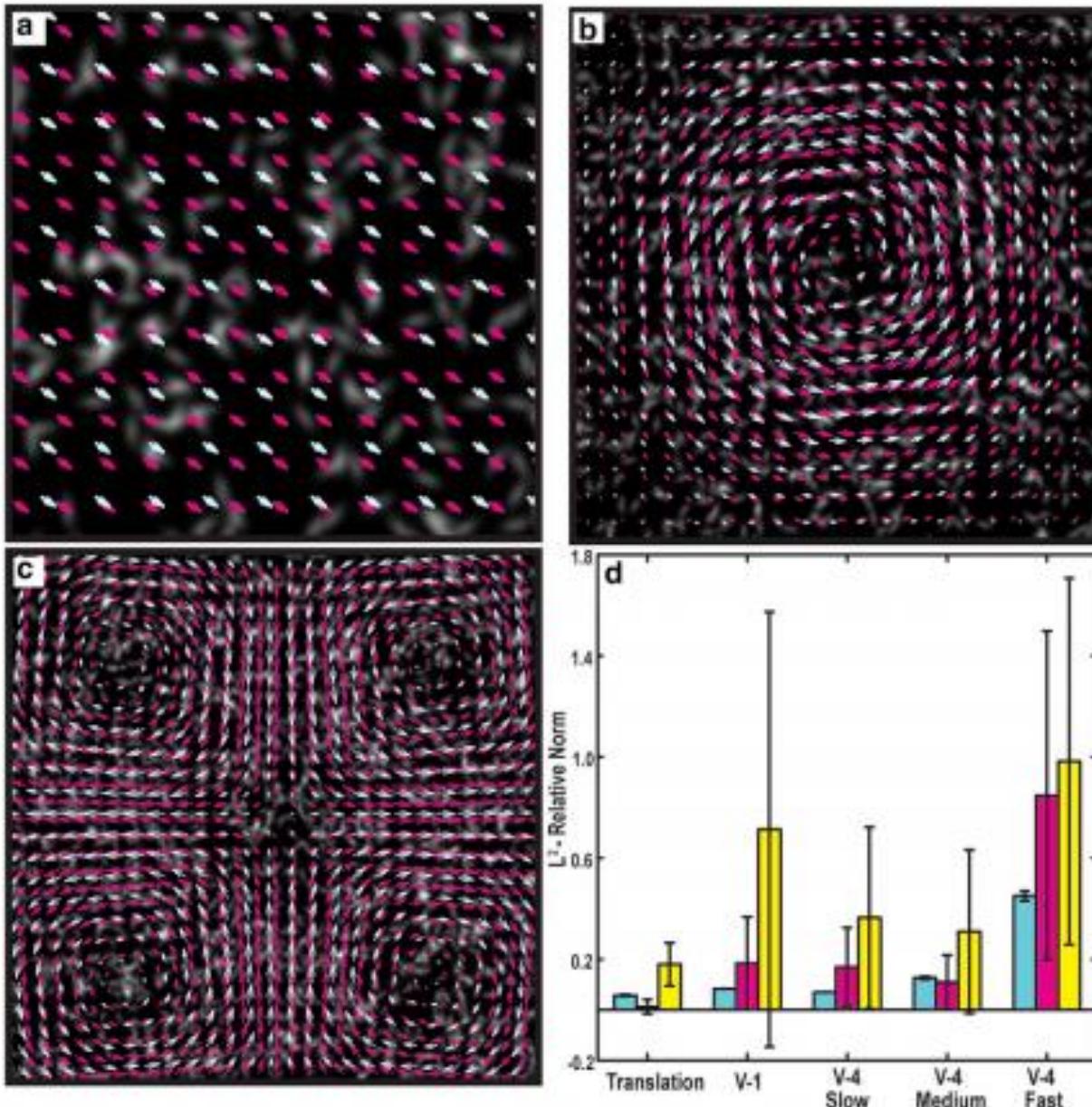
PIV for monolayer migration



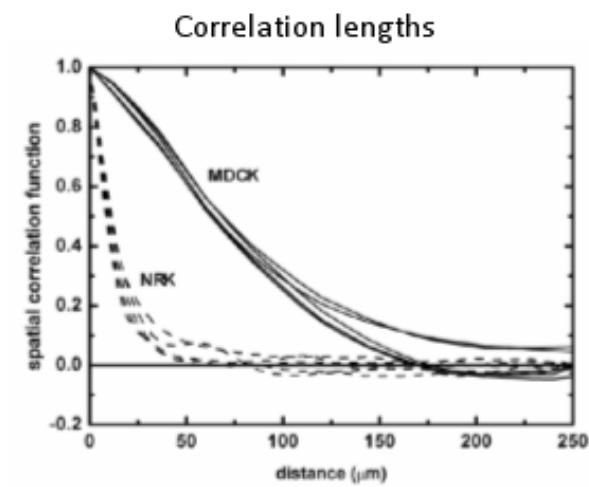
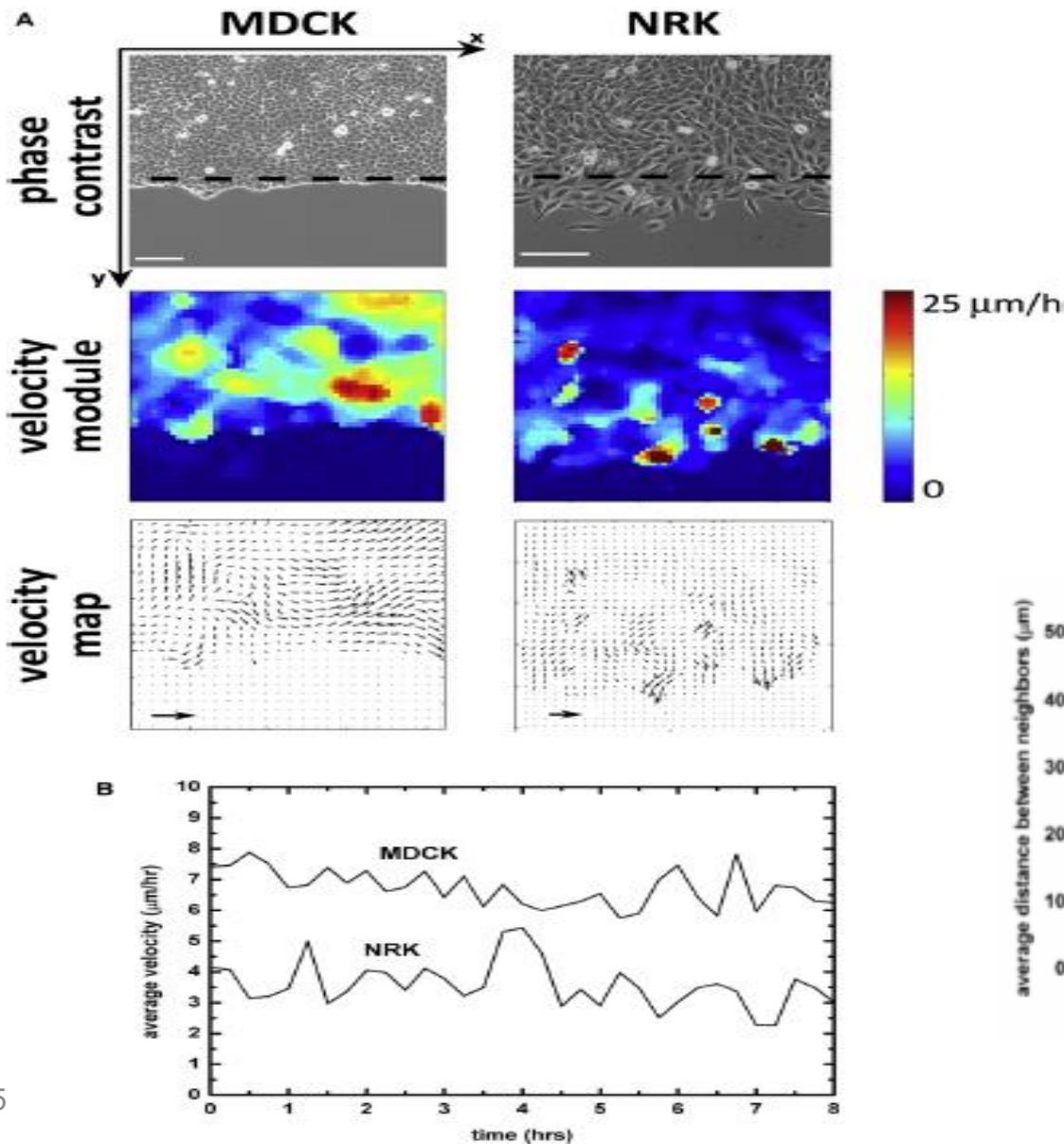
(gradient based) optical flow

- Partial derivatives with respect to the spatial and temporal coordinates
- Lucas–Kanade method
 - Assumptions:
 - motion is small
 - smooth change
 - Fast
 - Many extensions

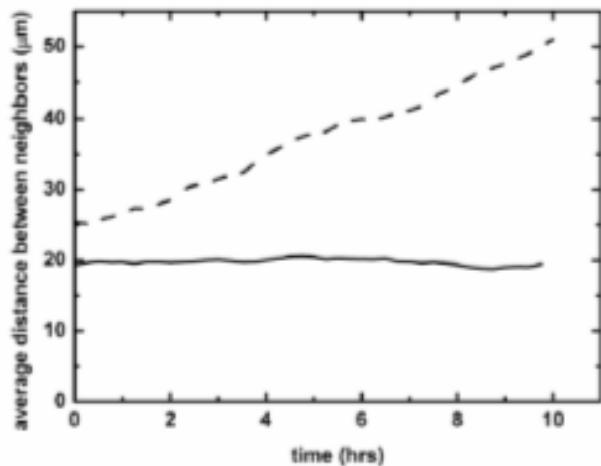
Optical flow versus PIV



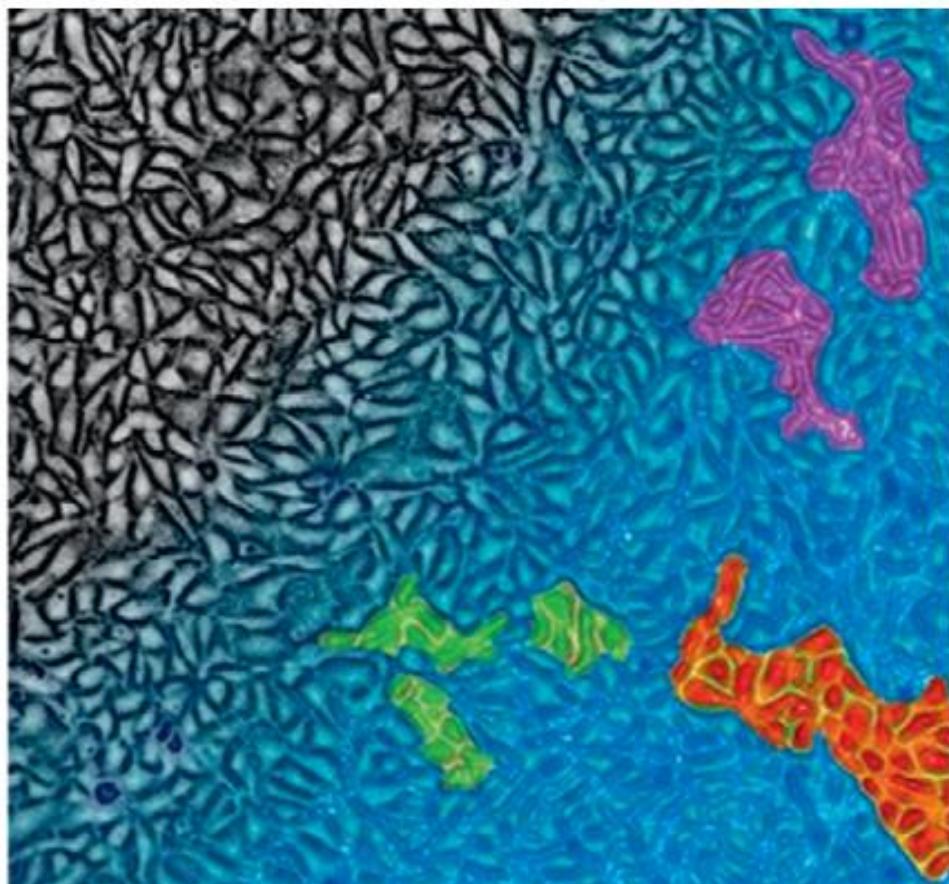
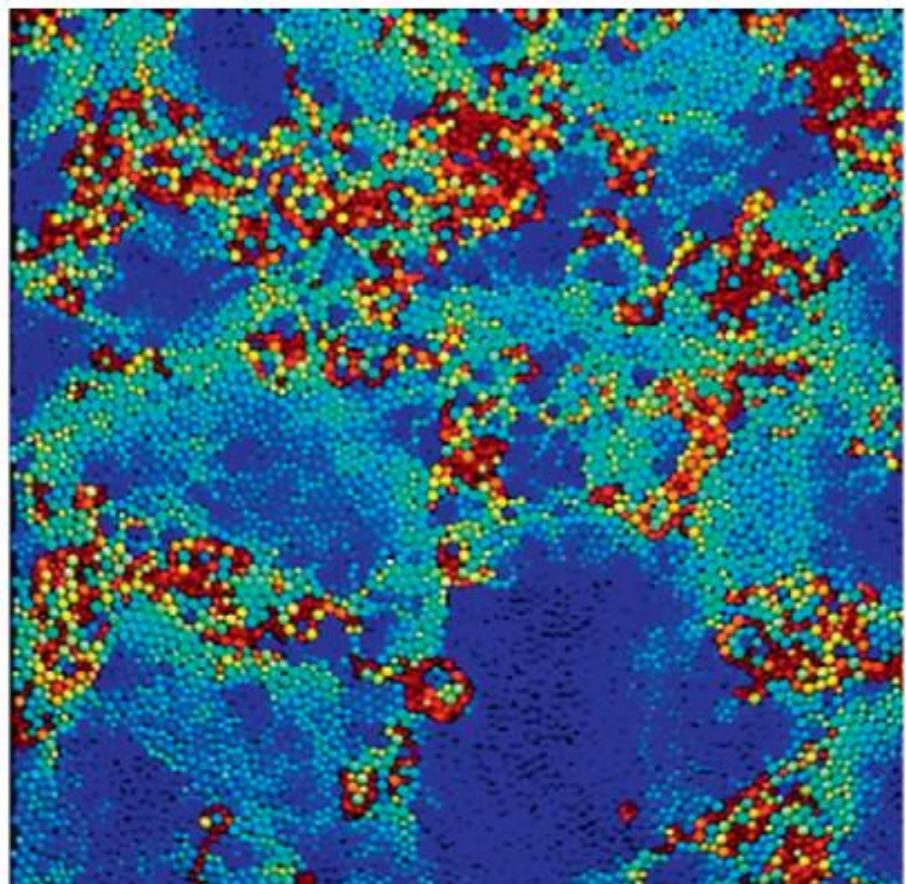
Measuring coordination



Distance between neighboring cells in time (NRK - separate)



Visualization (of cell speed)



Angelini (2011)
Trepat and Fredberg (2011)

Statistical Region Merging



Nock and Nielsen (2004)

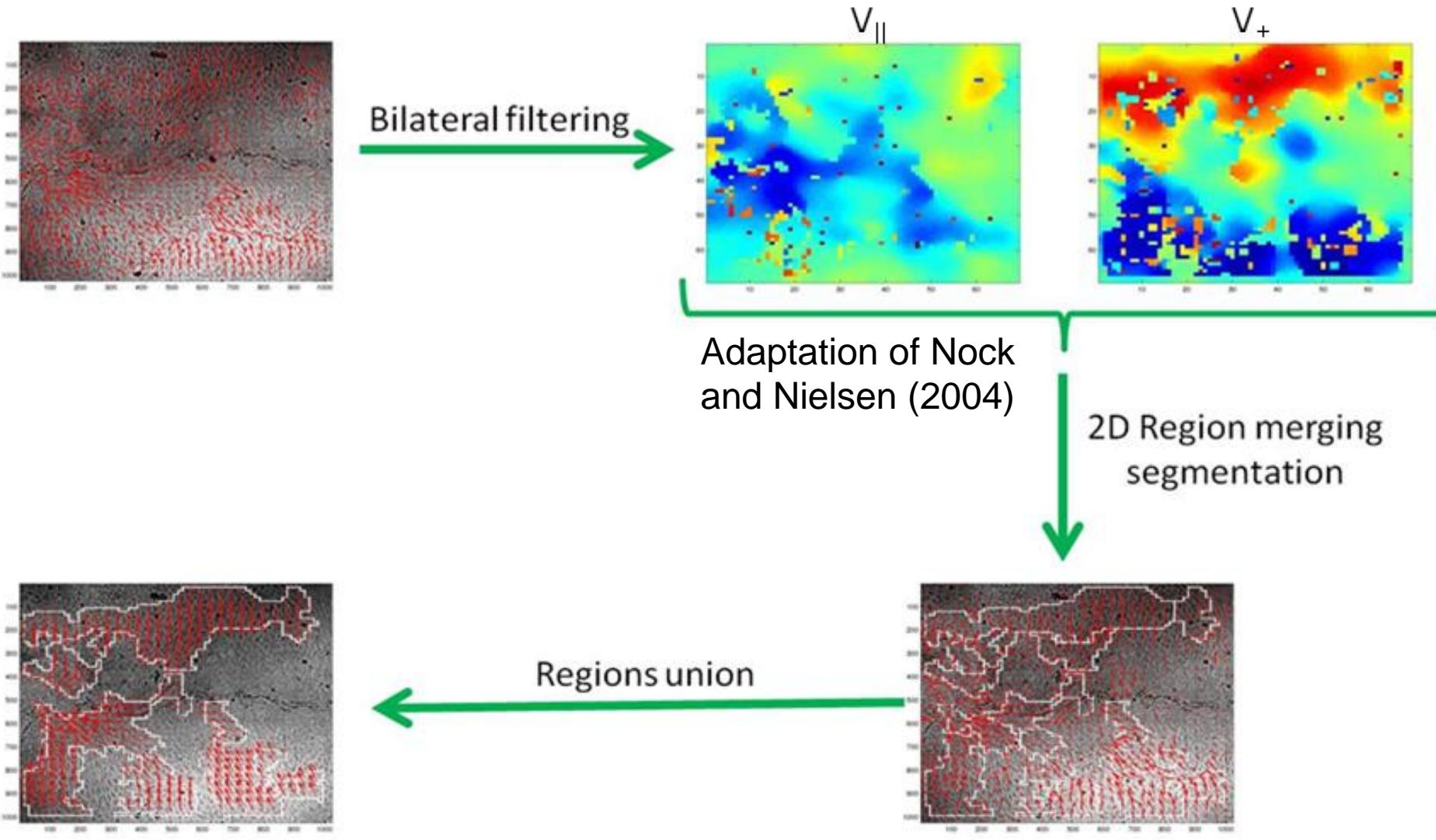
Region growing / merging

- Regions: sets of pixels with homogeneous properties
- Iteratively combining smaller regions (“growing”)
- Statistical test to decide whether to merge or not
- Balances sustainment of perceptual units vs. over-merging
- Here: implementation for velocity fields

Algorithmic components

- Initialization
- Metric for region similarity
- Merging predicate
- Merging order

Explicit detection of coordinated cells



Implementation

- Patch → region
- 4-connectivity neighbor patch-patch similarity
- Sort couples in ascending order
- Traverse couples by sorted order:
 - Find corresponding regions
 - Calculate region-region similarity
 - Merge if similarity < threshold (dependent of size + similarity)

Implementation (more detailed)

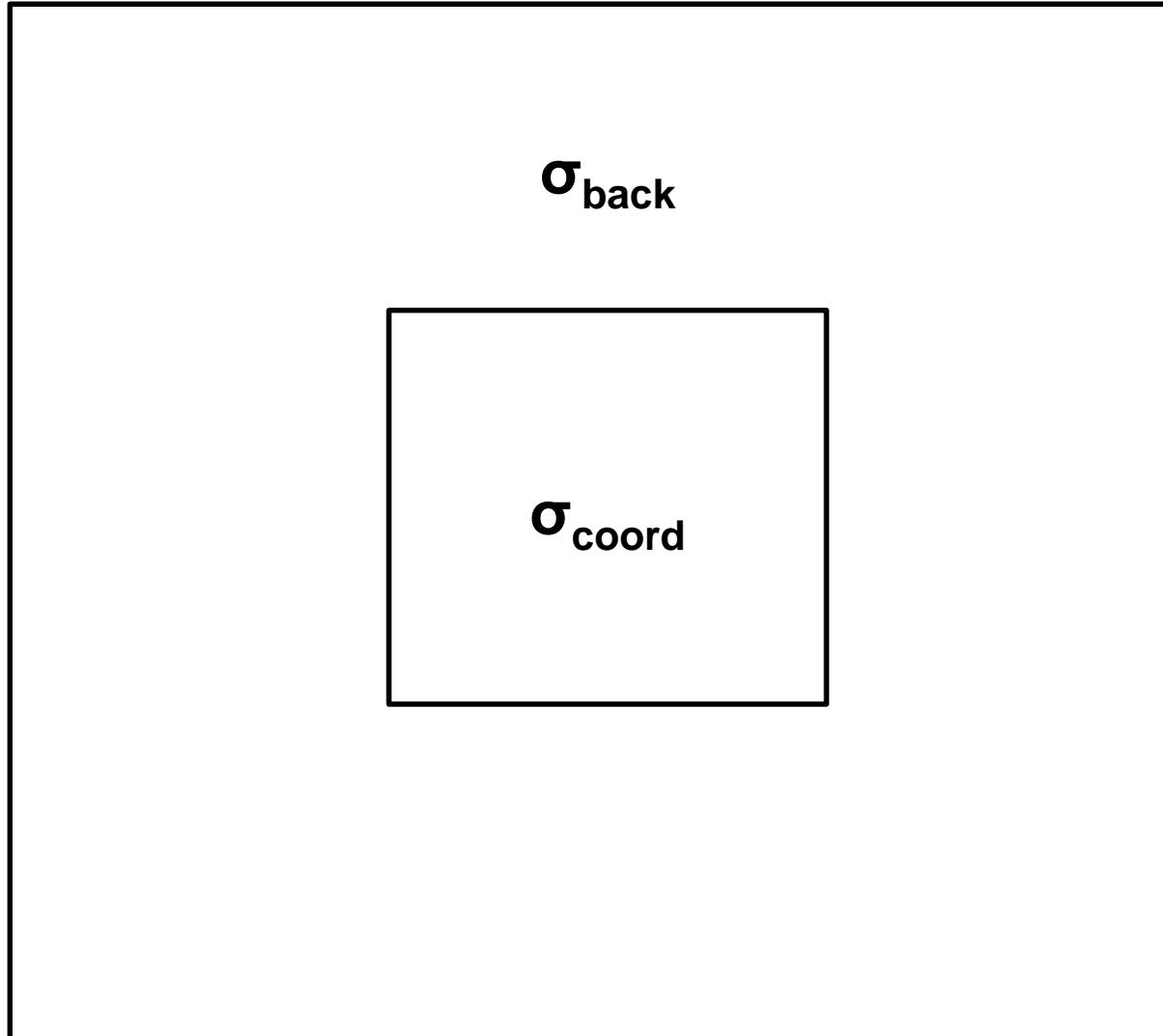
1. Start by defining a region for each patch containing its motion-estimation vector
2. Calculate the similarity for all 4-connectivity couples of adjacent motion-patches
3. Sort these couples in increasing order
4. Traverse this order once, for any current couple of pixels (p_1, p_2):
 - a. Find (r_1, r_2) the corresponding regions to (p_1, p_2)
 - b. Extract the average vector in r_1 and r_2 , calculate their similarity, $\text{sim}(r_1, r_2)$
 - c. Calculate the threshold for merging two regions $\text{TH} = b(r_1) + b(r_2)$, whereas $b(r) = \log(\text{size}(r)) * Q * \log(2.0/P)$
 - d. Merge r_1 and r_2 if and only if $\text{sim}(r_1, r_2) < \text{TH}$
5. Discard regions smaller than approximately 20 cells or where no significant motion was found
6. Unite touching-regions and report them as the final clusters

Pros and cons

- Pros:
 - Fast (not in my implementations..) and easily implementable
 - Can handle noise and occlusions
- Cons:
 - Does not capture “flow” patterns
 - Clusters are not sufficiently stable for tracking
 - Setting parameter/s to optimize similarity measure / merging predicate (for any method that explicitly segments)

Toy simulation (simulateCoordination)

Mean vector (0,1)



Mean vector $(dy, dx) = (0, 1)$

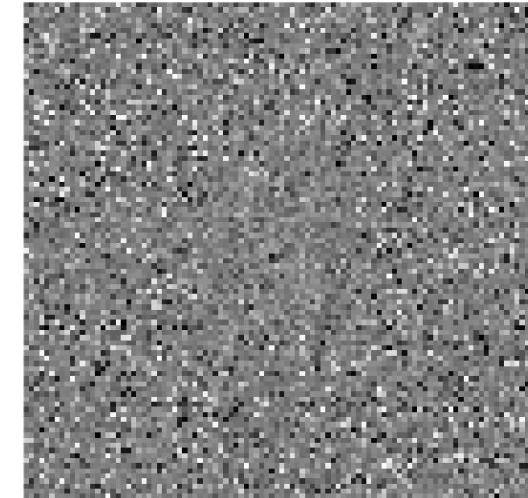
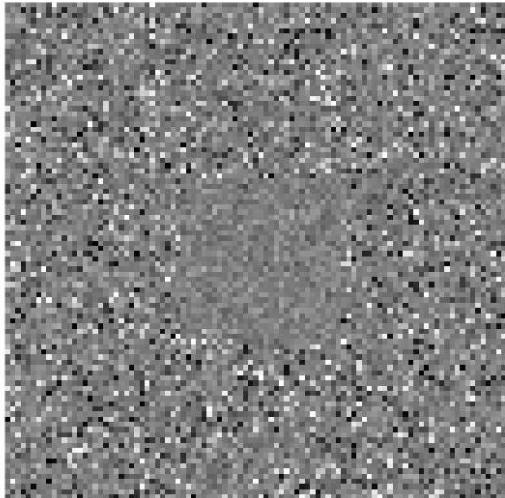
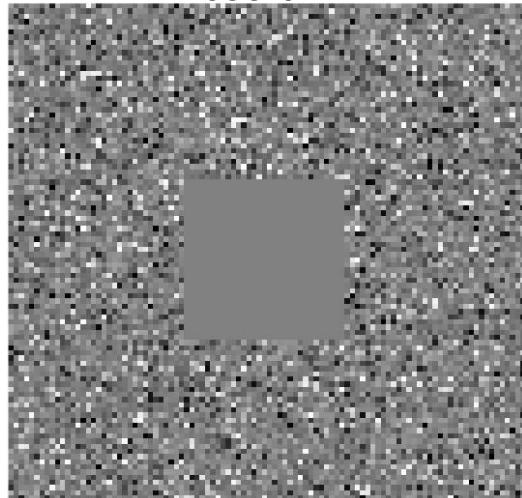
$\sigma x_{back} = 1$, $\sigma y_{back} = 0.3$

$\sigma x_{coord} = 0$,
 $\sigma y_{coord} = 0$

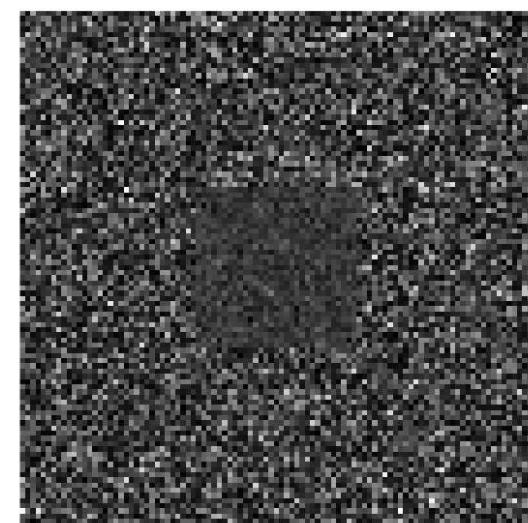
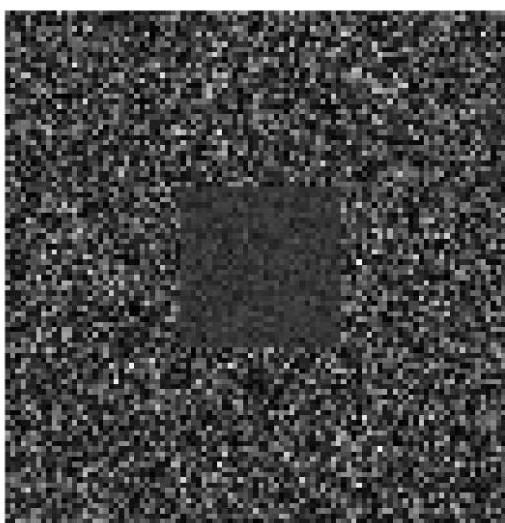
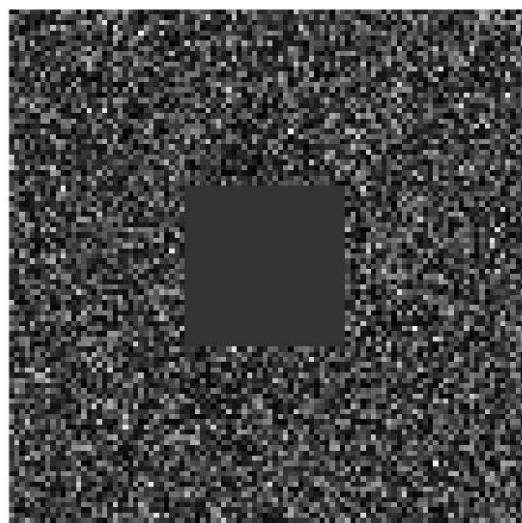
$\sigma x_{coord} = 0.2$,
 $\sigma y_{coord} = 0.2$

$\sigma x_{coord} = 0.3$,
 $\sigma y_{coord} = 0.3$

Orientation



Speed



Exercise: simulation

1. Download source code,

<https://github.com/assafzar/MonolayerKymographs>

- Set Matlab's path to code location (include subfolders)!

2. Toy simulation

- mainCoordination(outSimDname); Examine output
- Parameters:

```
params.pixelSize % um
params.patchSize % um - resolution is reduced!
params.nBilateralIter = 1;
params.minClusterArea = 500; % in um^2

% higher P,Q → more merging (Q more significant than P)
params.regionMerginParams.P = 0.03; % log(2/P)
params.regionMerginParams.Q = 0.005; % large Q

params.regionMerginParams.fVecSim =
@vecEuclideanSimilarity; ; % similarity
```

Toy simulation

- Explore parameters (params.regionMerginParams.P/Q) to optimally segment
- Explore similarity metric, uncomment %
params.fVecSim = @vecOrientationSimilarity;
- “Extra credit”:
 - Implement new similarity vecSpeedSimilarity, and assess
 - Segment by thresholding speed / orientation and assess (e.g., Otsu)
 - Use kmeans to segment (e.g., hack <https://goo.gl/kiQc4P>)
 - Implement automated assessment (I should have done this..)

Exercise: data

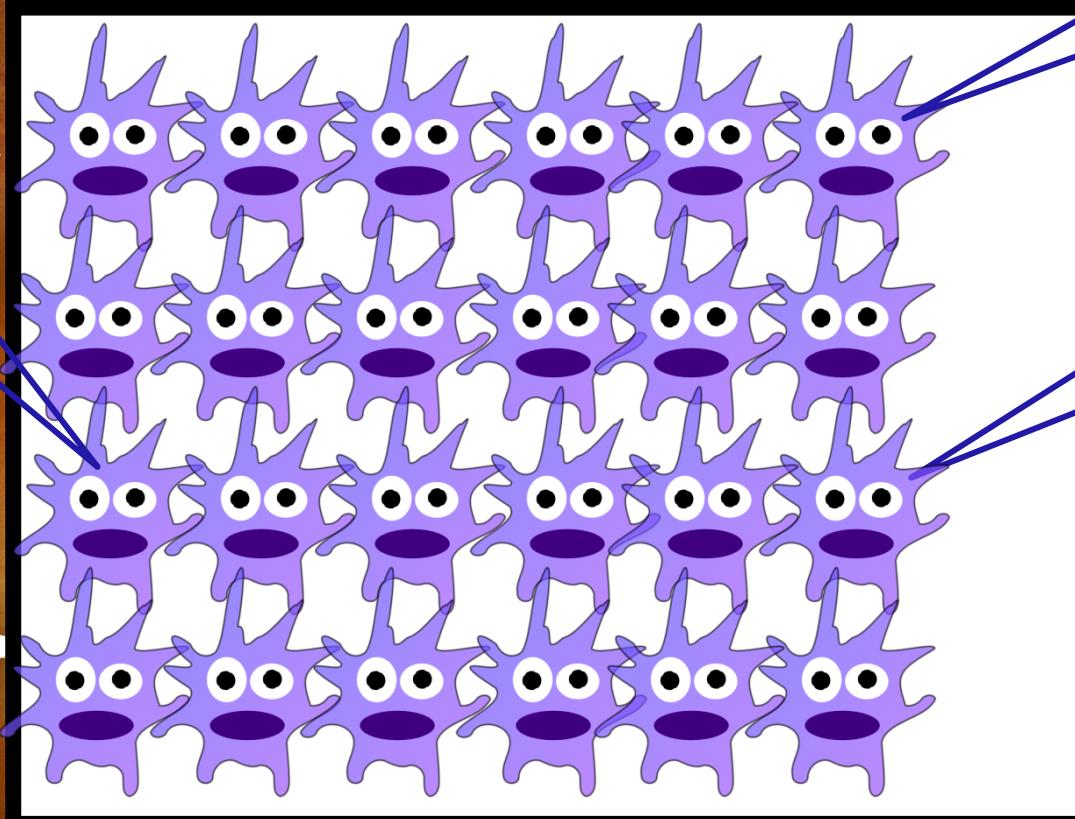
1. Download data [here](#)
2. Exercise:
 - Experiment folder is at (same code - mainCoordination):
 `Angeles_20140308_16hr_5min_0001_0002_AB01_03`
 - Check out coordinated clusters in the '**coordination**' folder
 - Delete / move the files in the coordination folder and recreate using, mainCoordination(outSimDname,inFname),
 inFname = 'Angeles_20140308_16hr_5min_0001_0002_AB01_03.tif'
 - Velocity fields are in the '**MF\mf**' folder, take a frame and calculate coordinated clusters, use
 doRegionGrowingSegmentCoordination and
 visualizeCoordinationSim
 - Switch similarity matric (see simulation exercise)
 - See params = setDefaultParams(pixelSize,timePerFrame)

Agenda

1. Collective cell migration
2. Detection of coordinated clusters (+ exercise)
3. **Example (data reuse)**
4. GEF screen (+ exercise)
5. DeBias – if times allow (co-localization)

WANTED!

MECHANISMS OF LONG RANGE
COMMUNICATION BETWEEN CELLS

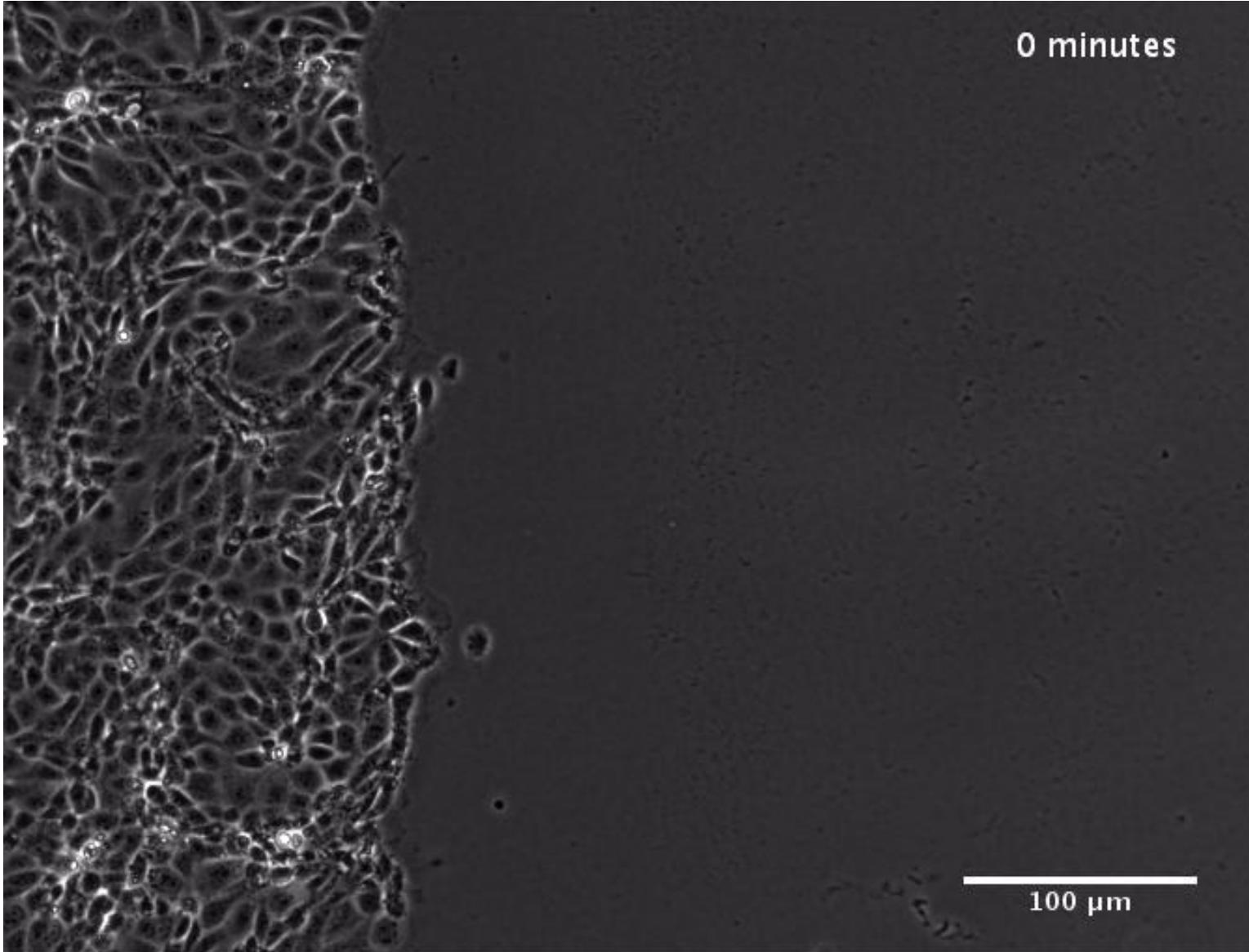


?

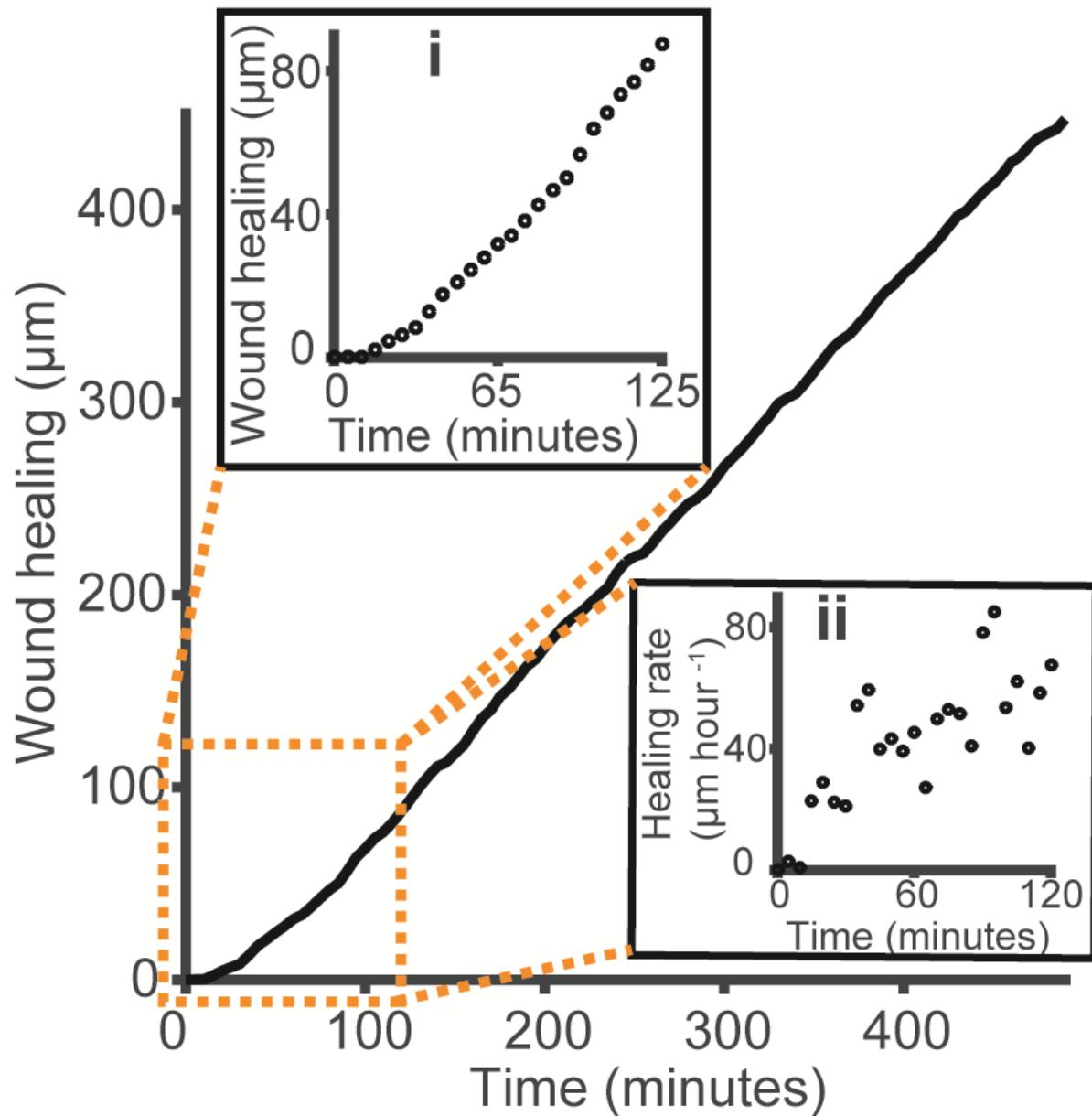
?



A simple model system to study intercellular long-range communication



The onset of monolayer migration



Two questions

- How intercellular long-range communication is induced by local mechanical fluctuations?
 - Spatial clustering of coordinated migrating cells
 - “old” data → new insight

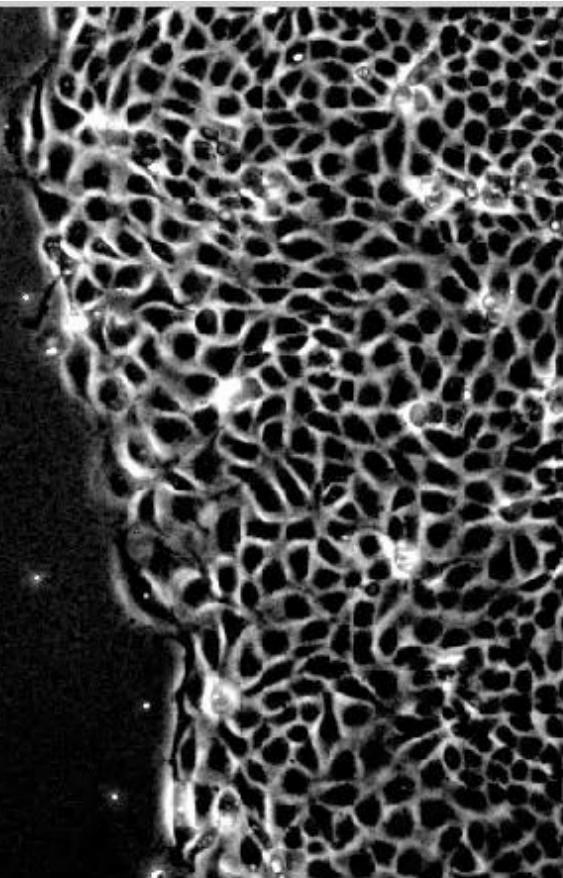
(Zaritsky et al. 2015)

- What are the molecular players driving long-range communication?
 - High-dimensional representation of spatiotemporal dynamics

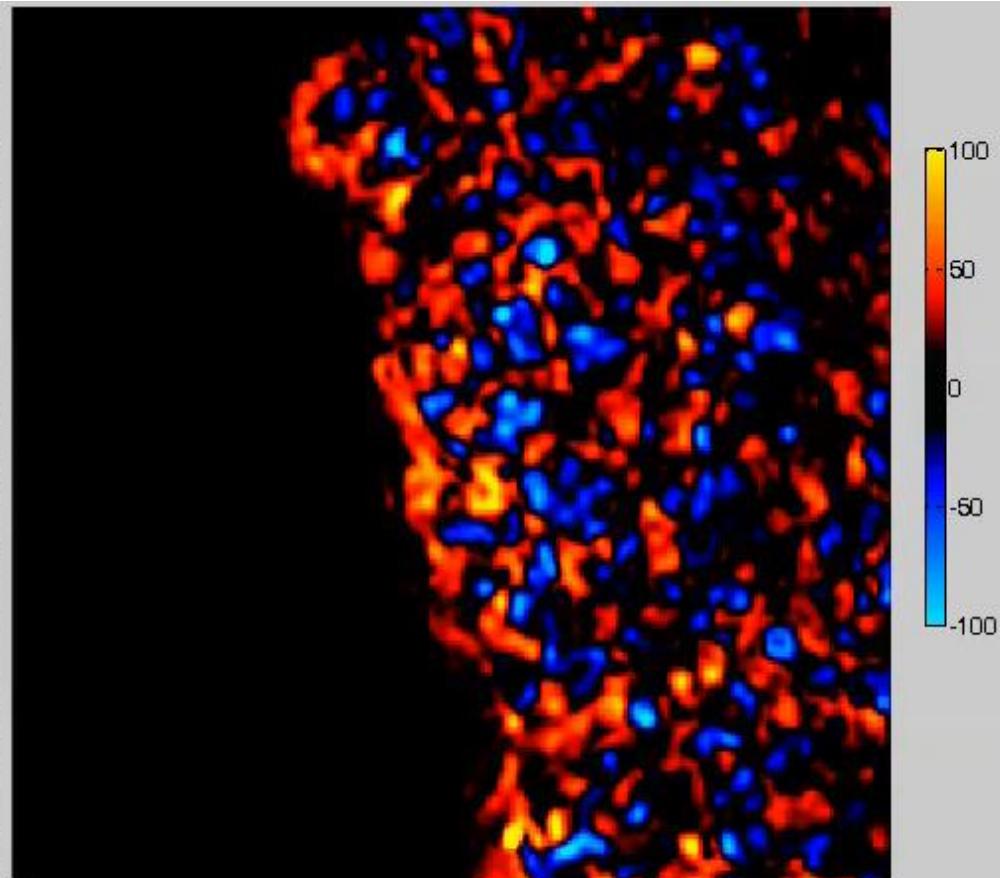
Zaritsky and Tseng et al. (2017)

How (global) coordination emerges from (local) heterogeneous traction forces?

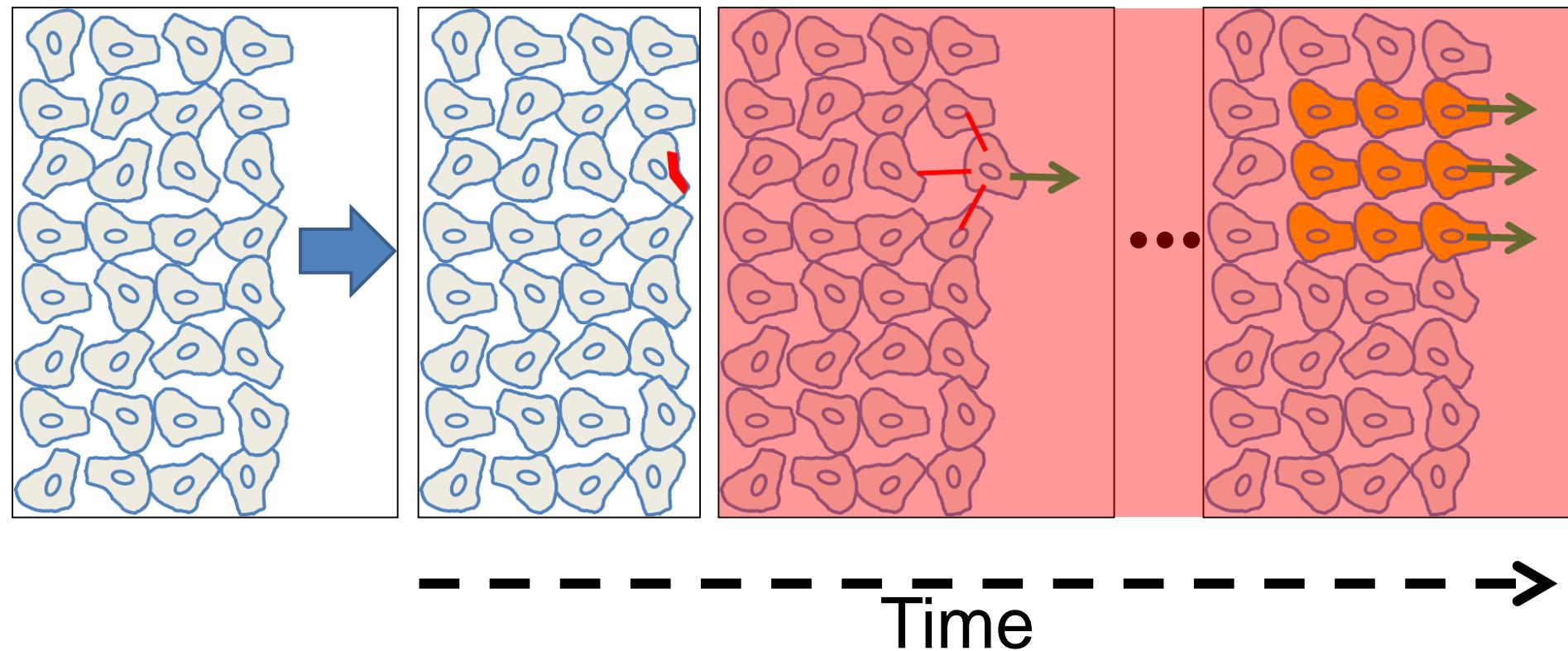
Phase Contrast



Traction T_x (P_a)



Suggested model



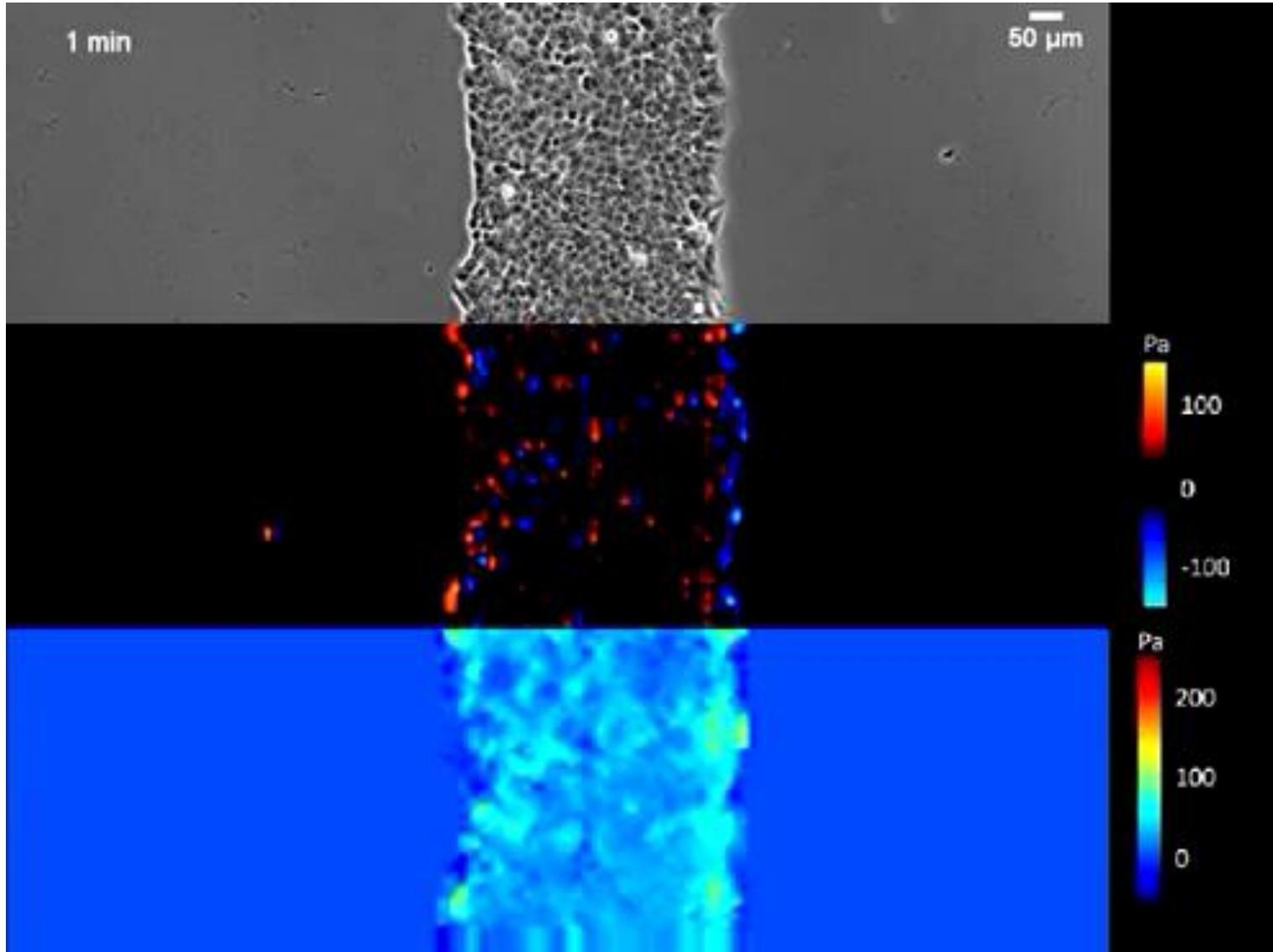
Stochastic force exertion transform to directional migration

Strain on neighbors coordinate their movement

Propagation in time and space to guide groups of cells

Measuring traction force, stress and velocity

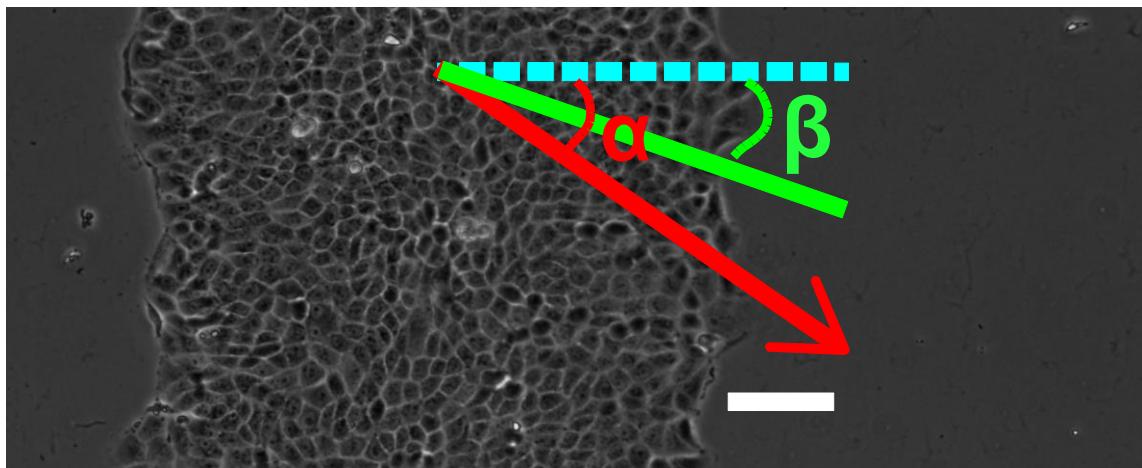
Phase contrast
Traction
 T_x
Average normal stress



Trepat et al. (2009)
Tambe et al. (2011)
Serra-Picamal et al. (2012)

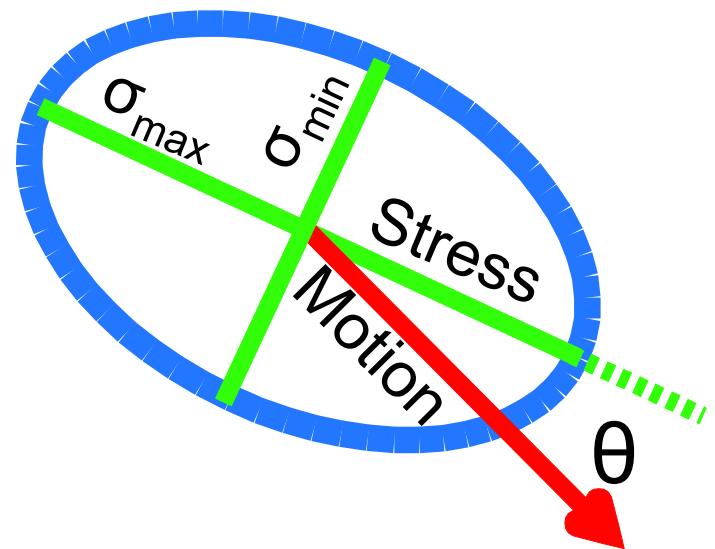
Motion-stress alignment

Velocity angle,
stress orientation



$$-90 \leq \alpha, \beta \leq 90$$

Motion-stress
alignment

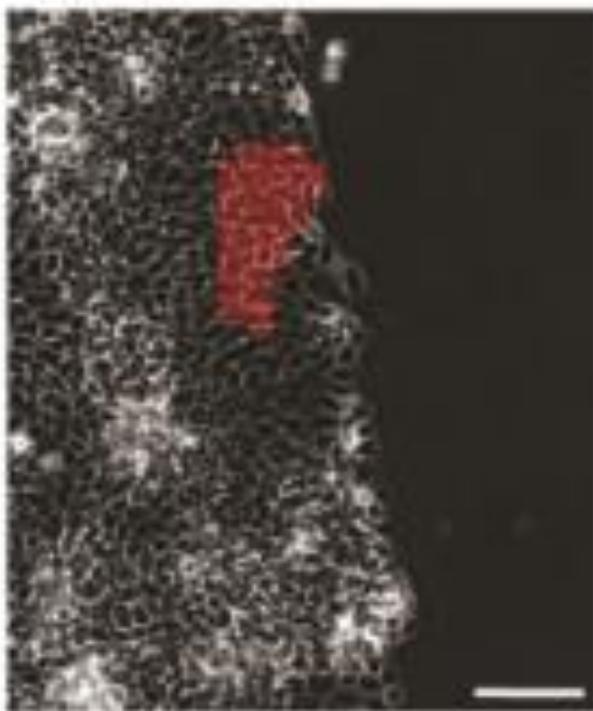


$$0 \leq \theta \leq 90$$

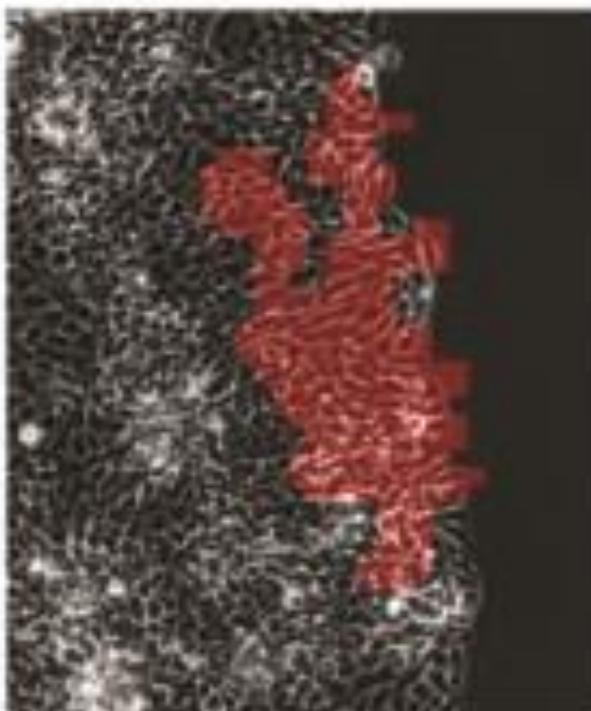
Tambe et al. (2011)
Trepat & Fredberg. (2011)

Region-growing segmentation for *explicit* detection of coordinated migration clusters

0 min



60 min

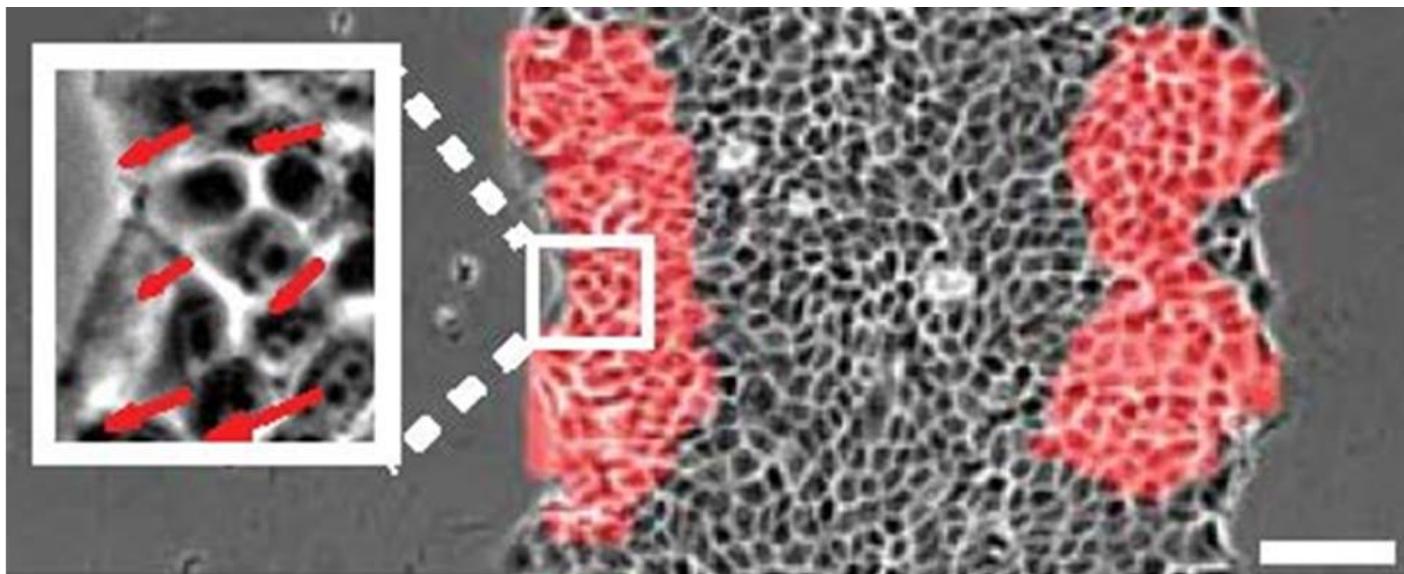


120 min

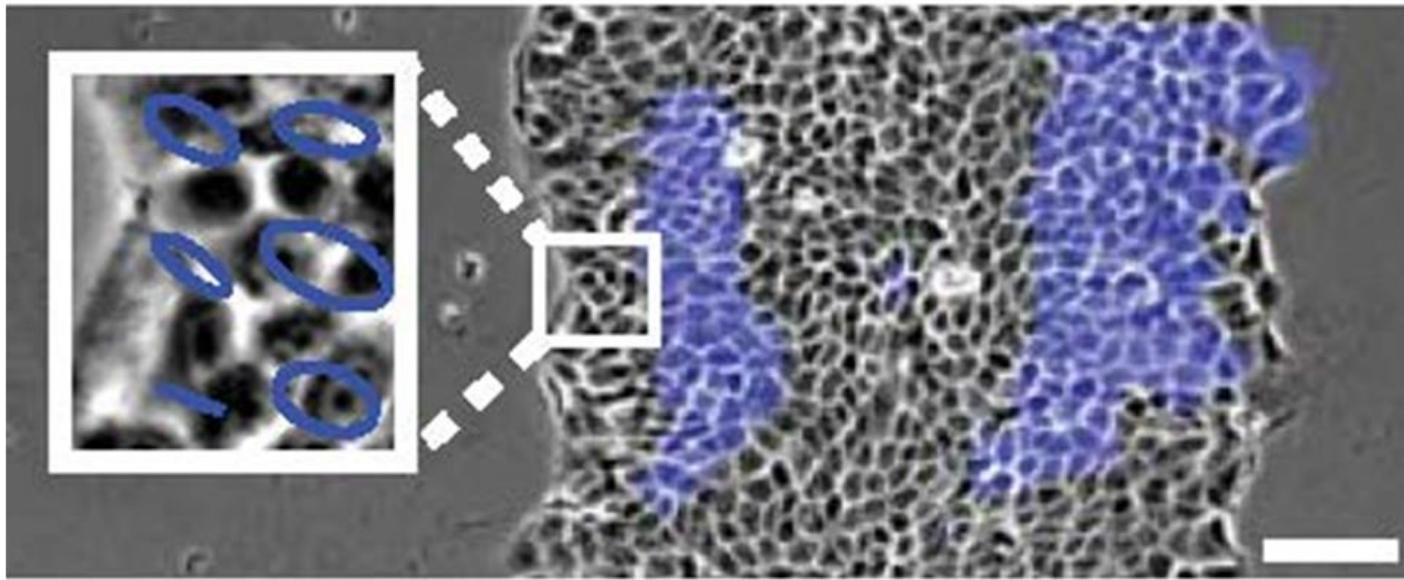


Associating coordinated stress and motion

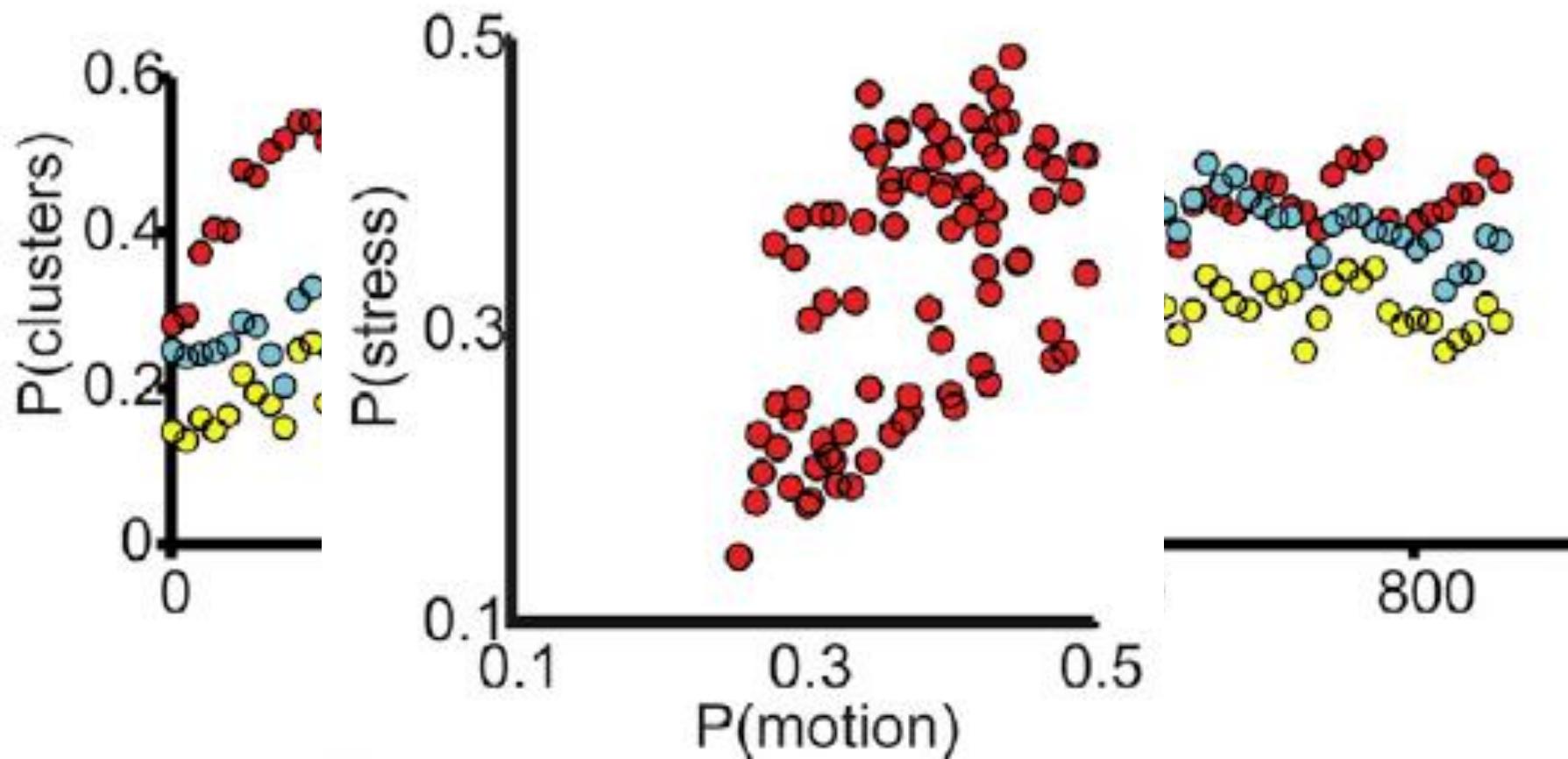
Motion



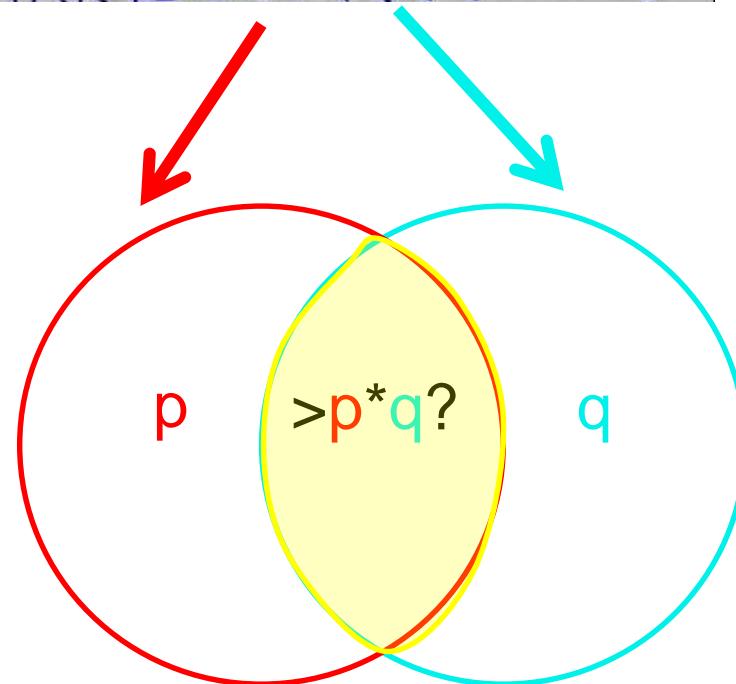
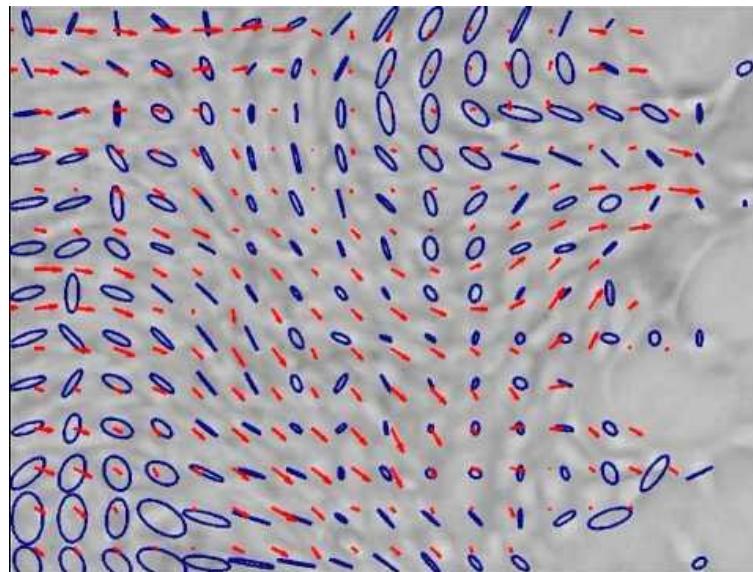
Stress



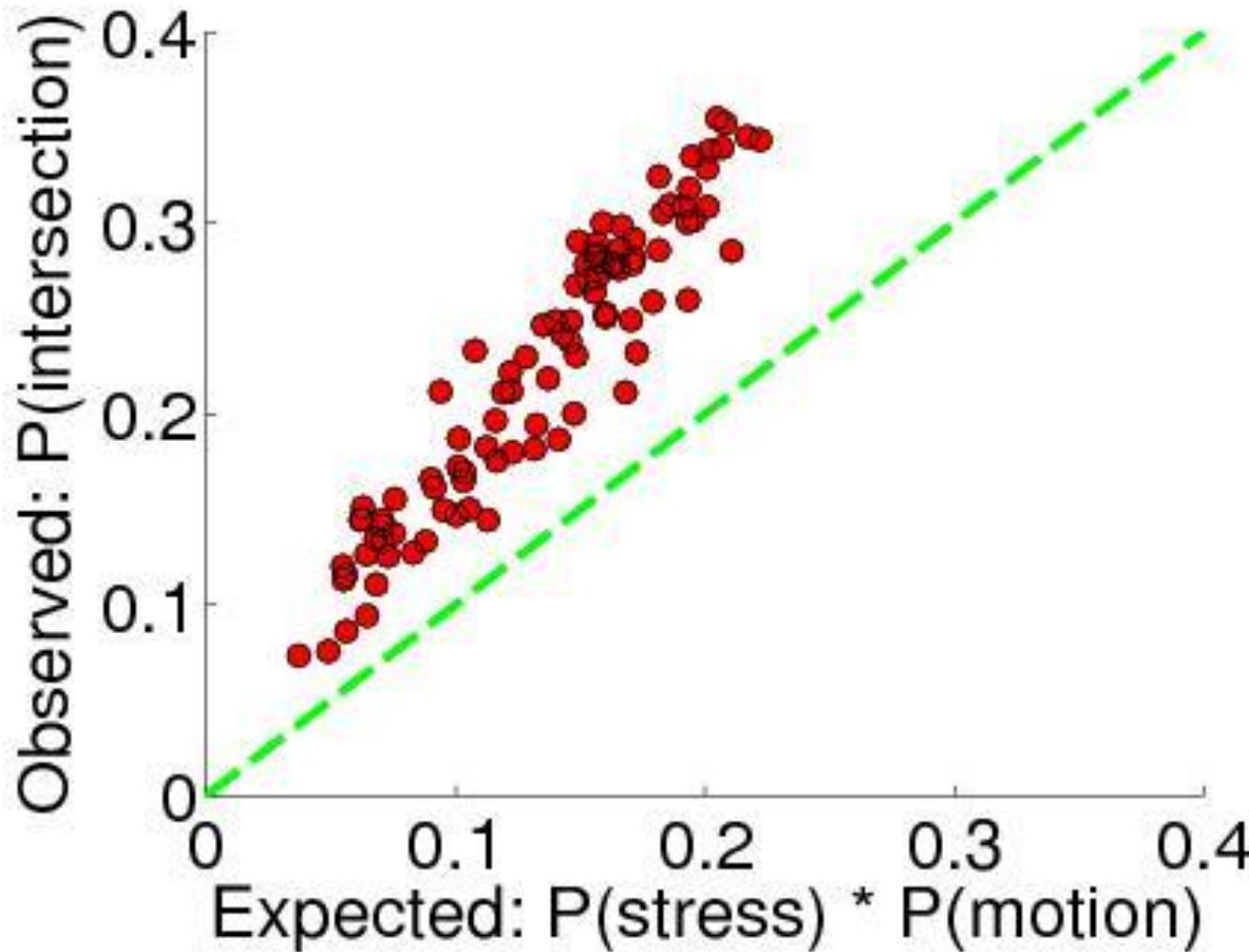
Coordinated motion is correlated to coordinated stress



Associating coordinated stress and motion

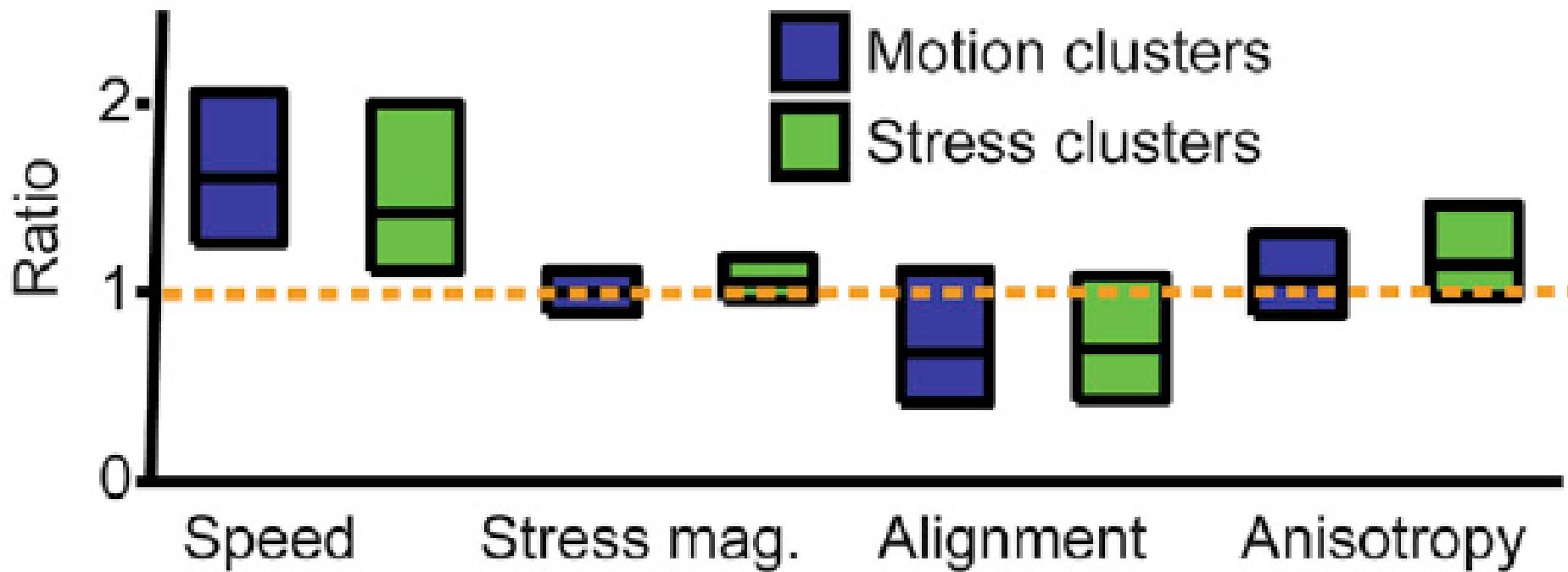


Motion- and stress- coordinated clusters are interlinked

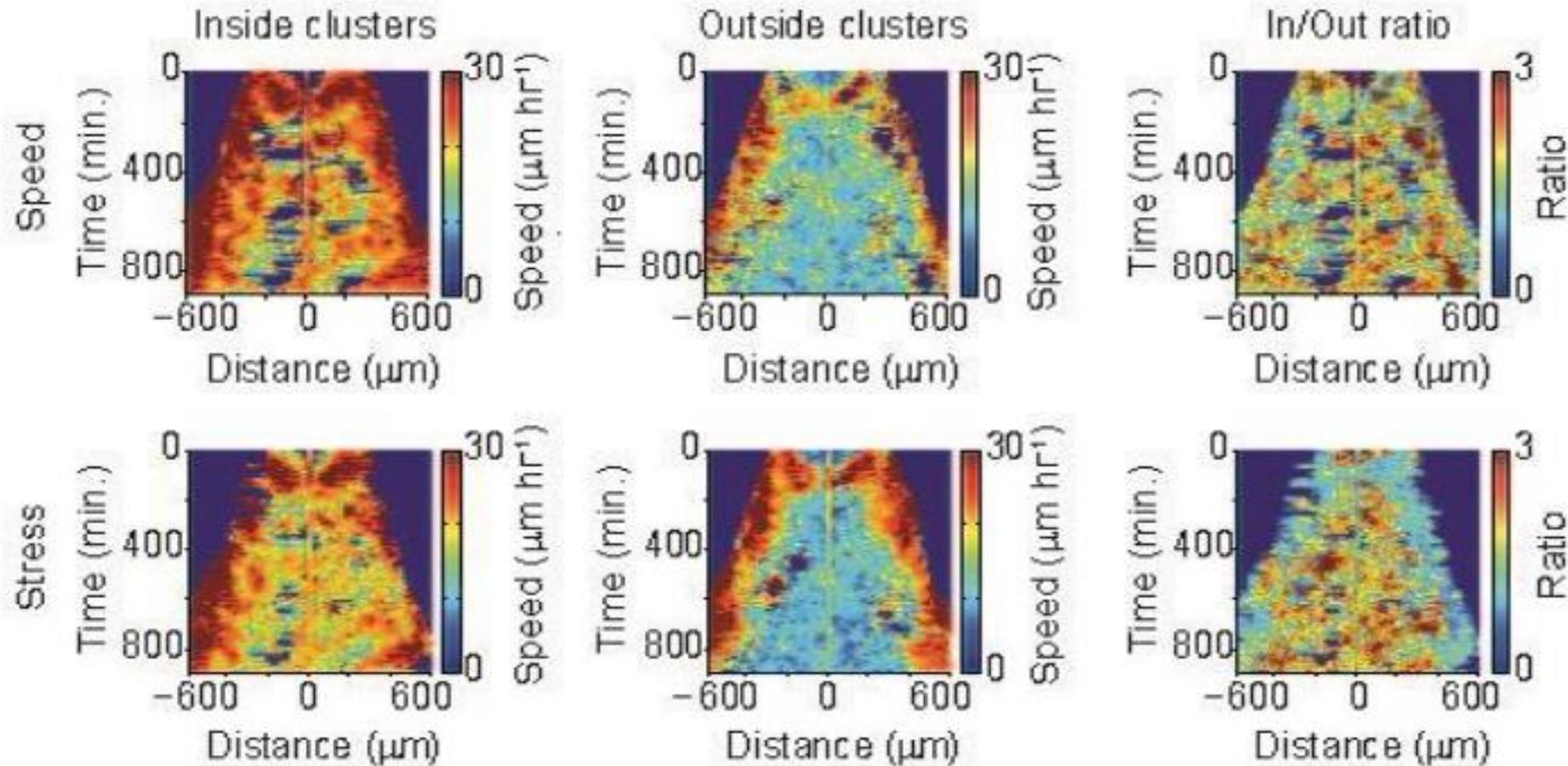


Cells in coordinated clusters move faster, with enhanced motion-stress alignment

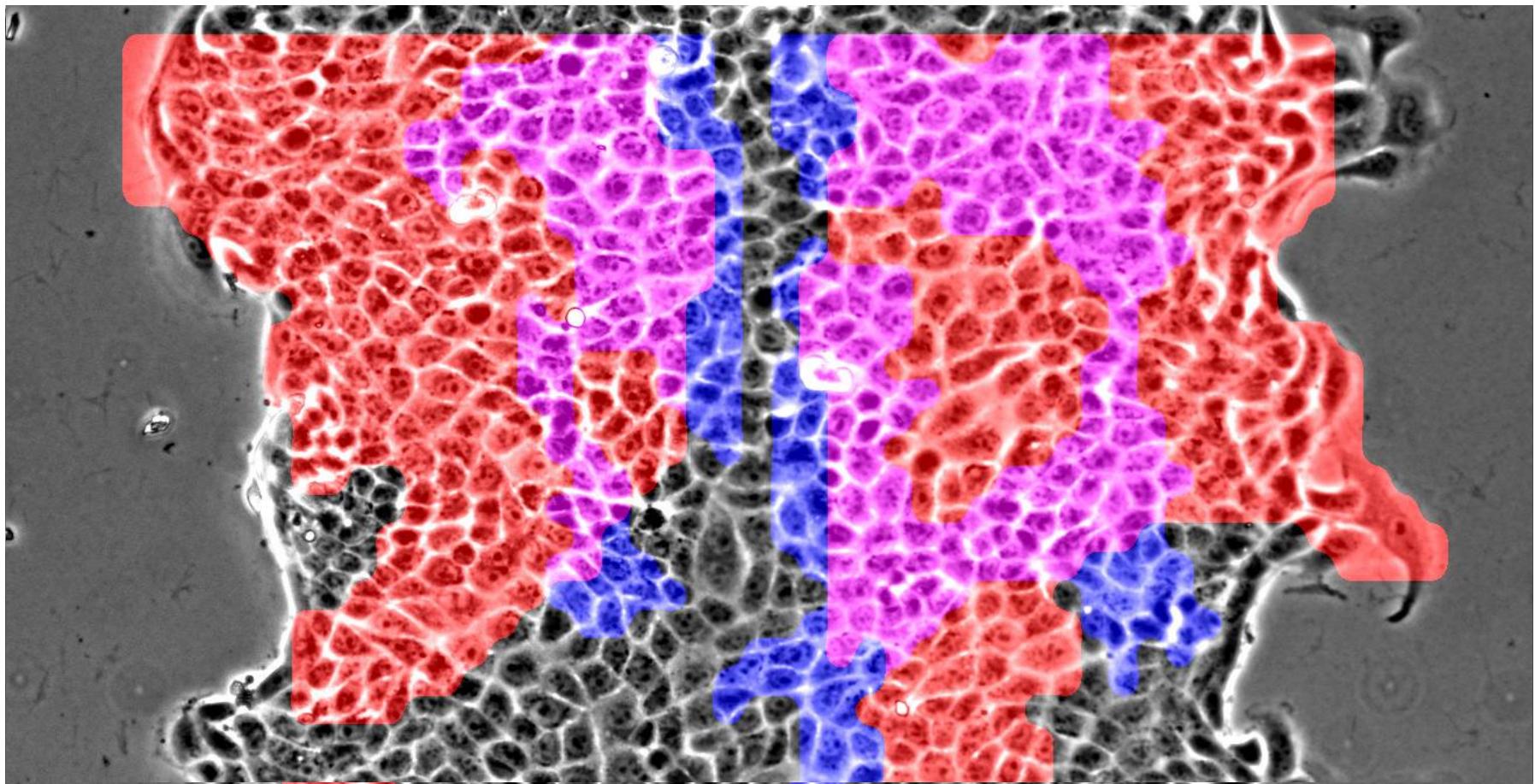
$$\text{Ratio}_{\text{property}} = \frac{\text{property}_{\text{in}}}{\text{property}_{\text{out}}}$$



Spatiotemporal analysis

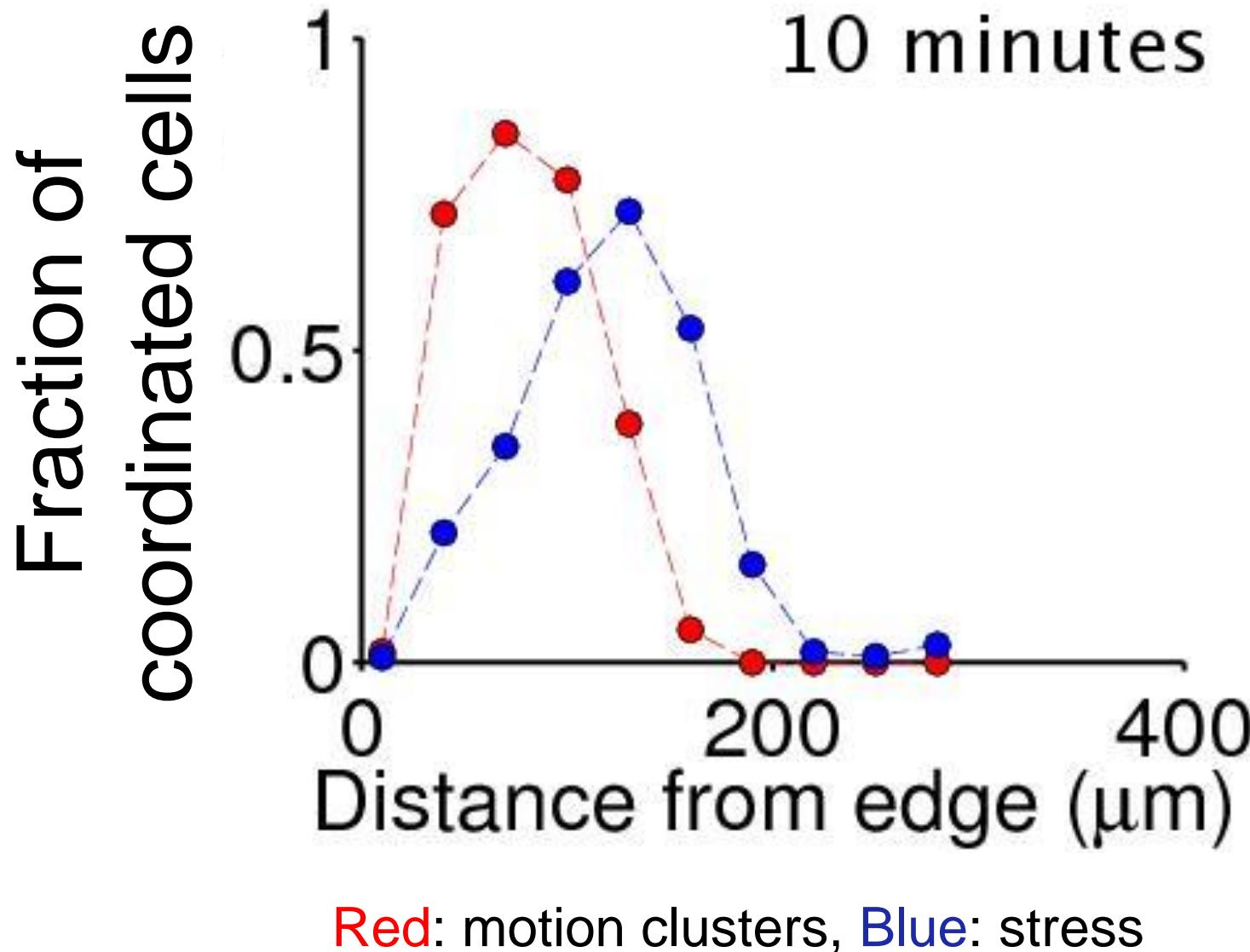


Strain-induced motion coordinates cluster's motility



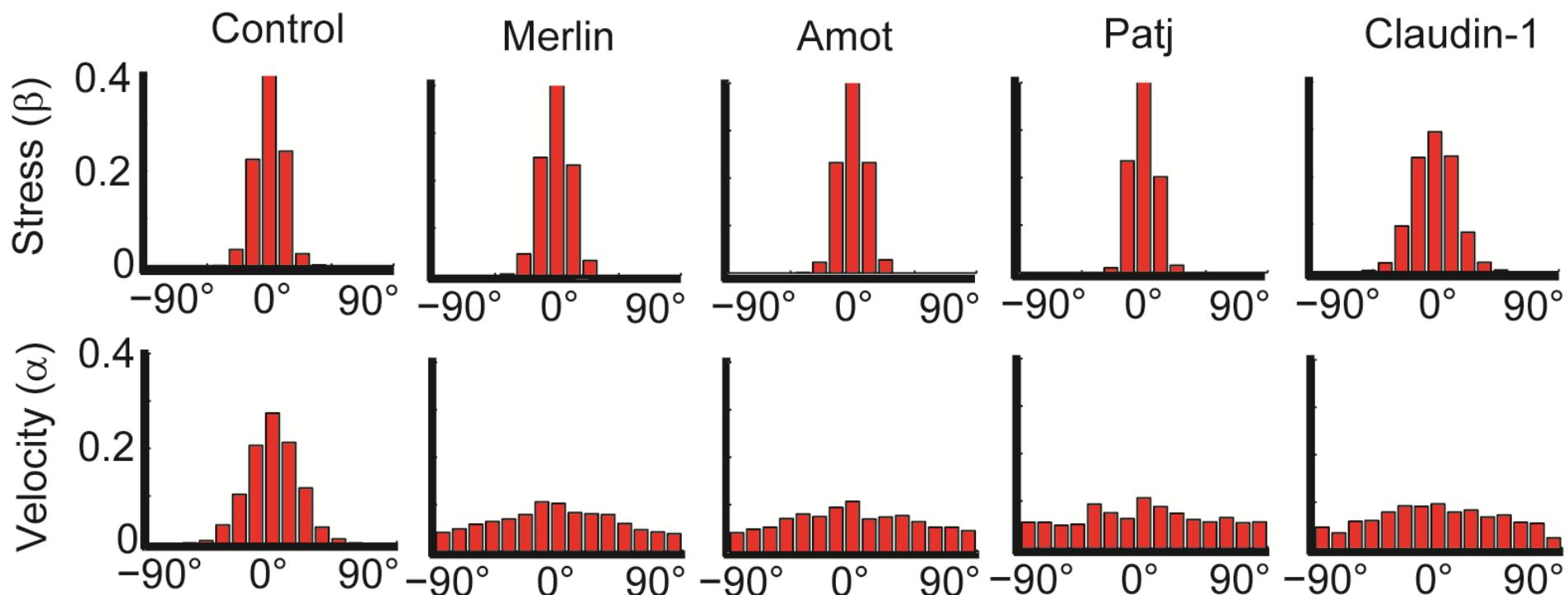
Red: motion clusters, Blue: stress, Magenta: both

Strain-induced motion coordinates cluster's motility

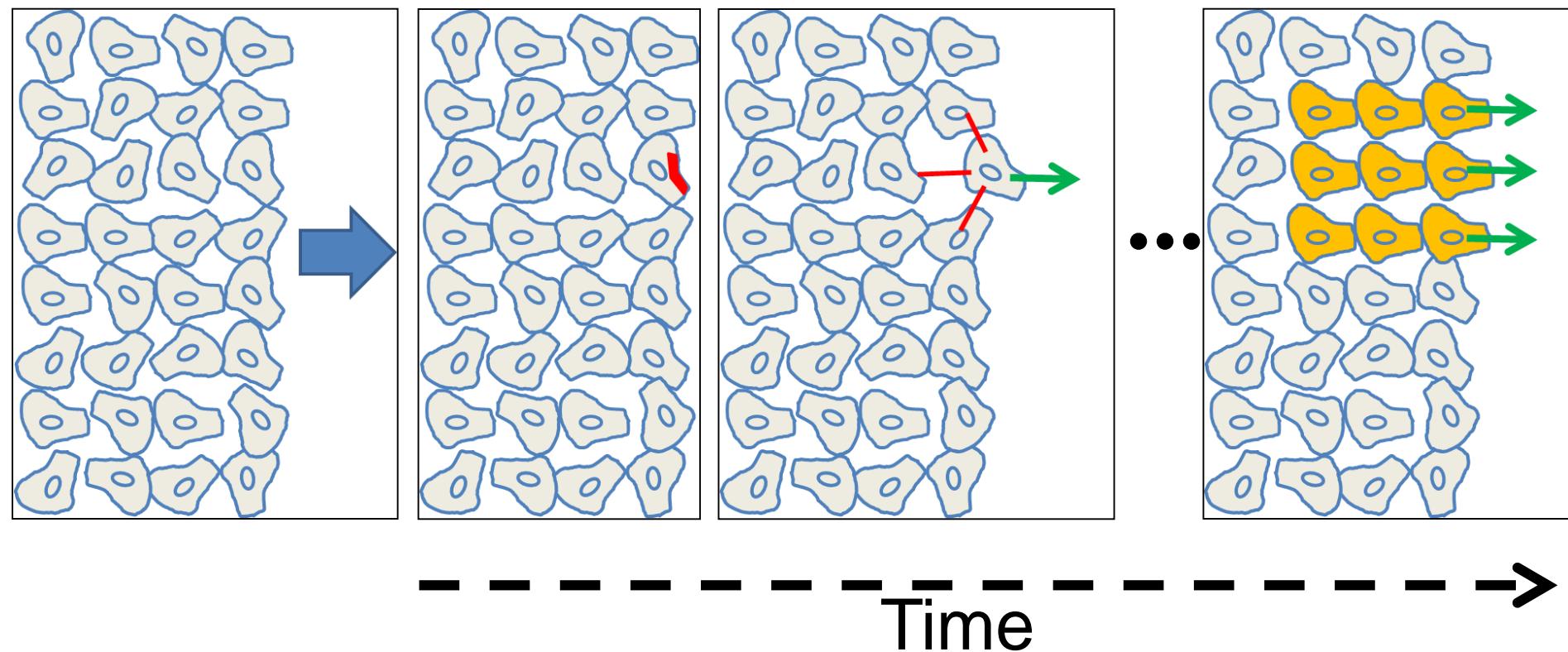


Stress aligns motion

Tight junction proteins play a role in effective transmission of aligned stress to aligned motion



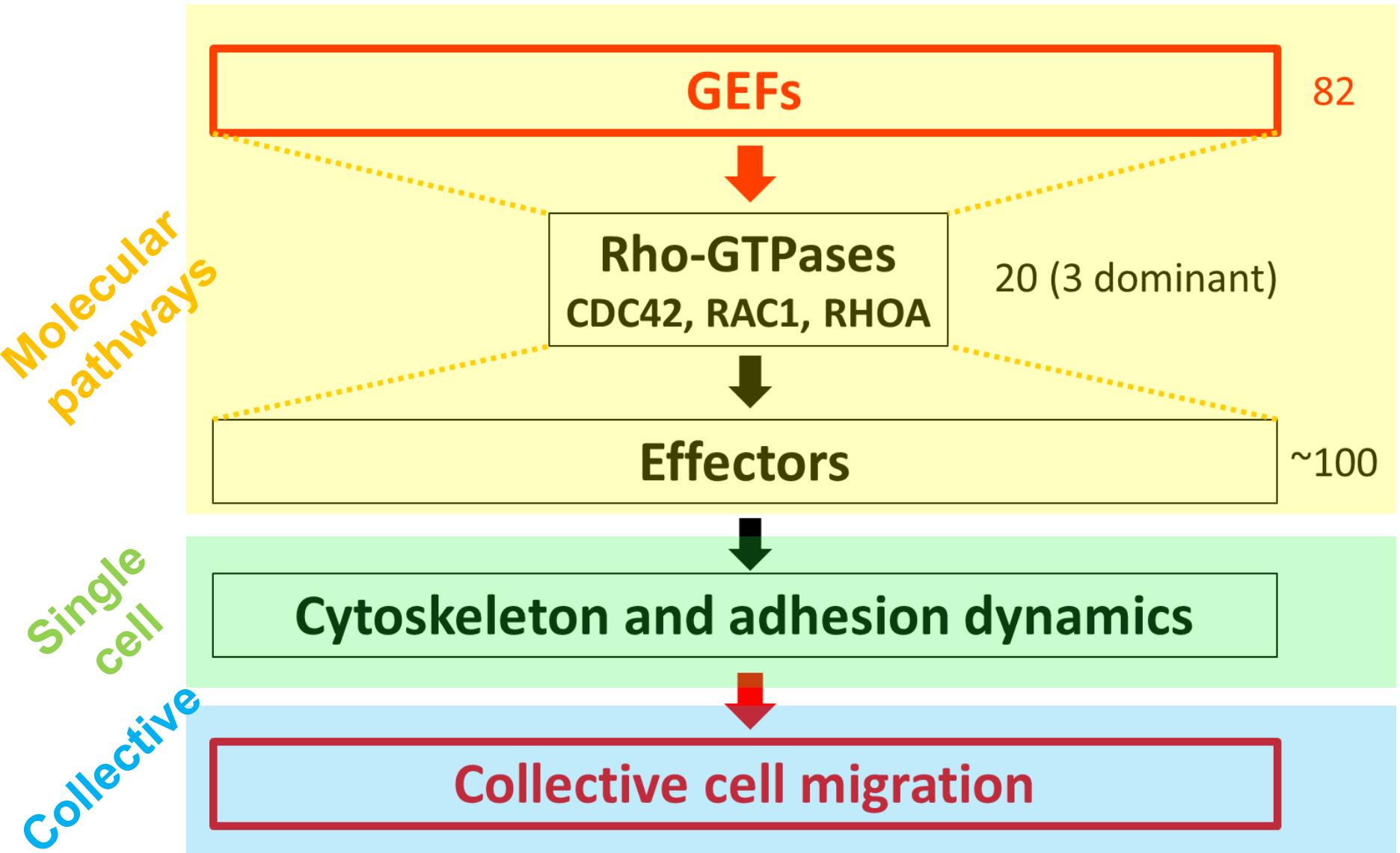
Coordination migration emerges from cell-cell junctional transmission of mechanical guidance cues



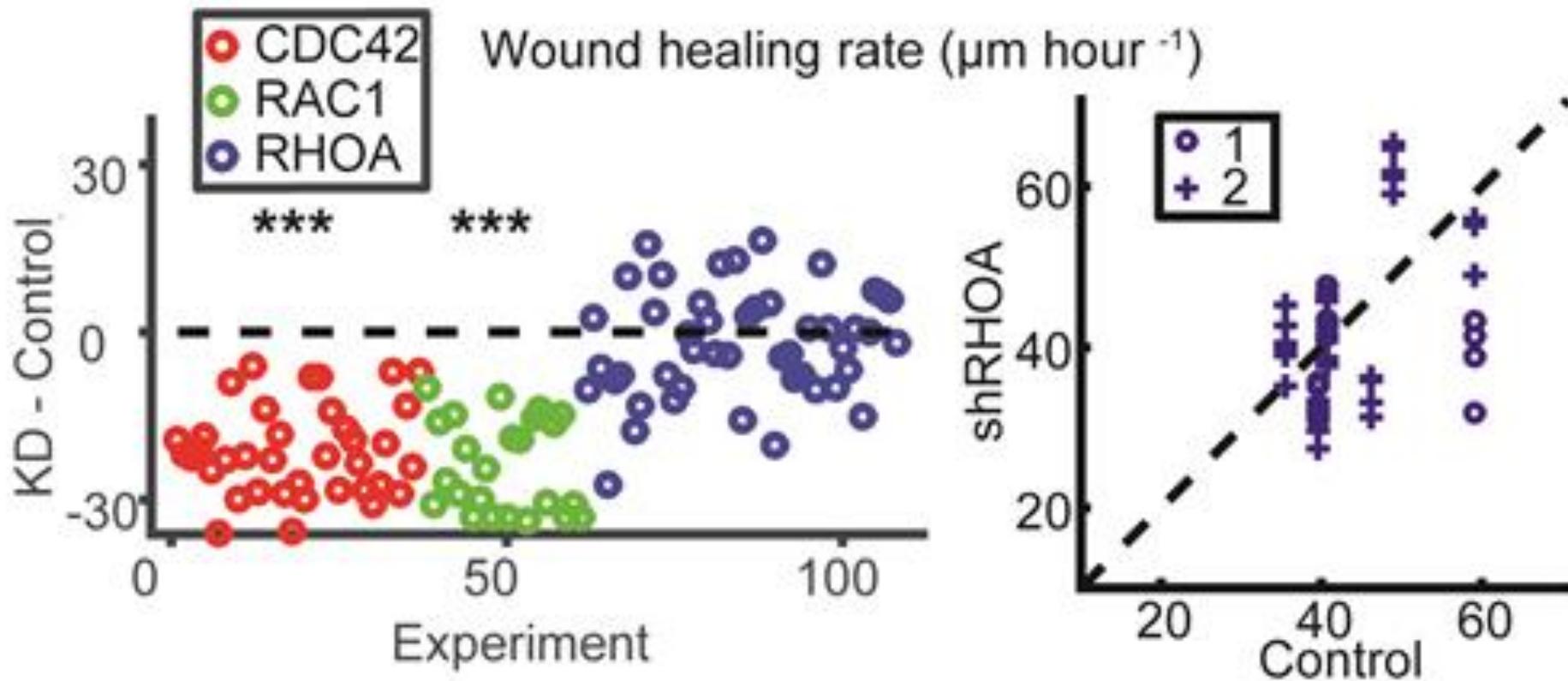
Agenda

1. Collective cell migration
2. Detection of coordinated clusters (+ exercise)
3. Example (data reuse)
4. **GEF screen (+ exercise)**
5. DeBias – if times allow (co-localization)

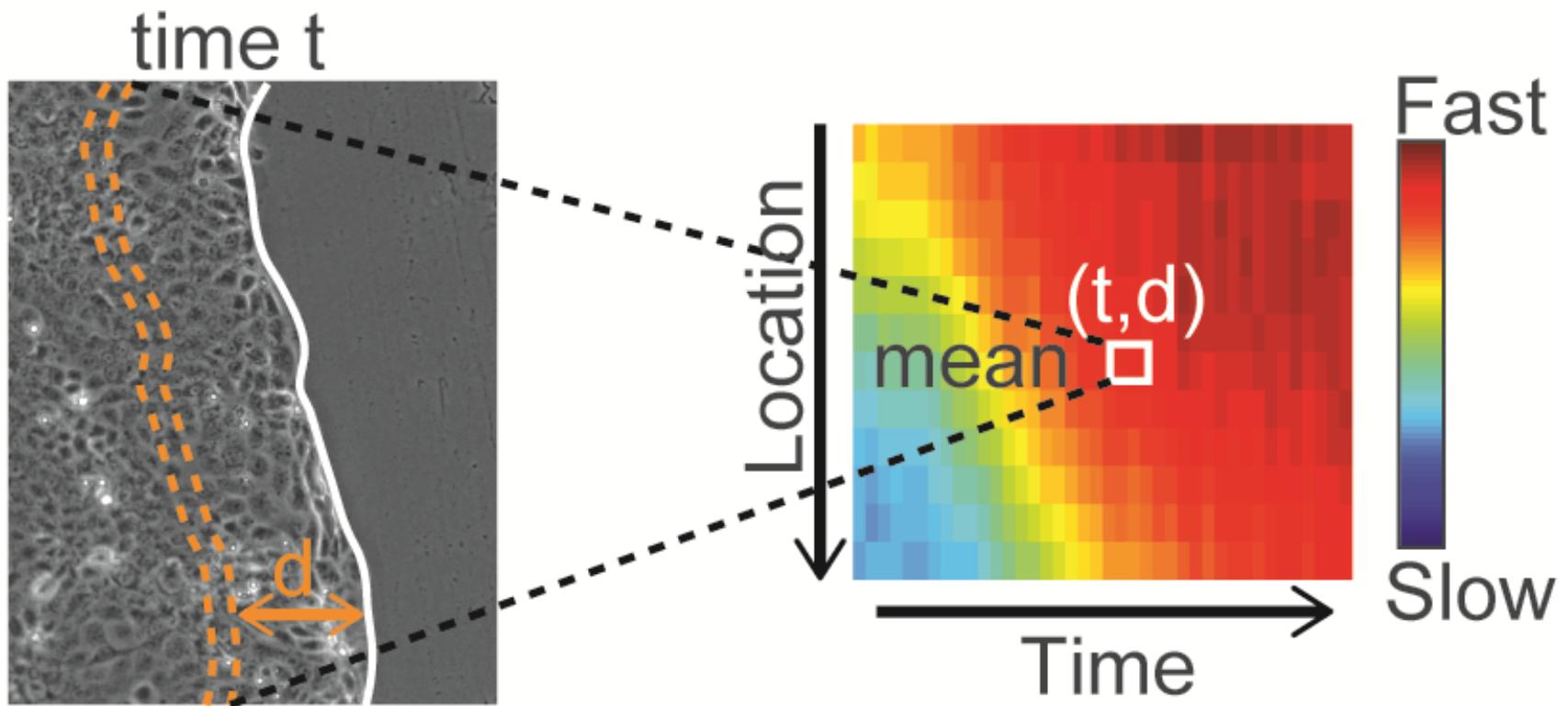
Rho-GTPases and their activators



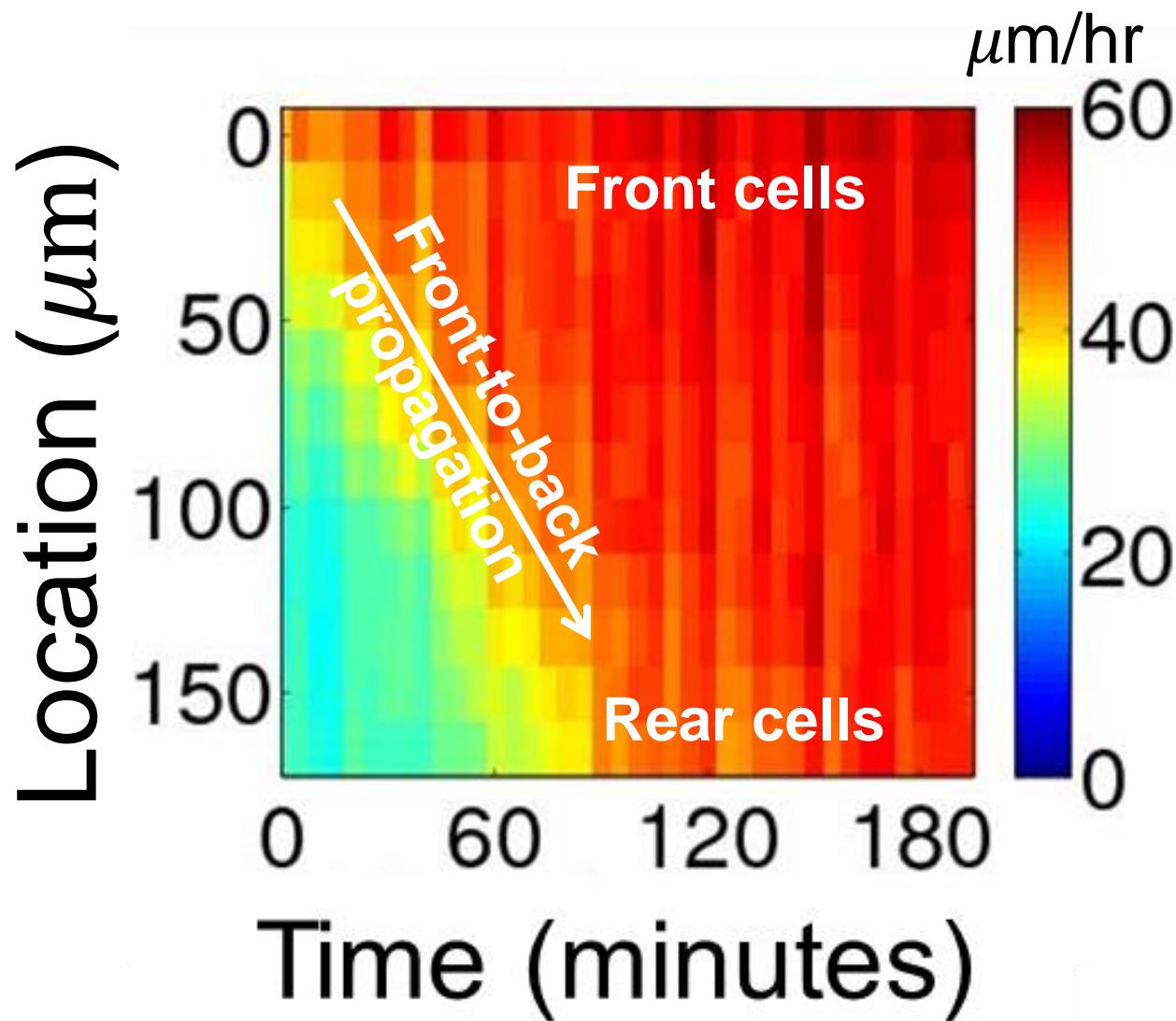
Down-regulation of Cdc42 and Rac1 but not RhoA disrupts monolayer migration



Spatiotemporal quantification



Visualizing front-to-back propagation



Workflow

```
processTimeLapse(filename, params);
```

```
[params, dirs] = initParamsDirs(filename, params); % set  
missing parameters, create output directories  
whLocalMotionEstimation(params, dirs); % velocity  
fields estimation  
whTemporalBasedSegmentation(params, dirs); % cellular-  
background segmentation  
whCorrectGlobalMotion(params, dirs); % correction of  
stage-location errors  
whSegmentationMovie(params, dirs); % segmentation movie  
whHealingRate(params, dirs); % wound healing rate over  
time  
whCoordination(params, dirs); % coordinated clusters  
whKymographs(params, dirs); % spatiotemporal kymographs
```

Parameters

```
params.pixelSize = 1.267428; % um
params.timePerFrame = 5; % minutes
params.nRois = 1; % 1 - advancing monolayer, 2 - wound healing
params.isDx = true; % main cell motion in x direction
params.always = false; % false - no reprocessing available results

params.patchSizeUm = 15.0; % 15 um
params.nTime = floor(200 / params.timePerFrame); % frames (200 min)
params.maxSpeed = 90; % um / hr (max cell speed)
% for kymographs display
params.kymoResolution.maxDistMu = 180; % how deep to go (um)

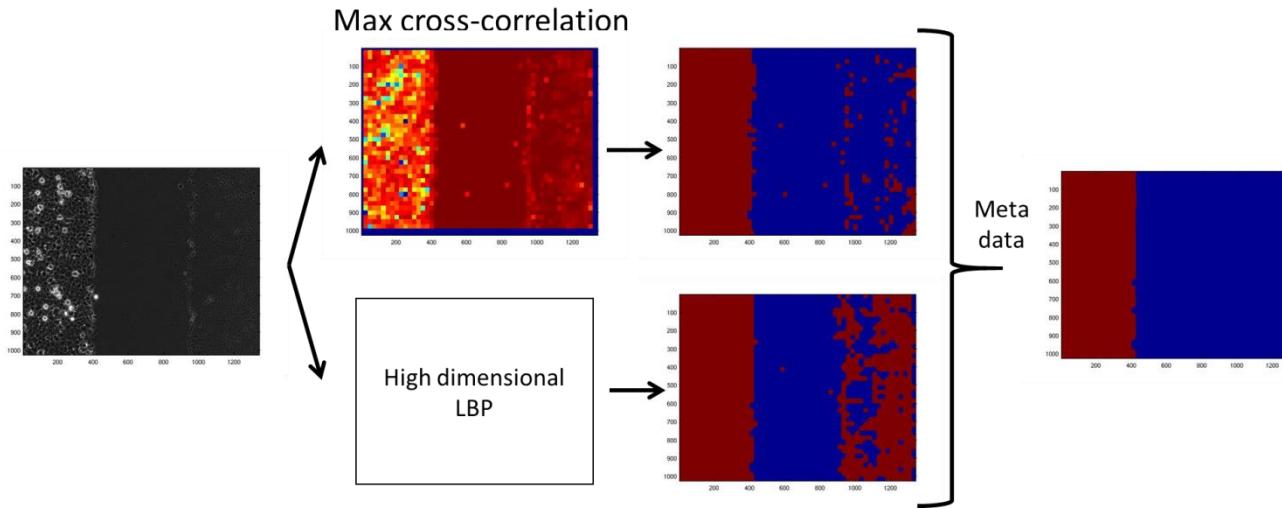
% Parameters that depend on previous params..
params.patchSize = ceil(params.patchSizeUm/params.pixelSize);%pixels
params.kymoResolution.min = params.patchSize;
params.kymoResolution.stripSize = params.patchSize;
params.kymoResolution.maxDistMu = 180; % um
```

Two side notes

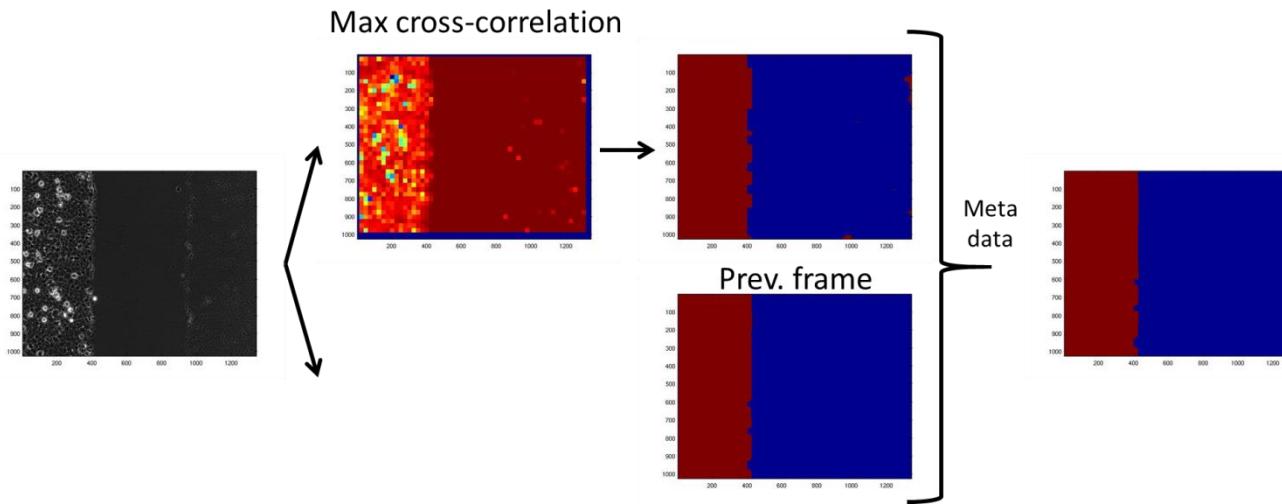
1. Segmentation
2. Flow fields vs. single cell tracking

Cellular/ background segmentation

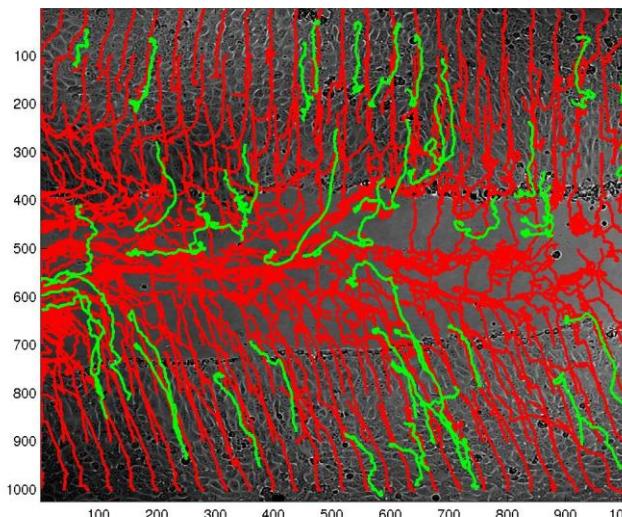
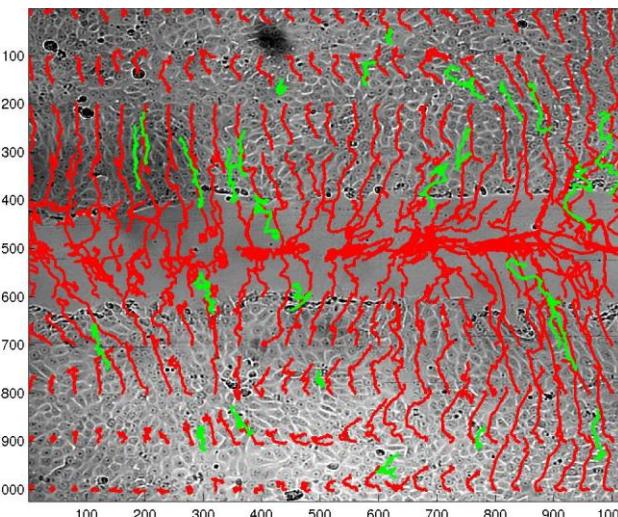
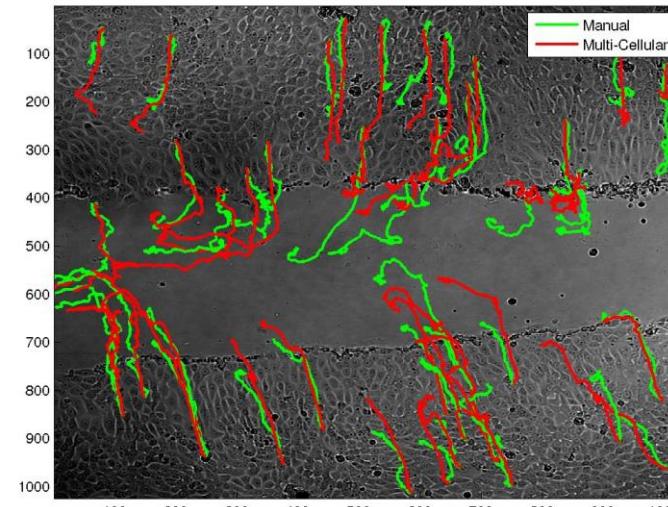
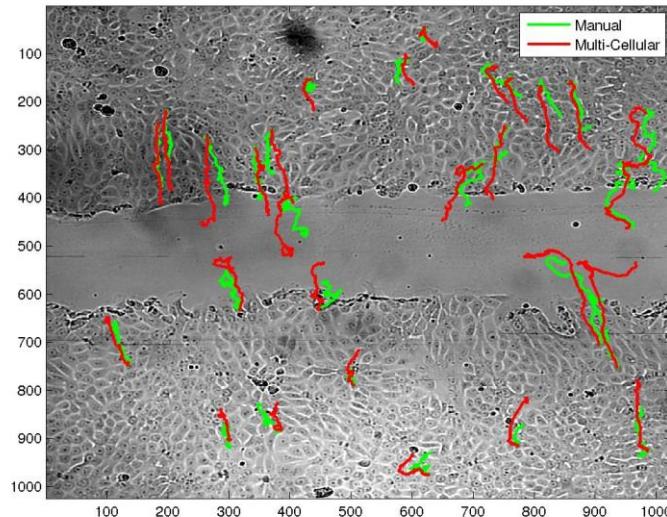
New Segmentation Algorithm: Frame 1



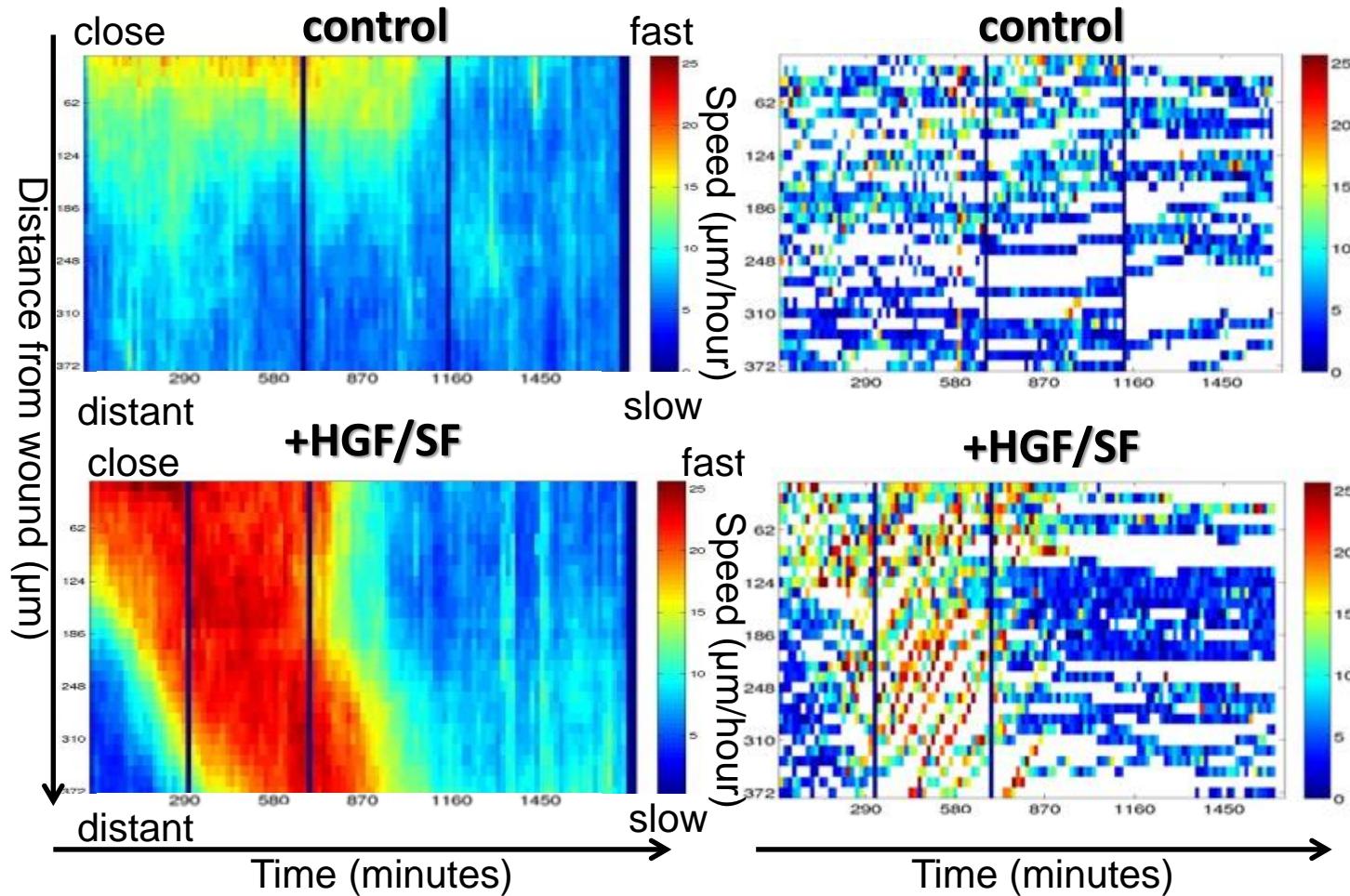
New Segmentation Algorithm: Frame > 1



PIV versus (partial) cell tracking - exploiting Information from **all cells**

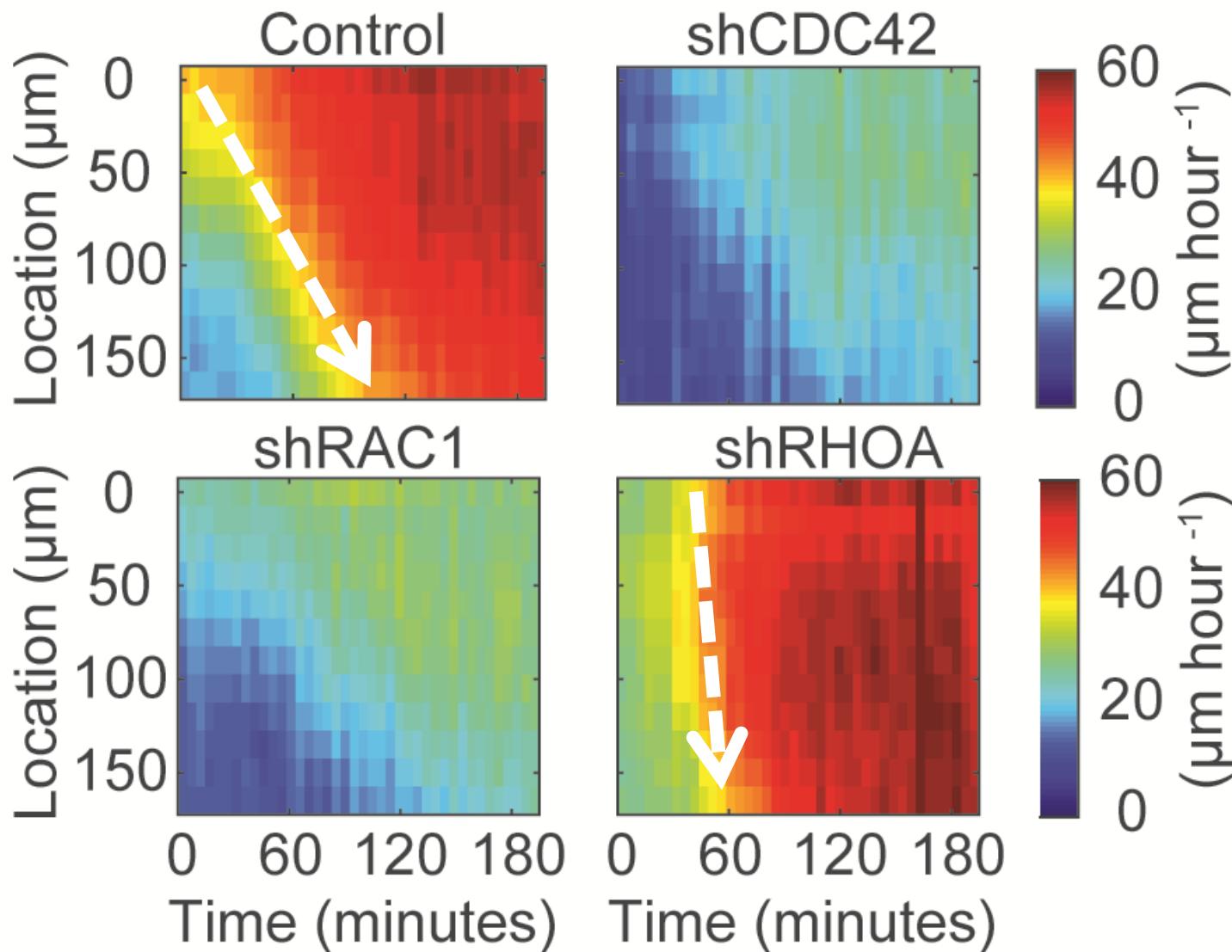


"Wisdom of Crowds"



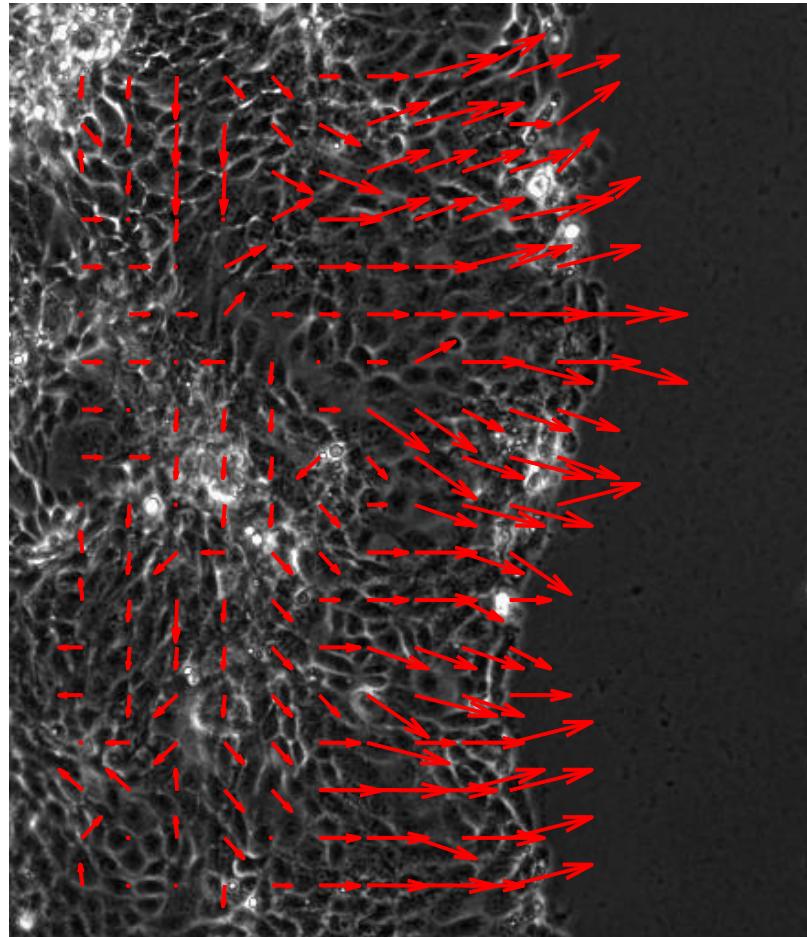
Zaritsky et al. (2012)

Depleted RhoA enhances long-range communication

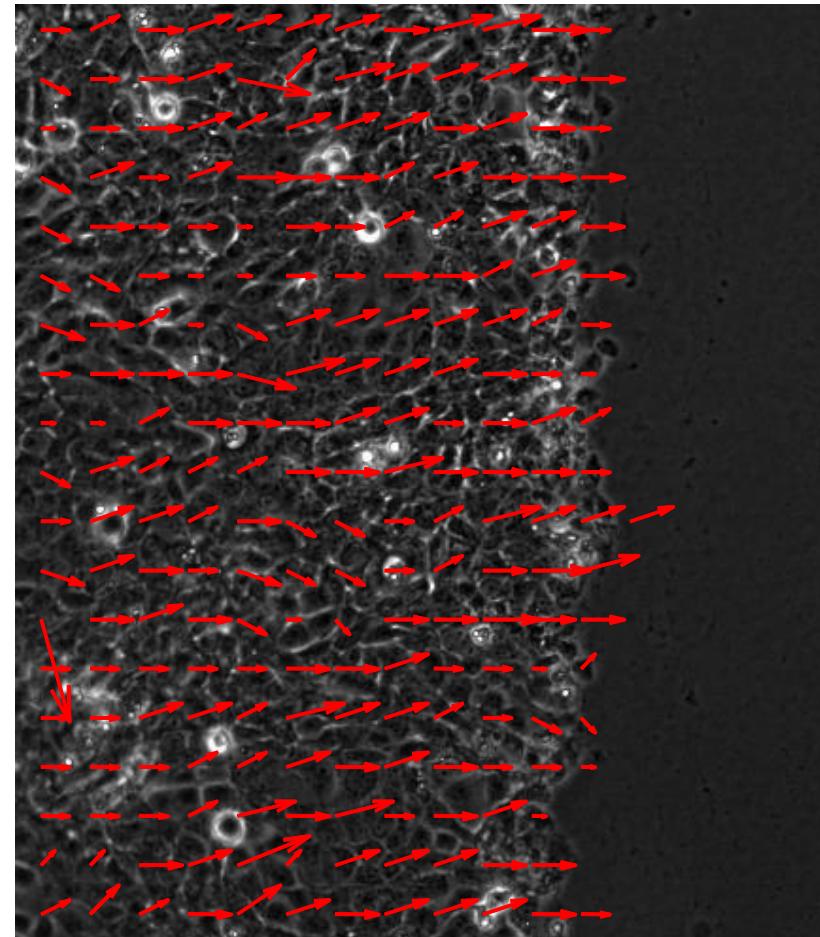


Depleted RhoA enhances long-range communication

Control



shRHOA



Comprehensive GEFs screen

- 81 GEFs, 3 (validated) hairpins, > 3 locations per condition
- Control and follow-up experiments
- > 3,000 videos to analyze
- Robust algorithmic pipeline
- Variability
- Measures for screening

Comprehensive GEFs screen

Library of pSUPER shRNA
retroviruses (3 hairpins per GEF)
targeting 80/81 GEFs



75/80 GEFs expressed in
16HBE cells (PCR/WB)



Hairpin knockdown efficiency
(WB/qPCR): 16 GEFs had KD <
50% for all 3 hairpins

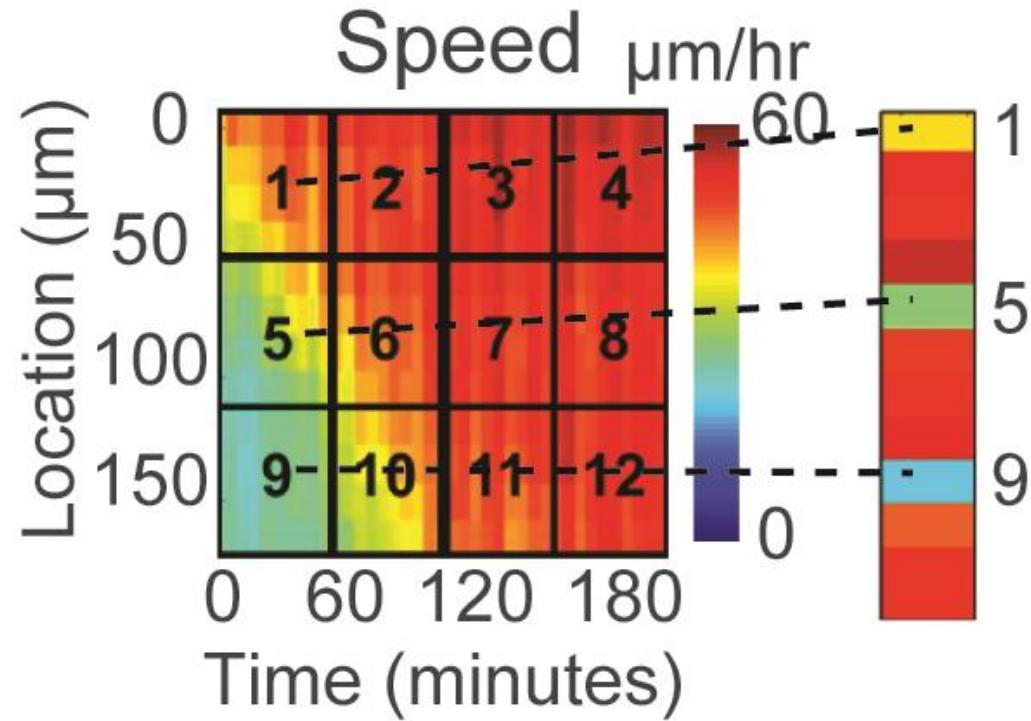
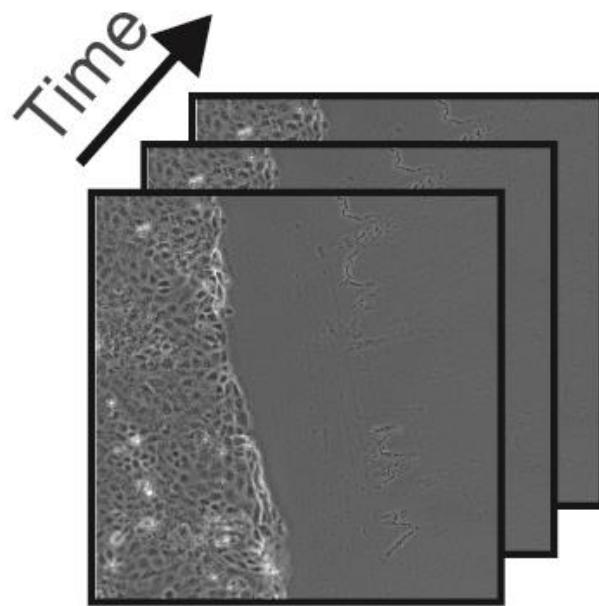


Live imaging analysis screening
for 59 remaining GEFs

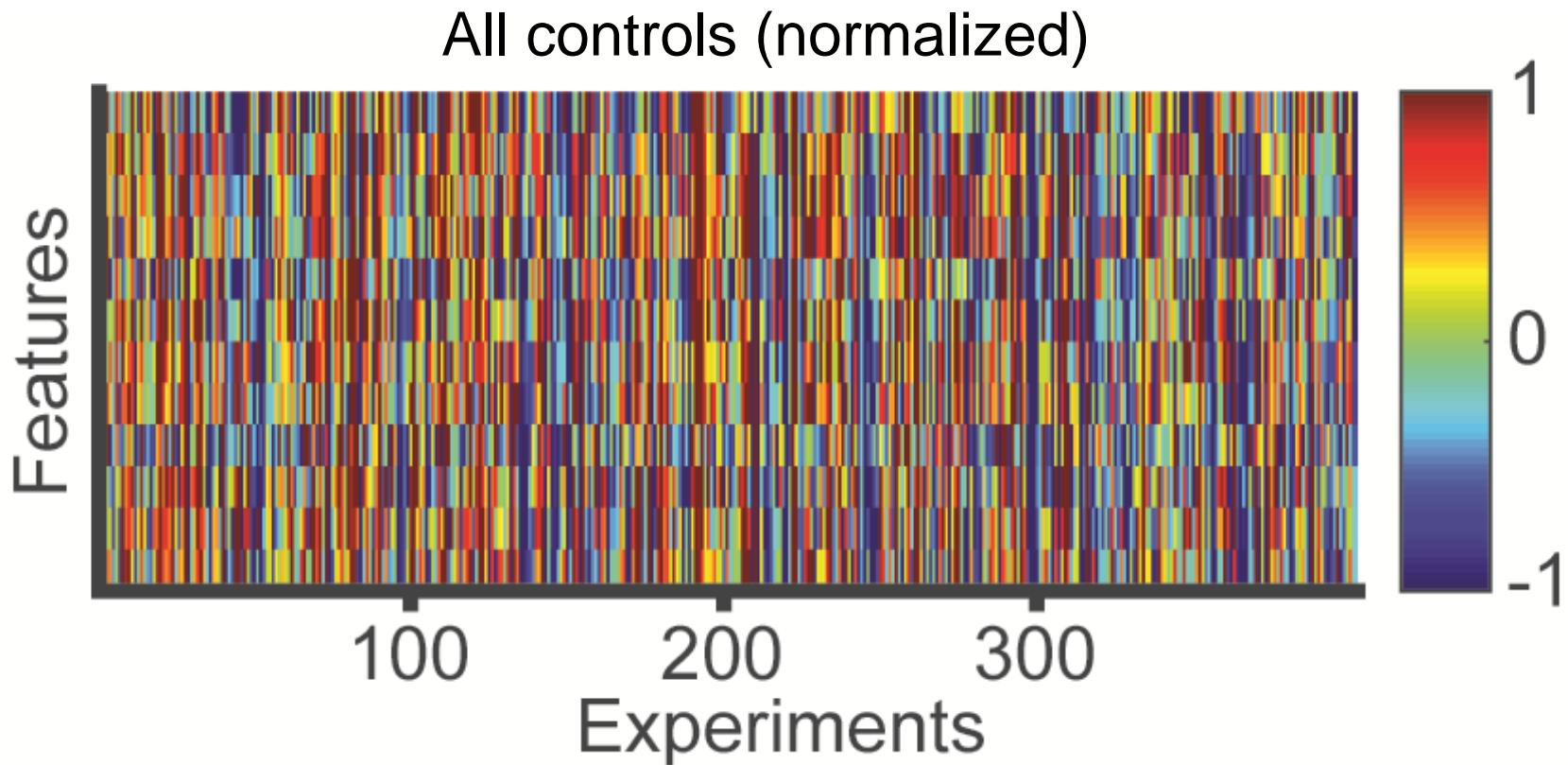
Documenting the dataset (almost 3 years of experiments)

Mutation gene	File name	File format	Pixel size (um)	Knockdown efficiency (%)	Date
pSuper	Yunyu_20131123_20hr_5min_2013_0001_AA01_01	.zvi	0.624951		20131123
pSuper	Yunyu_20131123_20hr_5min_2013_0001_AA01_02	.zvi	0.624951		20131123
pSuper	Yunyu_20131123_20hr_5min_2013_0001_AA01_03	.zvi	0.624951		20131123
pSuper	Yunyu_20131123_20hr_5min_2013_0001_AA01_04	.zvi	0.624951		20131123
CDC42_1	Yunyu_20131123_20hr_5min_2013_0001_AA02_01	.zvi	0.624951	0.95	20131123
CDC42_1	Yunyu_20131123_20hr_5min_2013_0001_AA02_02	.zvi	0.624951	0.95	20131123
CDC42_1	Yunyu_20131123_20hr_5min_2013_0001_AA02_03	.zvi	0.624951	0.95	20131123
CDC42_1	Yunyu_20131123_20hr_5min_2013_0001_AA02_04	.zvi	0.624951	0.95	20131123
pSuper	Yunyu_20131206_20hr_5min_0001_AB01_01	.zvi	1.249902		20131206
pSuper	Yunyu_20131206_20hr_5min_0001_AB01_02	.zvi	1.249902		20131206
pSuper	Yunyu_20131206_20hr_5min_0001_AB01_03	.zvi	1.249902		20131206
pSuper	Yunyu_20131206_20hr_5min_0001_AB01_04	.zvi	1.249902		20131206
CDC42_1	Yunyu_20131206_20hr_5min_0001_AB02_01	.zvi	1.249902	0.95	20131206
CDC42_1	Yunyu_20131206_20hr_5min_0001_AB02_02	.zvi	1.249902	0.95	20131206
CDC42_1	Yunyu_20131206_20hr_5min_0001_AB02_03	.zvi	1.249902	0.95	20131206
CDC42_1	Yunyu_20131206_20hr_5min_0001_AB02_04	.zvi	1.249902	0.95	20131206
pSuper	Yunyu_20131214_20hr_5min_0003_AA01_01	.zvi	1.249902		20131214
pSuper	Yunyu_20131214_20hr_5min_0003_AA01_02	.zvi	1.249902		20131214
pSuper	Yunyu_20131214_20hr_5min_0003_AA01_03	.zvi	1.249902		20131214
CDC42_1	Yunyu_20131214_20hr_5min_0003_AA02_01	.zvi	1.249902	0.95	20131214
CDC42_1	Yunyu_20131214_20hr_5min_0003_AA02_02	.zvi	1.249902	0.95	20131214
CDC42_1	Yunyu_20131214_20hr_5min_0003_AA02_03	.zvi	1.249902	0.95	20131214

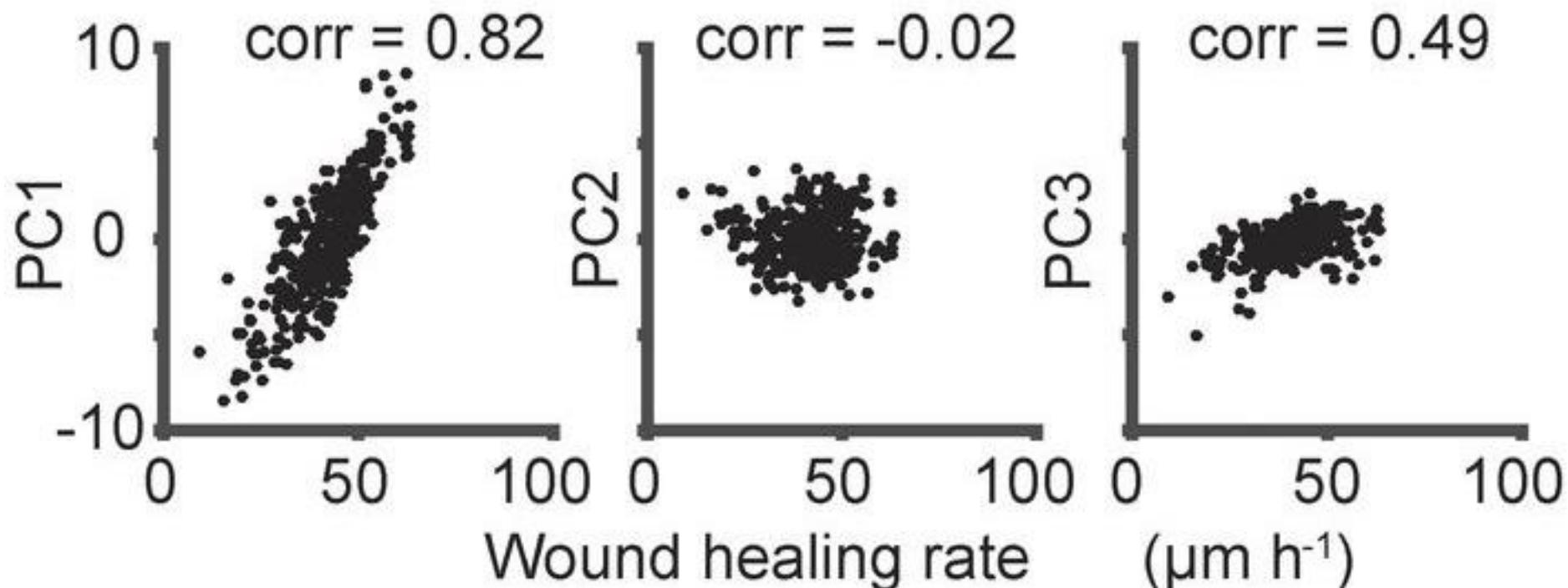
Encoding spatiotemporal dynamics



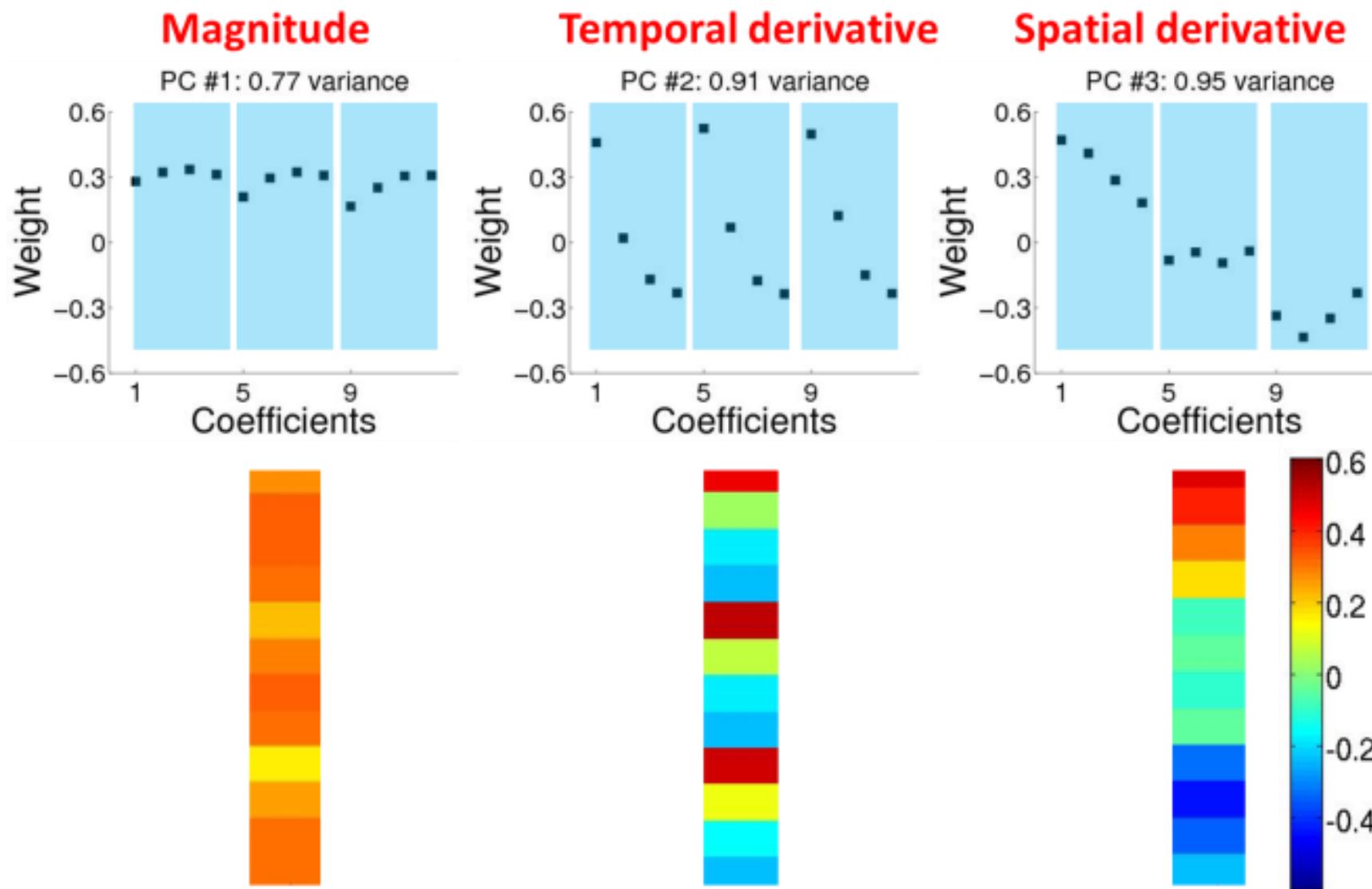
Information encoded in a wound healing experiment



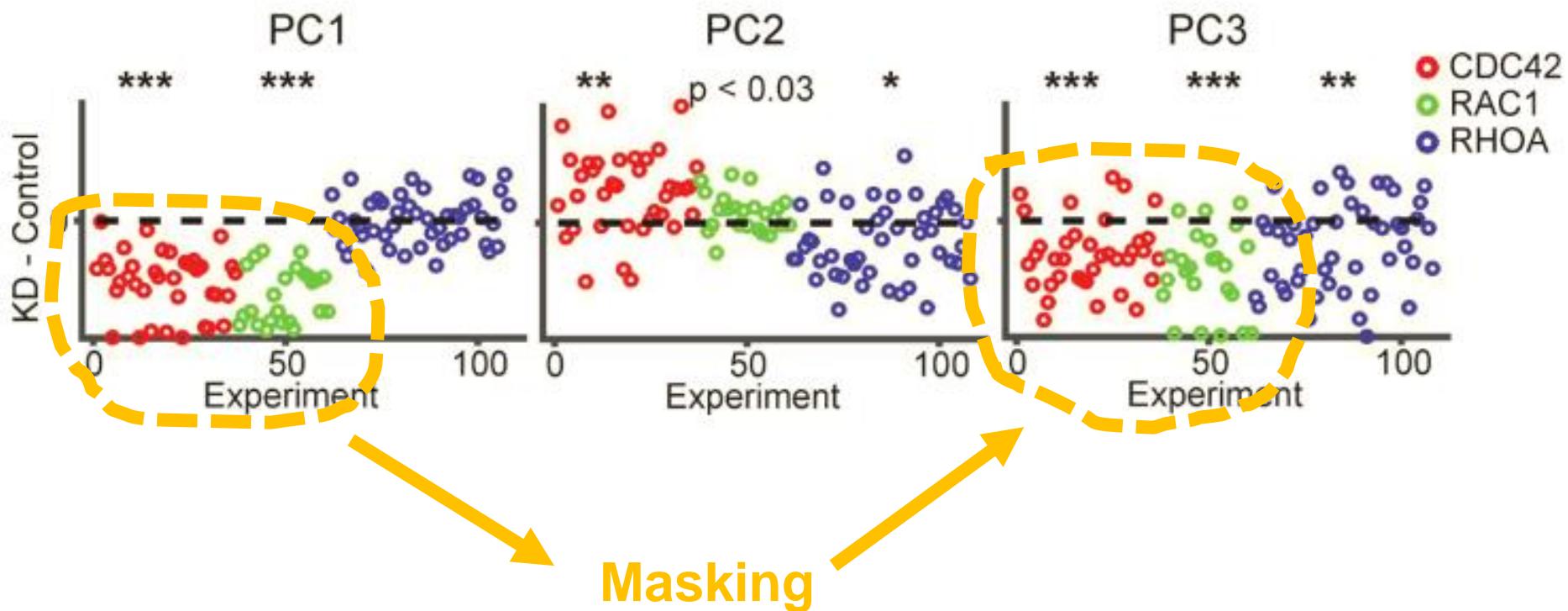
Association with monolayer migration rate



Reverse engineering: what information is encoded in an experiment?

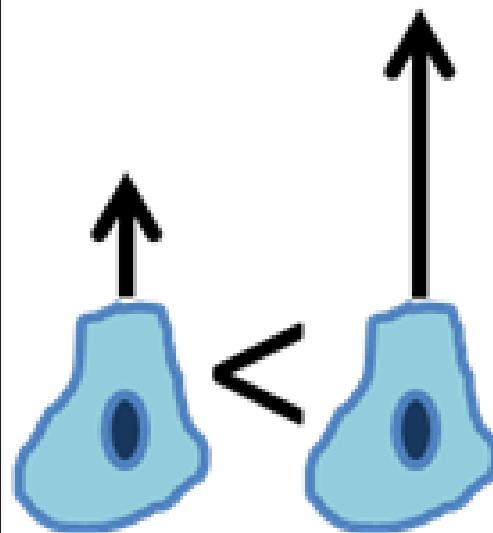


Effect on PCs by depletion of canonical Rho GTPases

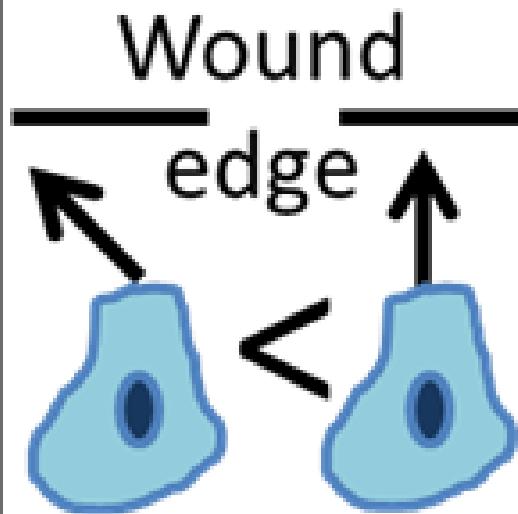


Measures

Speed

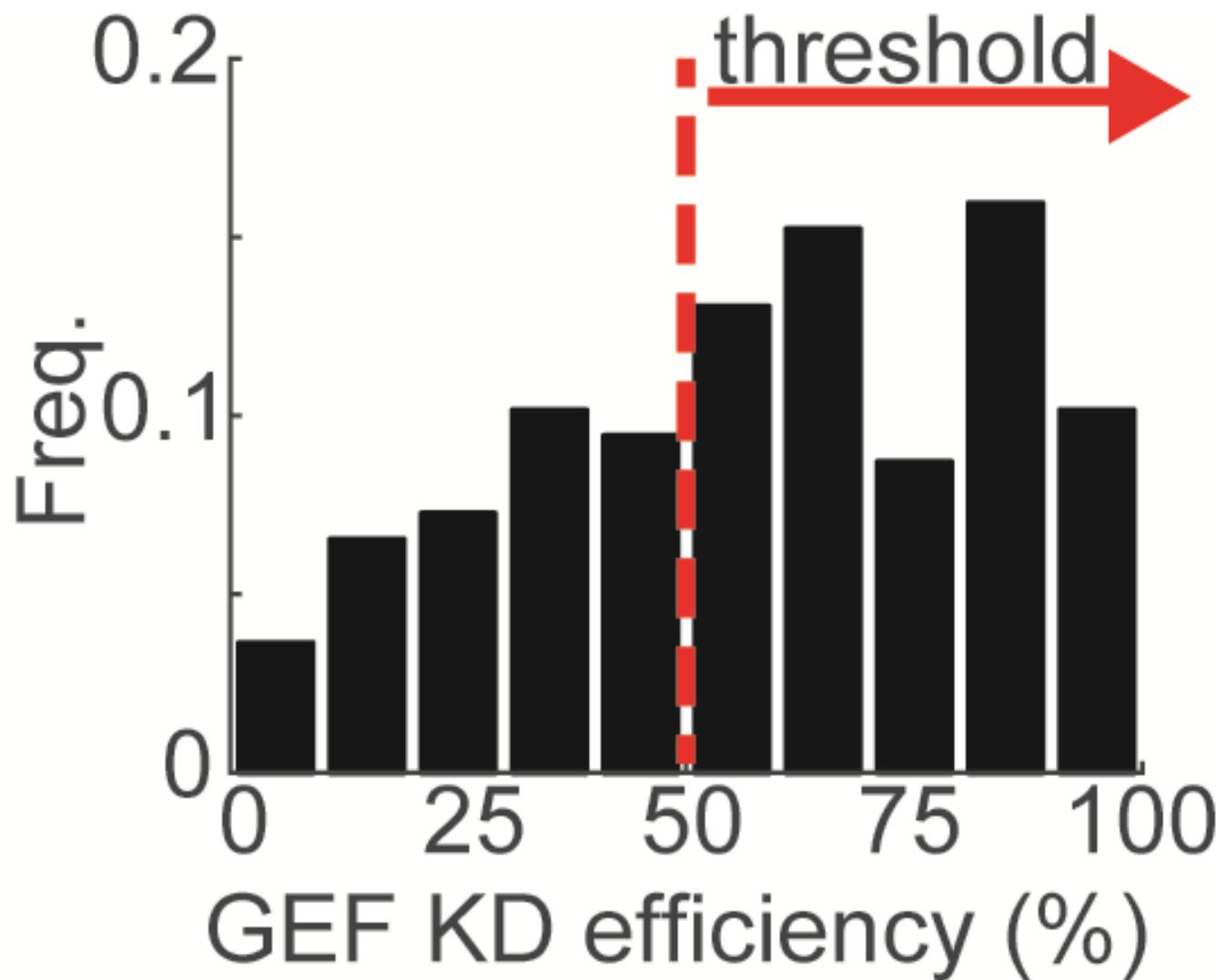


Directionality

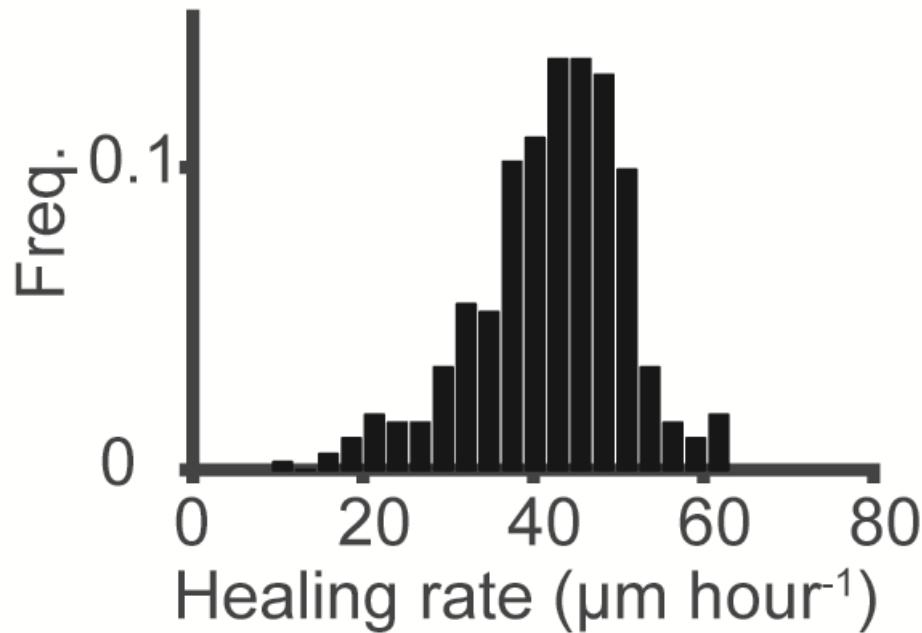


Screening methodology

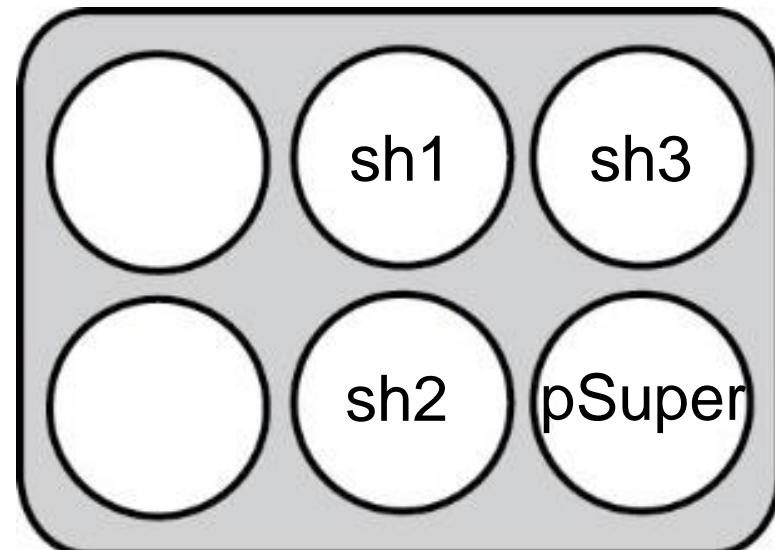
Knockdown efficiencies



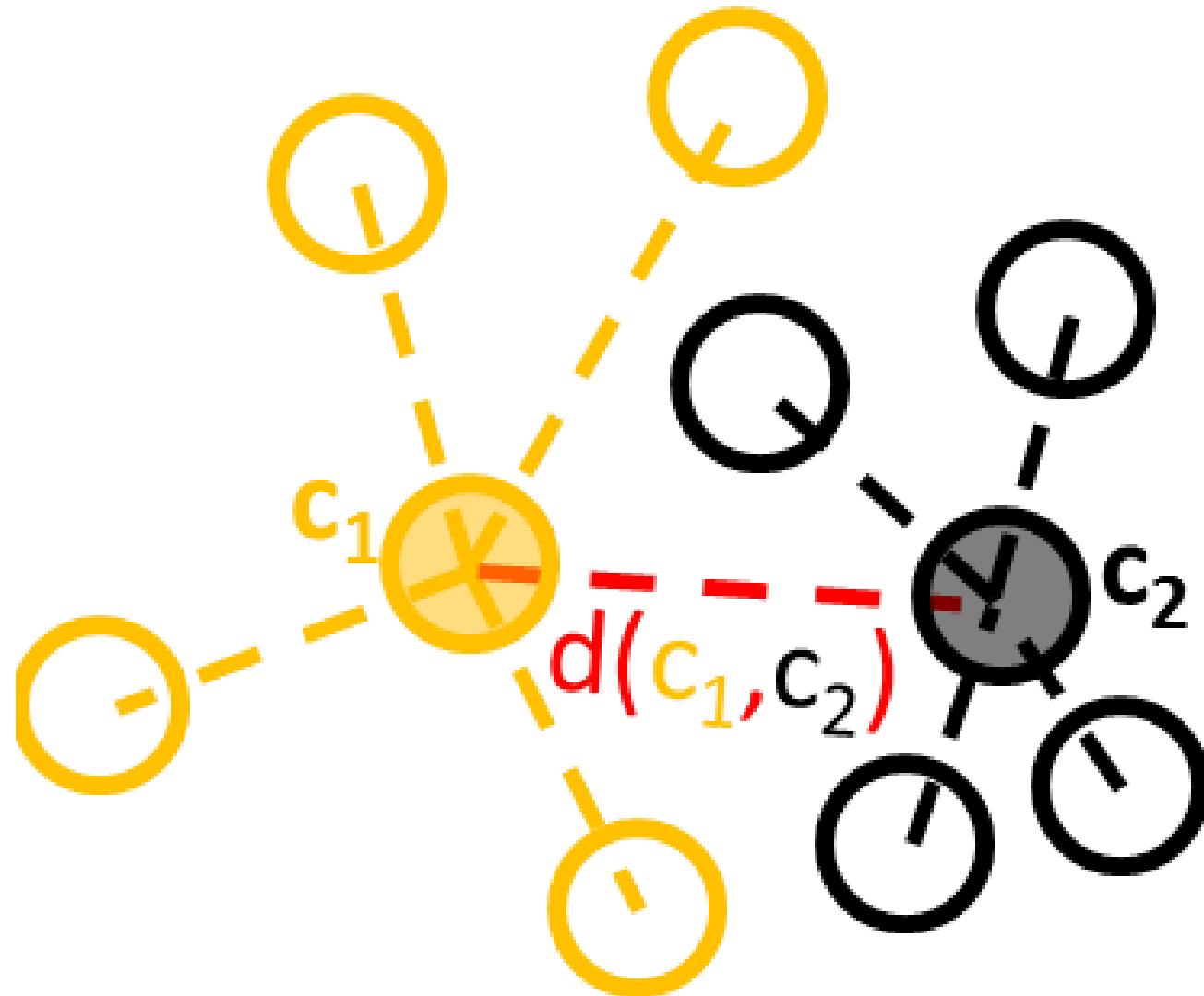
Inter-day variability in controls



- 6 well plates
- 3 shRNAs + 1 control (pSuper)
- 4-6 locations imaged per well



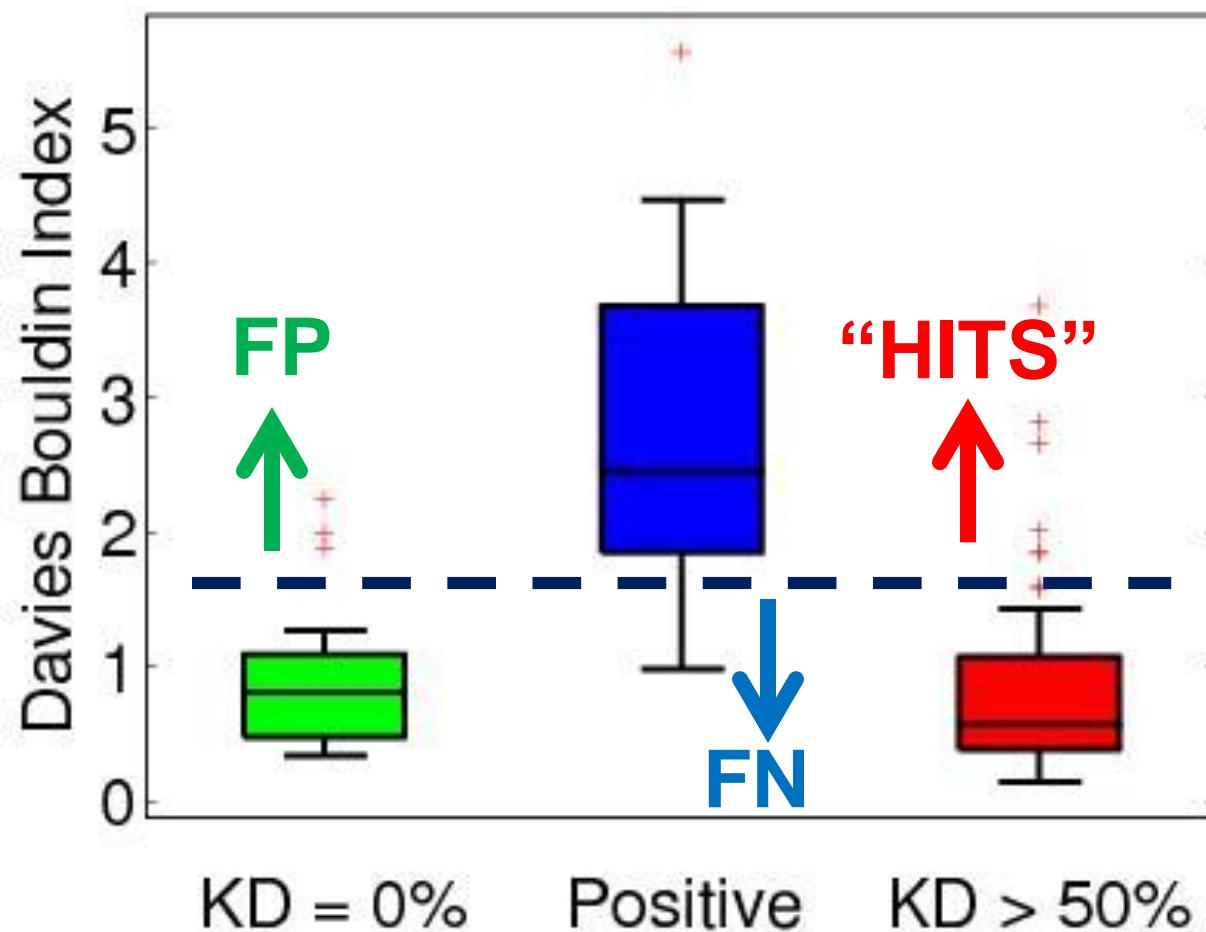
Scoring a knockdown phenotype



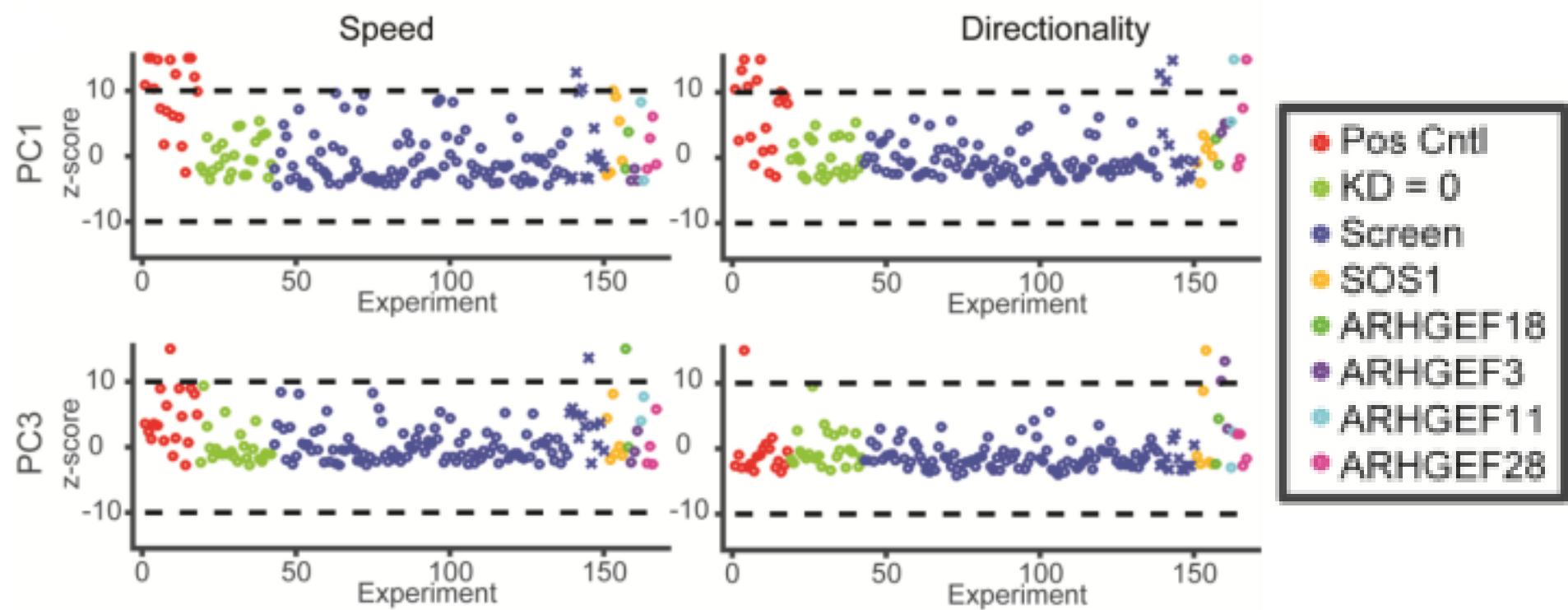
Dunn index, Davies-Bouldin index, silhouette coefficient

Quantifying off-target effects

- Exploiting 0% KD Experiments & “Known” Targets
 - 0% KD as **off-target controls**
 - CDC42, RAC1, β -PIX as **positive controls**



Screen

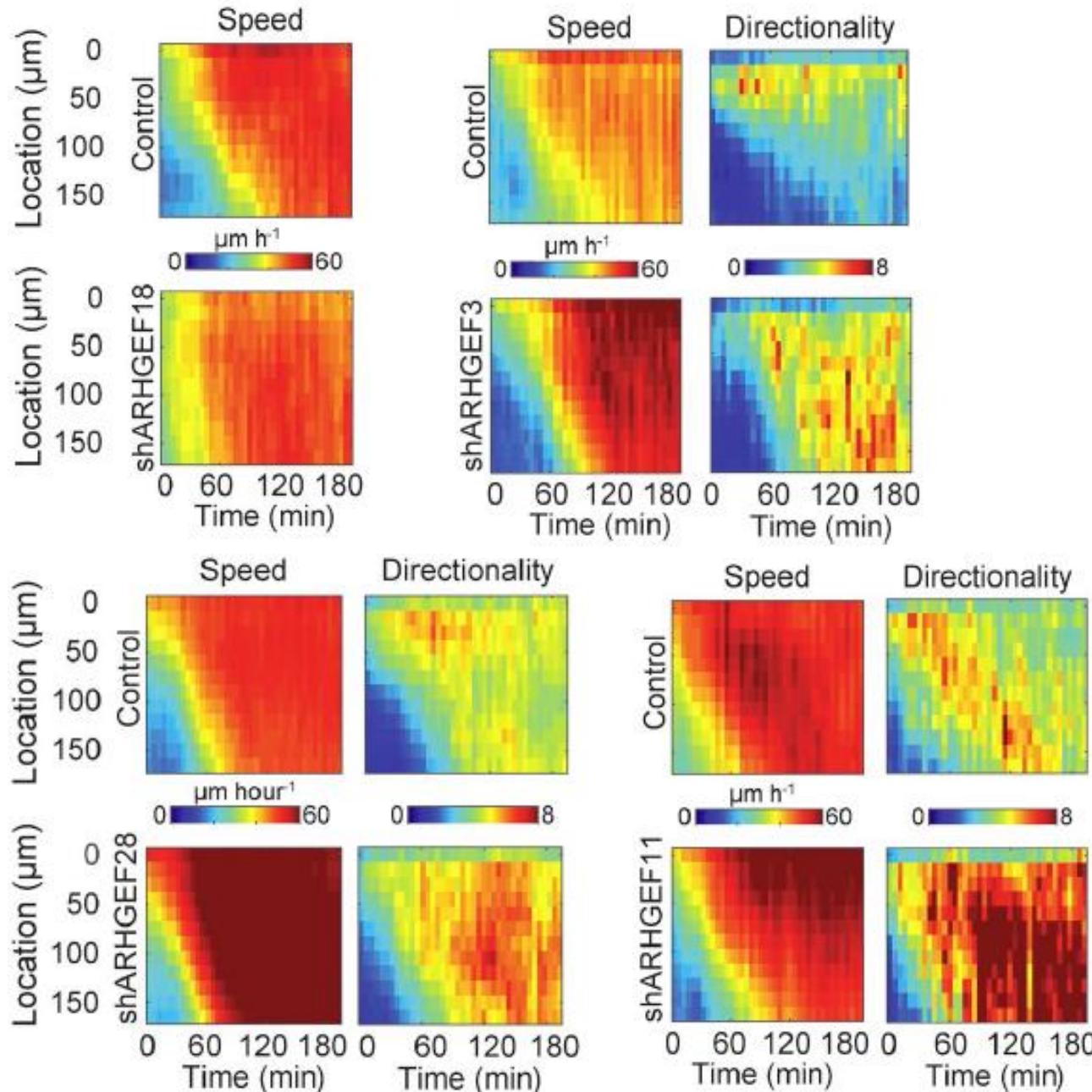


Screen hits

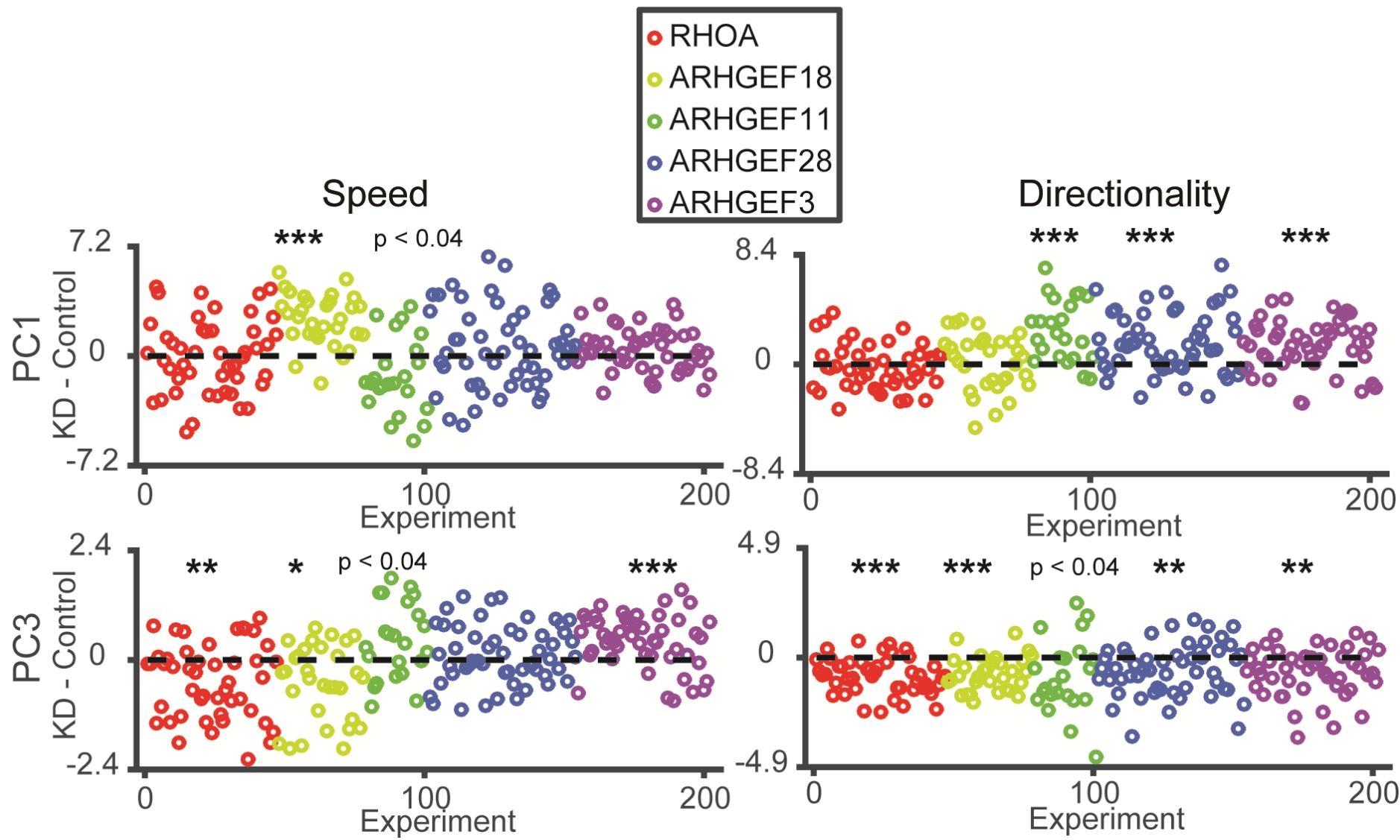
Condition	Long range communication (PC3)	Speed (PC1)	Directionality (PC1)
β -PIX		↓	↓
SOS1	✗	↓	↓
ARHGEF18	↑		
ARHGEF11			↑
ARHGEF28			↑
ARHGEF3	↑		
ARHGEF10			↑
TRIO		↓	↓
TUBA		↓	↓
ARHGEF9	✗		
DOCK10*			

* - hit in directionality PC2, details in legend

Screen hits - visualization



Validation (and discovering new phenotypes)

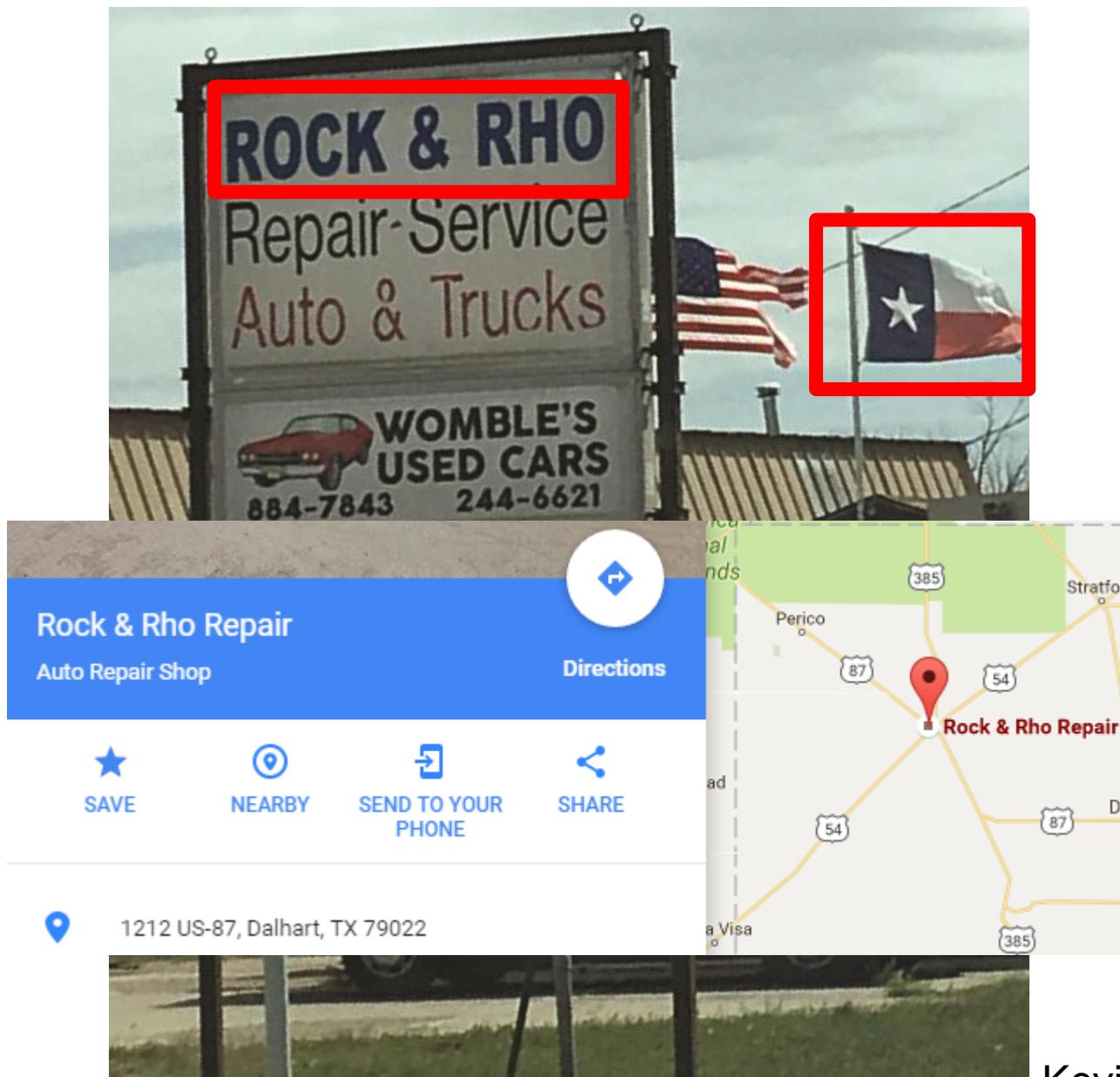


Greatest hits

RhoA GEFs inhibit long-range communication

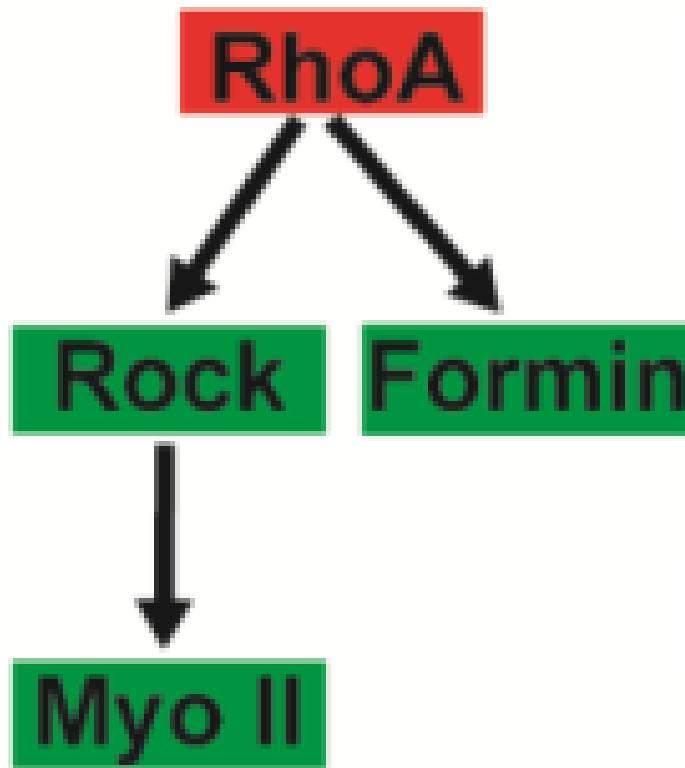
Condition	Short range communication (PC1 coordination)	Long range communication (PC3)	Speed (PC1)	Directionality (PC1)
SOS1-RAS	↓↓↓		↓↓↓	↓↓↓
ARHGEF3		↑↑		↑↑↑
ARHGEF11				↑↑↑
ARHGEF28	↑↑↑	↑↑		↑↑↑
ARHGEF18		↑↑↑	↑↑↑	
RHOA		↑↑↑		

What about Myosin?



Kevin Dean, [@kD3AN](https://twitter.com/kD3AN)

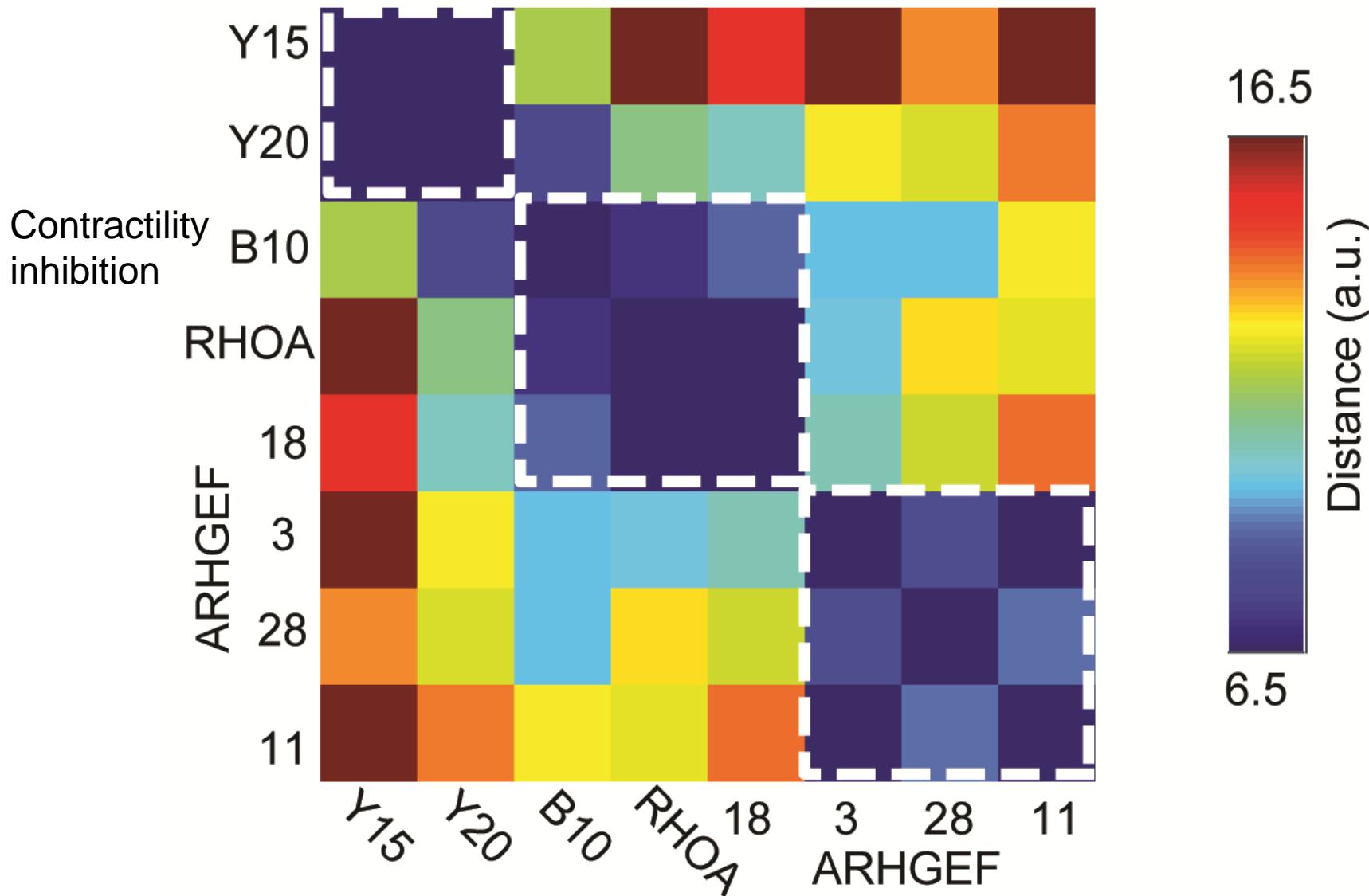
Downstream effectors of RhoA



Actomyosin contractility disturbs intercellular communication downstream of the ARHGEF18 - RHOA pathway

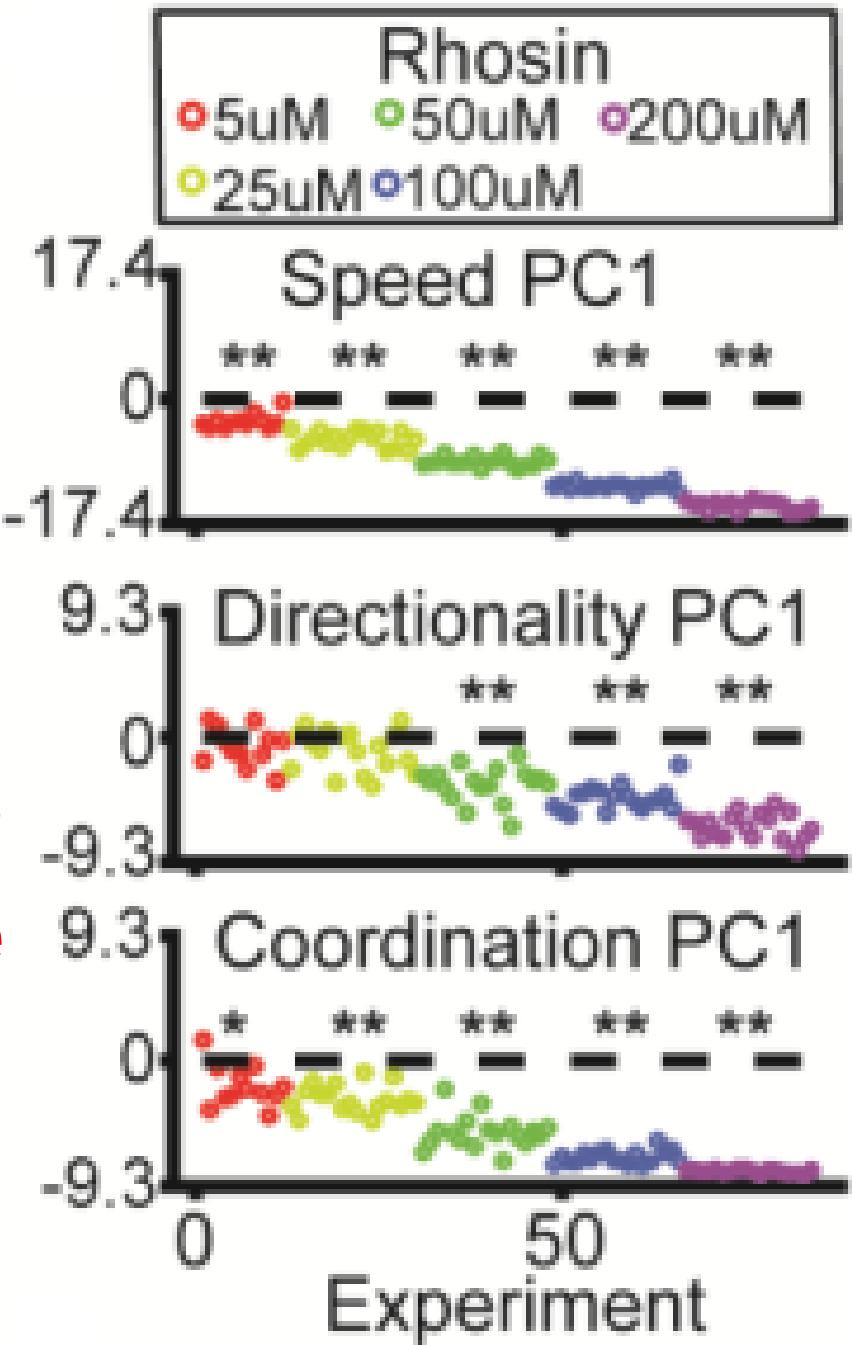
Condition	Short range communication (PC1 coordination)	Long range communication (PC3)	Speed (PC1)	Directionality (PC1)
SOS1-RAS	↓↓↓		↓↓↓	↓↓↓
ARHGEF3		↑↑		↑↑↑
ARHGEF11				↑↑↑
ARHGEF28	↑↑↑	↑↑		↑↑↑
ARHGEF18		↑↑↑	↑↑↑	
RHOA		↑↑↑		
MyosinII (low)	↑	↑		
ROCK (low)	↑↑	↑↑	↑↑	↑
ROCK (high)	↓↓↓		↓↓↓	↓↓↓

Differential functional roles of RhoA-GEFs down-stream of RhoA signaling

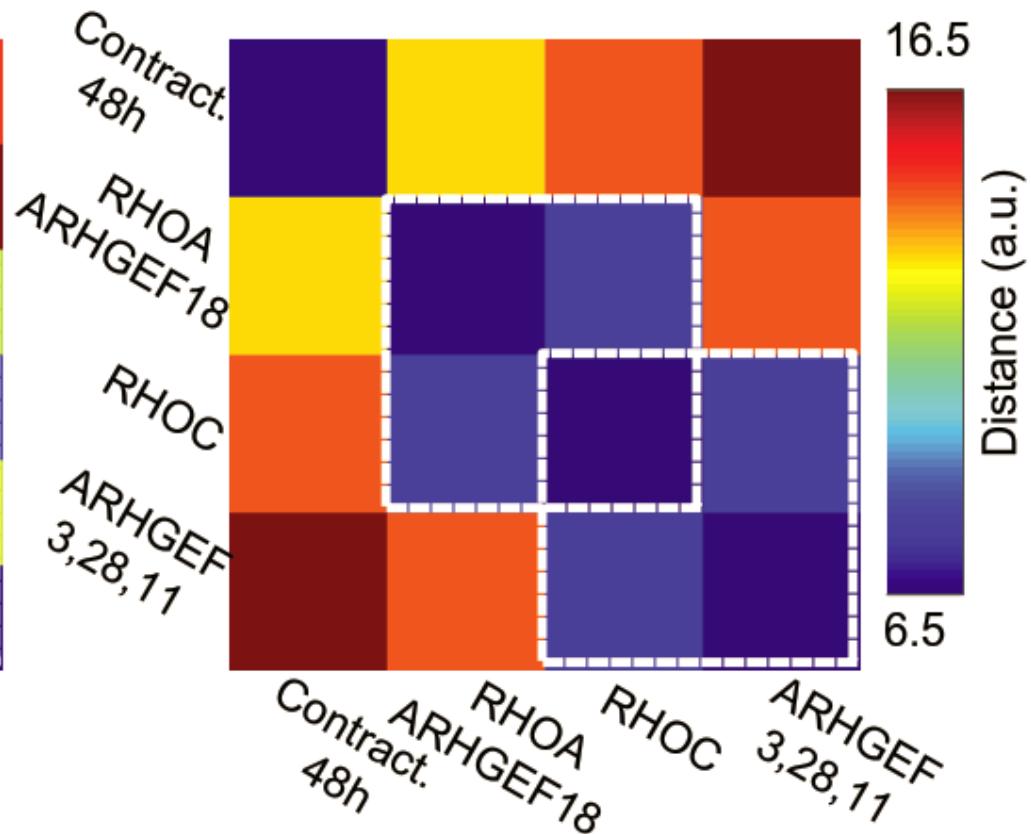
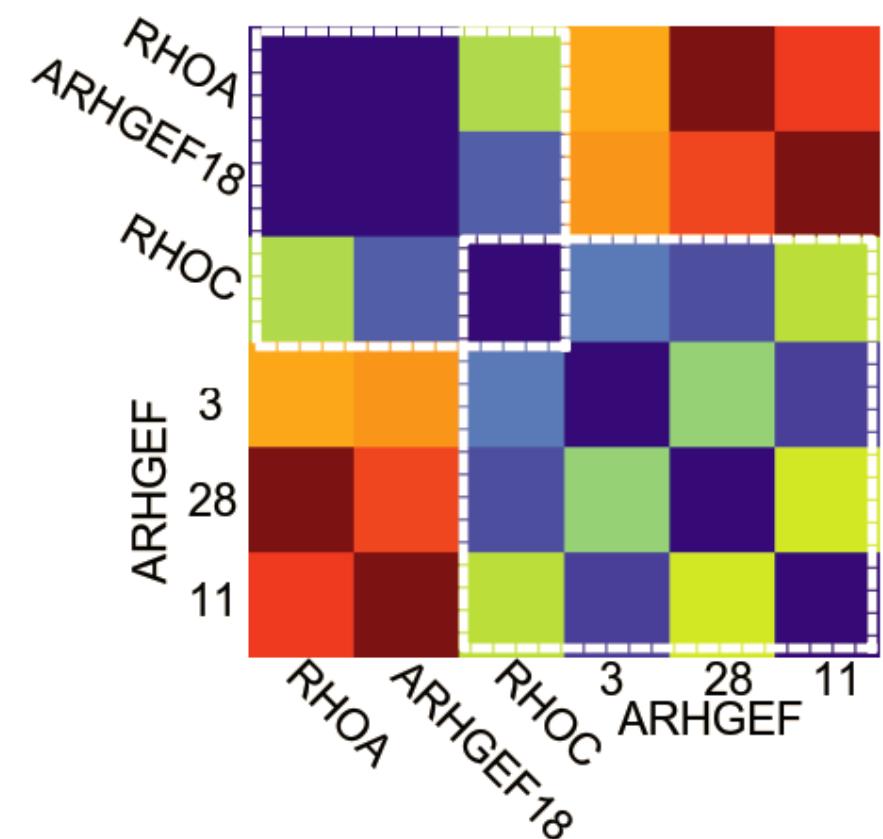


Rho isoforms are required for collective migration

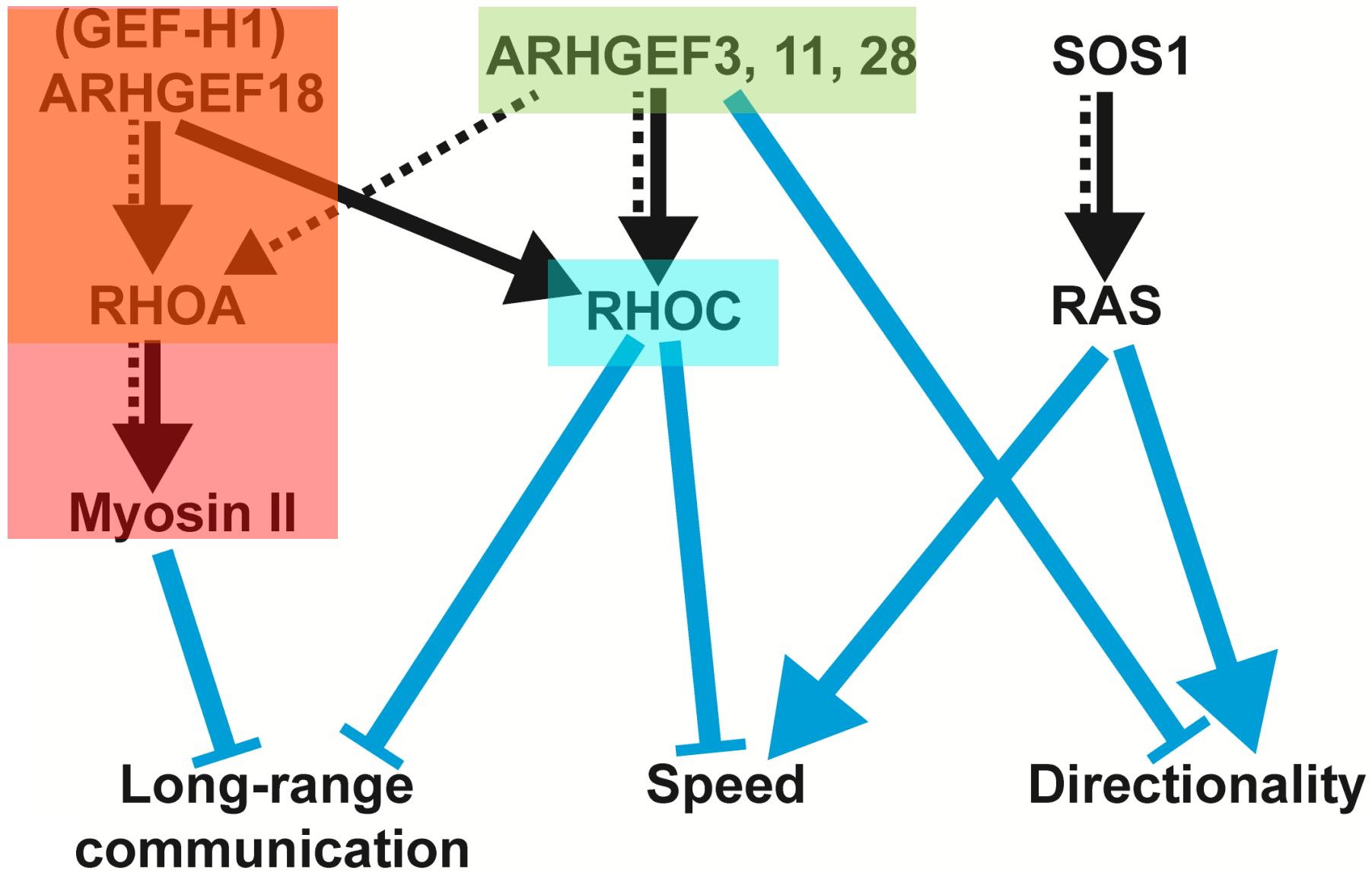
You will reproduce
(part of) this figure
as exercise



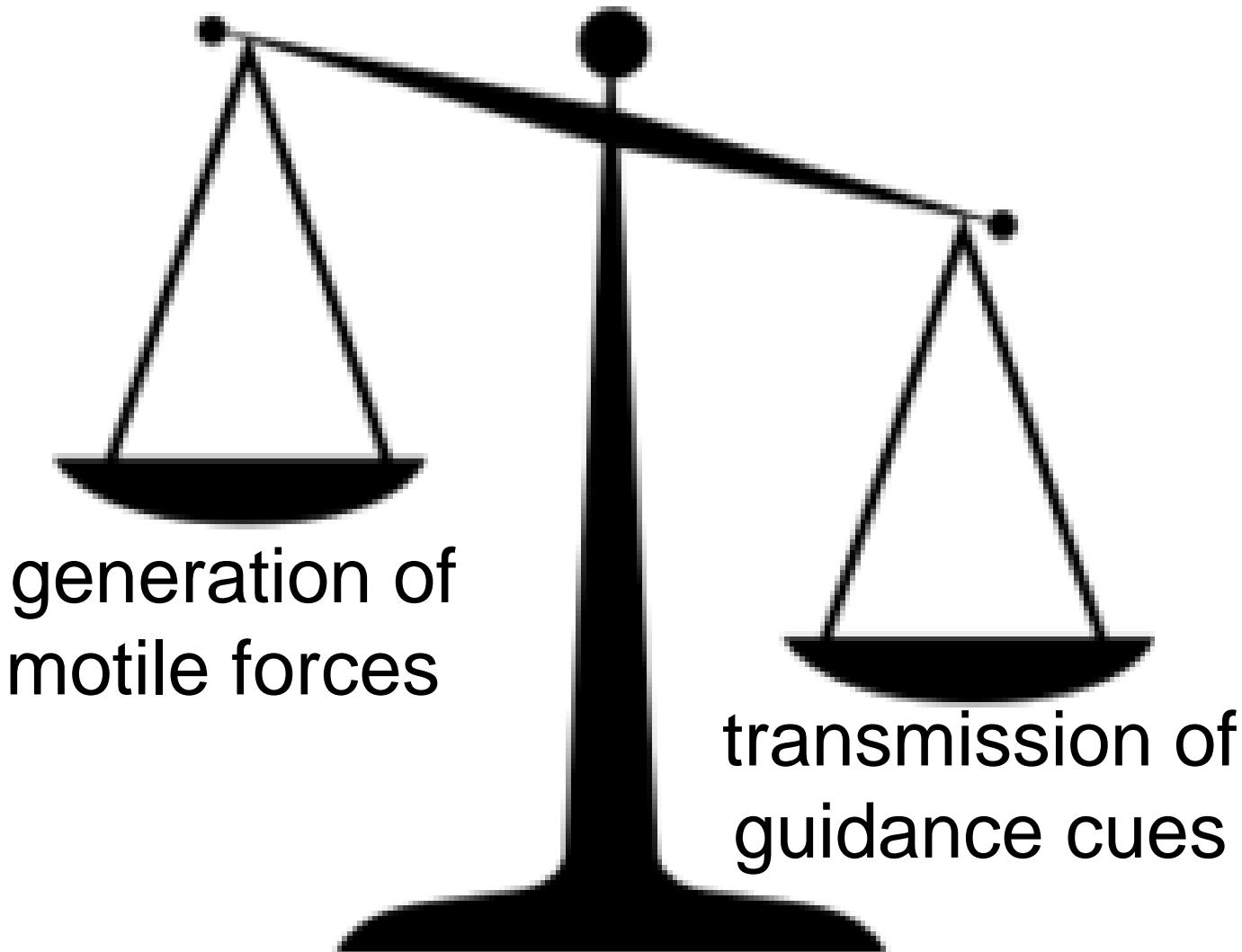
RhoC has an intermediate phenotype



Diverse roles of GEFs in regulating collective cell migration



RhoA-GEFs/RhoA/Myosin-II balances motile forces vs. mechanical guidance



References, resources

References:

- Zaritsky et al. Propagating waves of directionality and coordination orchestrate collective cell migration (2014)
<http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1003747>
- Zaritsky et al. Seeds of locally aligned motion and stress coordinate a collective cell migration (2015)
<http://jcb.rupress.org/content/early/2017/05/15/jcb.201609095>
- Zaritsky, Tseng et al. Diverse roles of guanine nucleotide exchange factors in regulating collective cell migration (2017)
[www.cell.com/biophysj/abstract/S0006-3495\(15\)01123-6](http://www.cell.com/biophysj/abstract/S0006-3495(15)01123-6)

Source code:

- <https://github.com/DanuserLab/MonolayerKymographs>

Acknowledgments

Yun-Yu Tseng



Ángeles Rabadán



Shefali Krishna



Mike Overholtzer



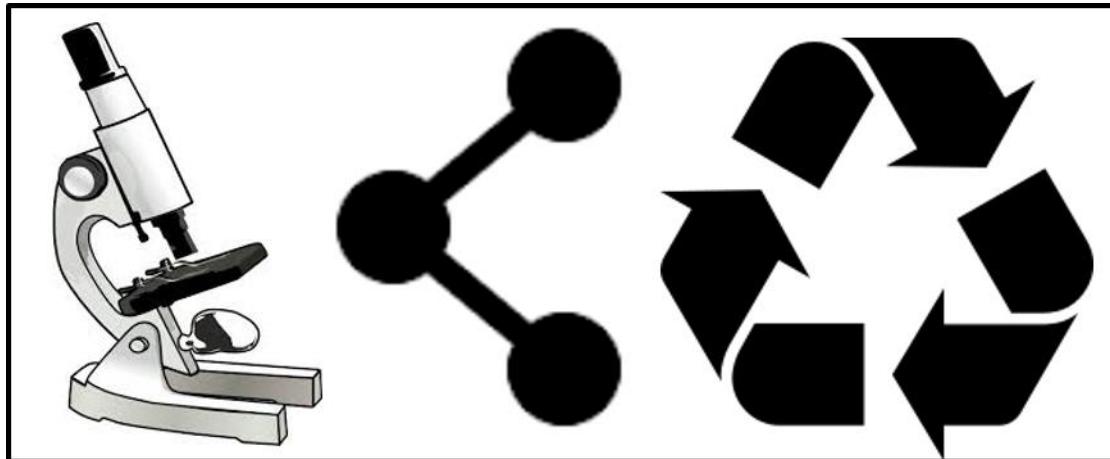
Gaudenz Danuser



Alan Hall



Reusing cell image data for new biological insight (and tool development, and reproducibility)



Subgroup @ASCB:
<https://assafzar.wixsite.com/ascb2017-subgroup>

Thanks for sharing your data!



Institute for bioengineering
of Catalonia



Xavier Serra-
Picamal



Xavier Trepaut



Memorial Sloan Kettering
Cancer Center..



Yun-Yu Tseng



Angeles
Rabadan



Joachim Spatz



Tamal Das



Exercise

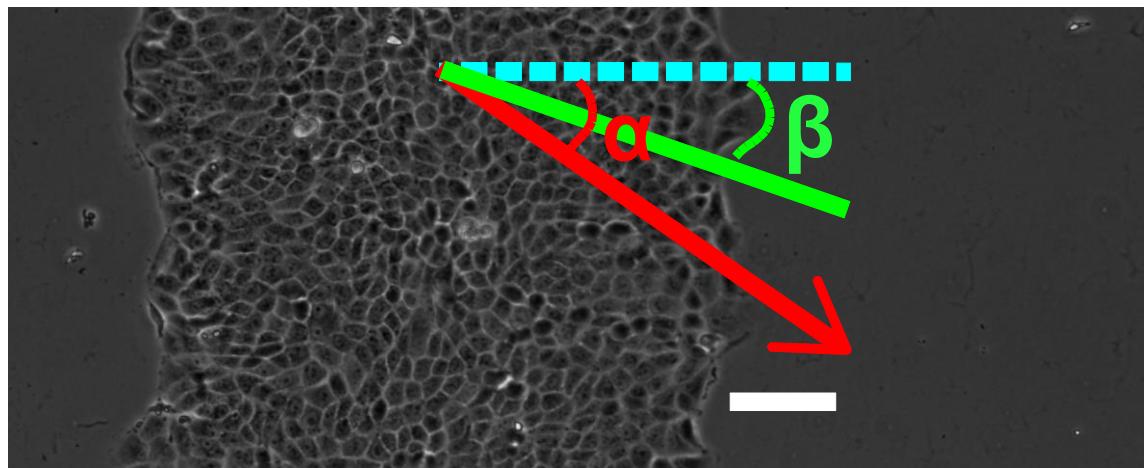
1. Execute the workflow on a single video, visualize kymographs
 - Download data [here](#) (intermediate data, no kymographs)
 - filename = [path filesep 'Angeles_20150402_14hrs_5min_AA01_.7tif'];
 - mainTimeLapse(filename); Examine kymographs
2. Rhosin data
 - Download Rhosin data [here](#) (only kymographs and meta data)
 - Set RhosinDname to directory
 - mainRhosin(RhosinDname)
 - Transform using pre-determined PCA and compare KD to control
 - “Extra credit”: direct calculation of spatial / temporal derivative
 - “Homework”:
 - Tweak one component (PIV / cell-background segmentation)

Agenda

1. Collective cell migration
2. Detection of coordinated clusters (+ exercise)
3. Example (data reuse)
4. GEF screen (+ exercise)
5. **DeBias – if times allow (co-localization)**

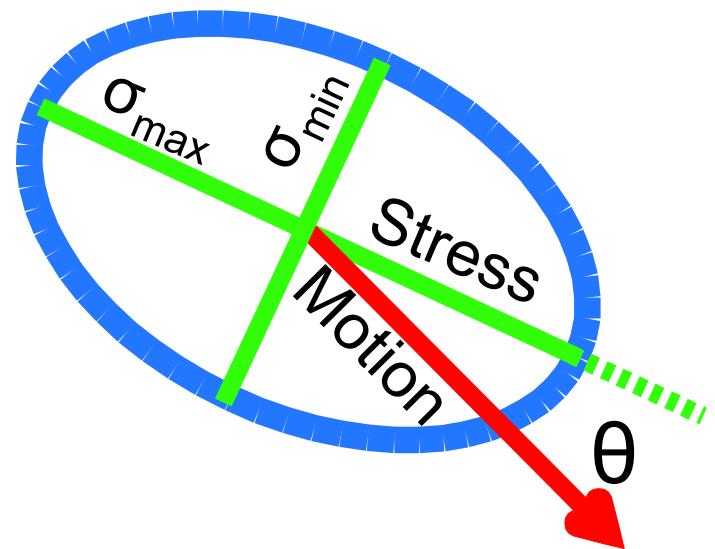
Motion-stress alignment

Velocity angle,
stress orientation



$$-90 \leq \alpha, \beta \leq 90$$

Motion-stress
alignment

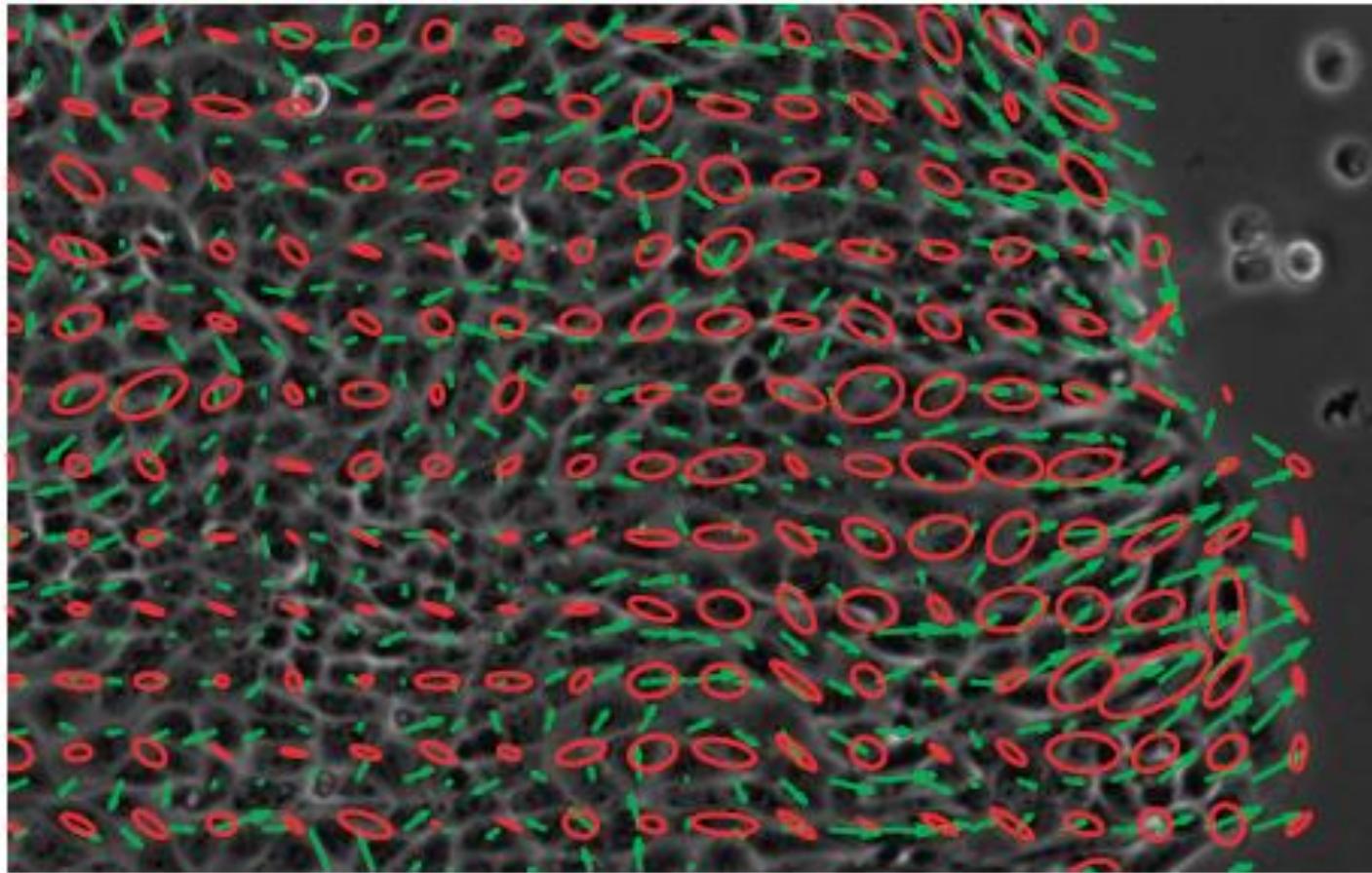


$$0 \leq \theta \leq 90$$

Tambe et al. (2011)
Trepat & Fredberg. (2011)

Plithotaxis

“tendency for each individual cell within a monolayer to migrate along the local orientation of the maximal principal stress.”



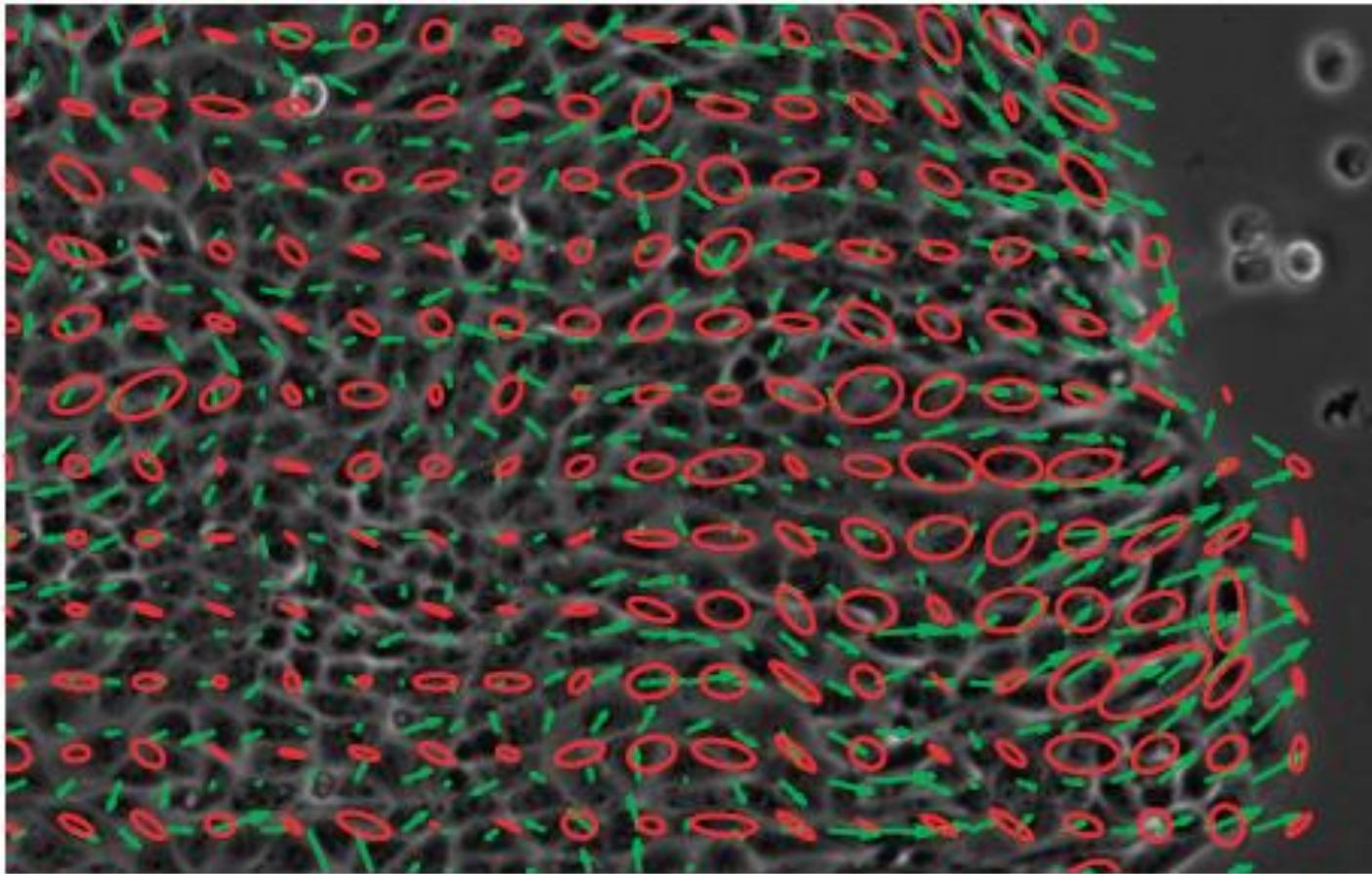
Tambe et al. (2011)

Trepat and Fredberg (2011)

Serra-Picamal and Conte et al. (2012)

Plithotaxis

“tendency for **each individual** cell within a monolayer to migrate along the **local orientation** of the maximal principal stress.”



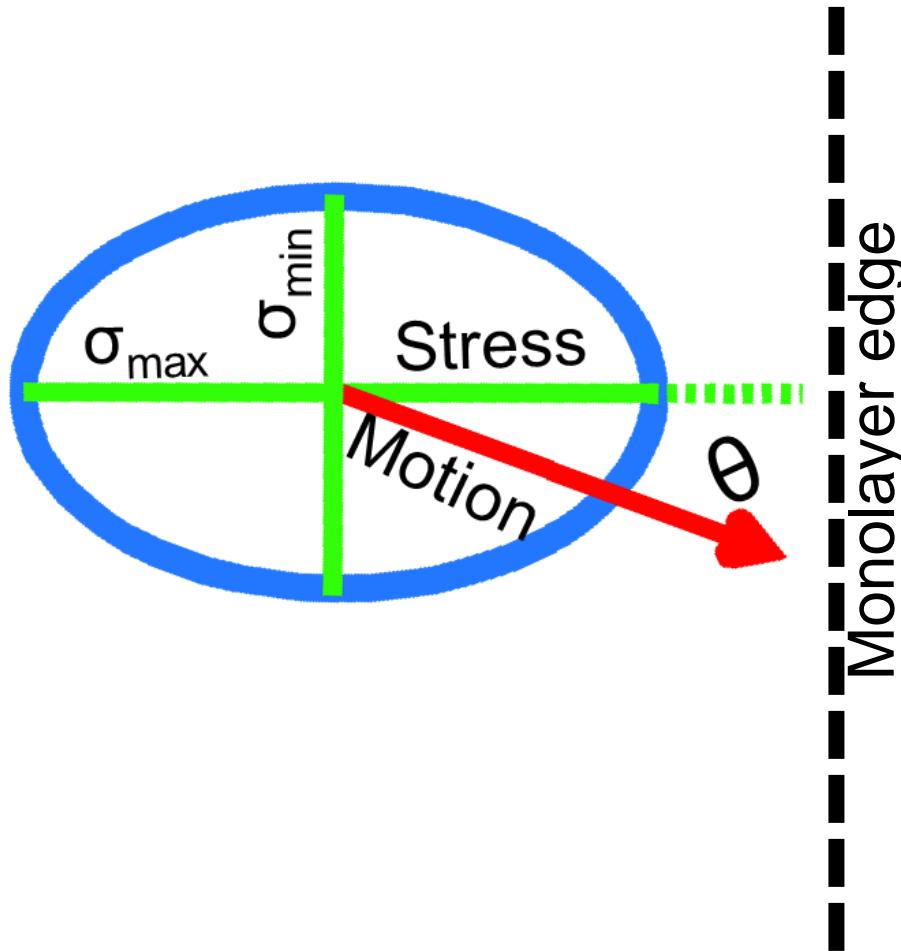
Tambe et al. (2011)

Trepaut and Fredberg (2011)

Serra-Picamal and Conte et al. (2012)

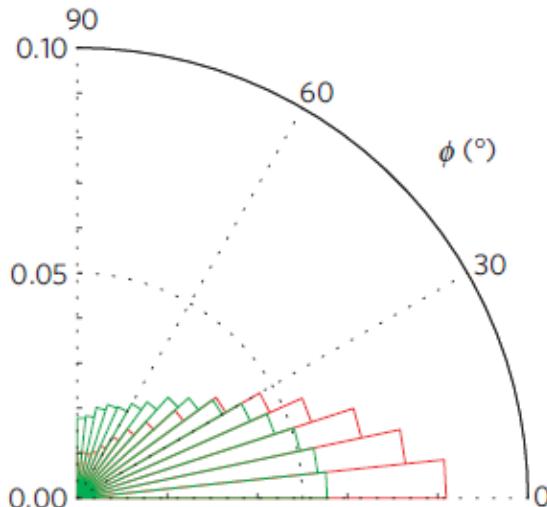
Plithotaxis?

“tendency for **each individual** cell within a monolayer to migrate along the **local orientation** of the maximal principal stress.”

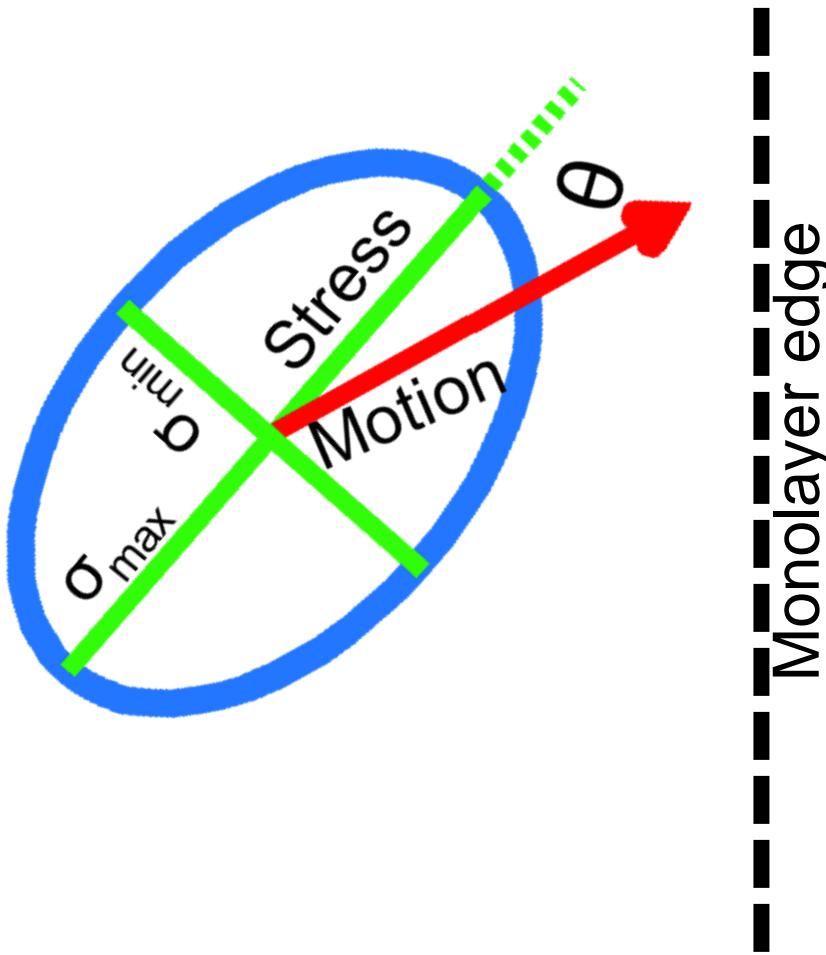


Plithotaxis?

“tendency for each **individual** cell within a monolayer to migrate along the **local orientation** of the maximal principal stress.”

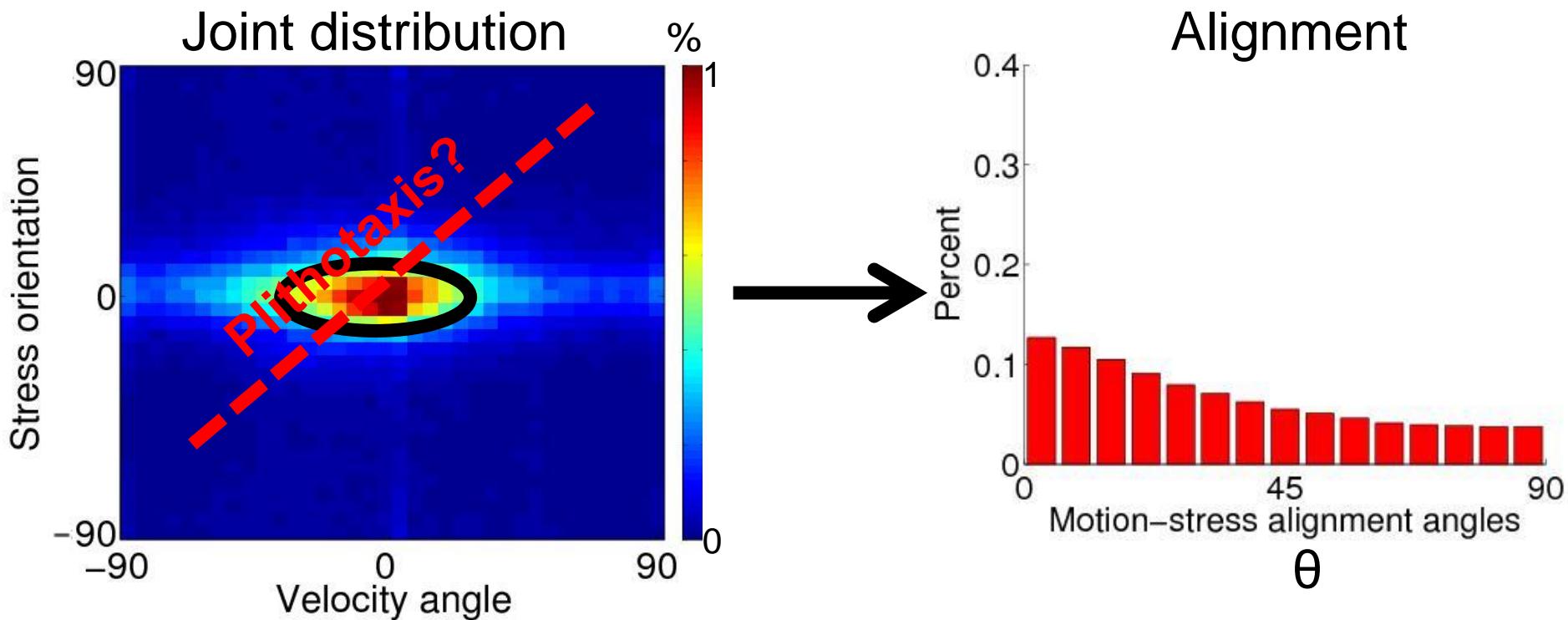


Serra-Picamal and Conte
et al. (2012)

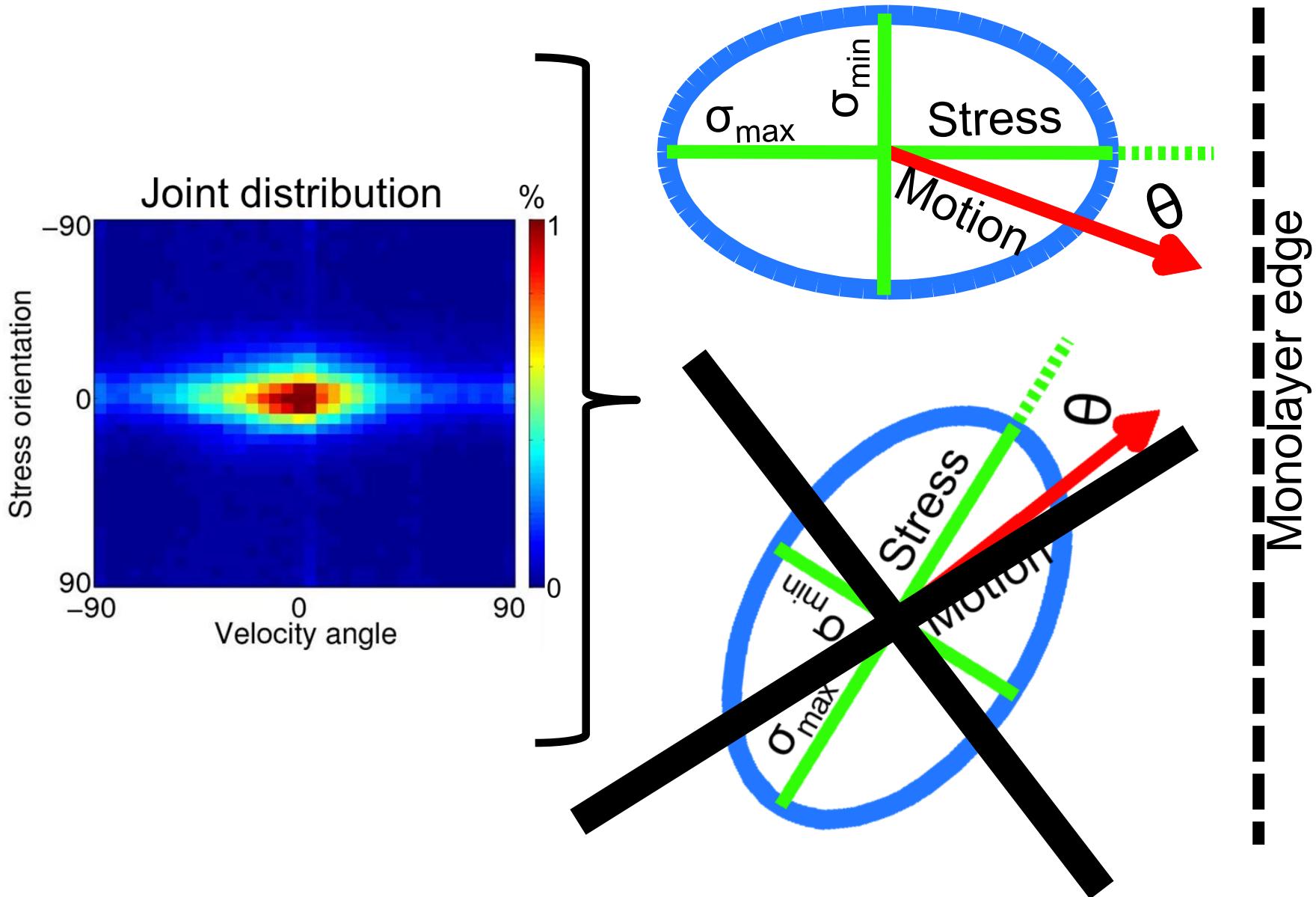


The roles of (global) monolayer
geometry versus (local) plithotaxis
in inducing motion-stress alignment

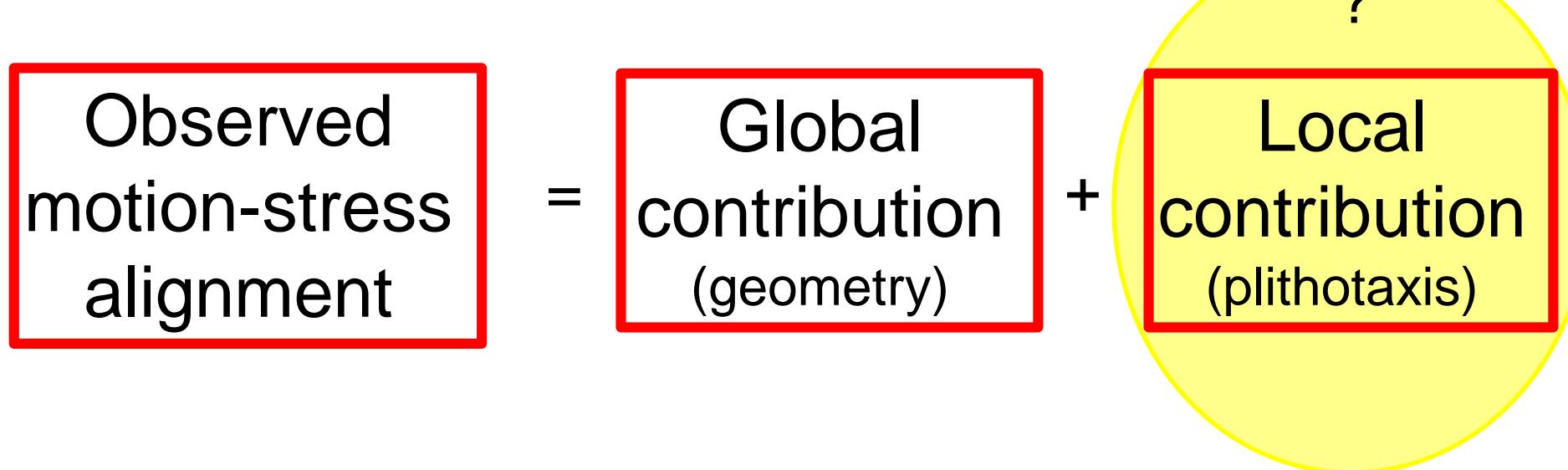
Motion-Stress Alignment \neq Plithotaxis



No Evidence for Plithotaxis!

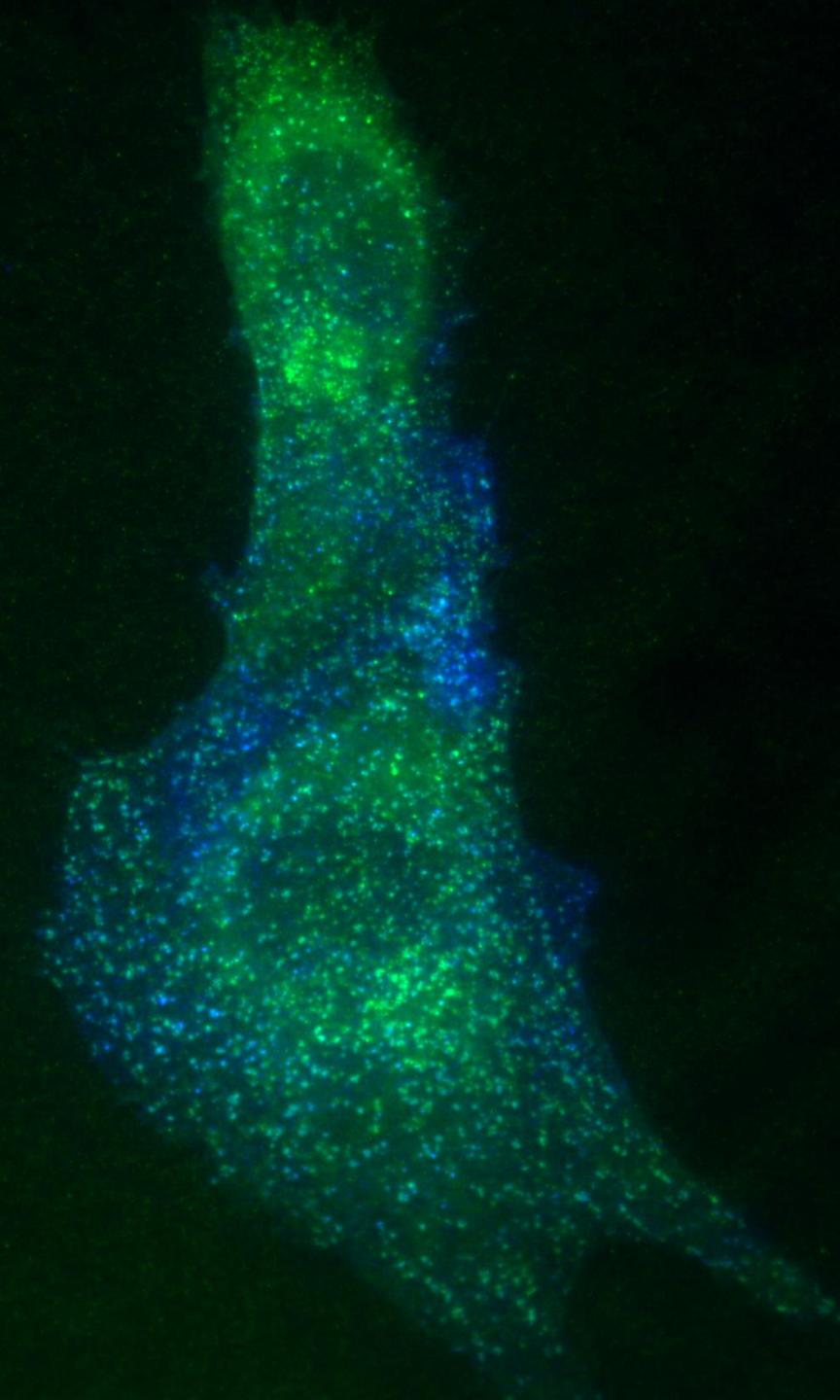


Components of motion-stress alignment



The interplay between development of quantitative tools ("hammers") and identifying open important questions in cell biology ("nails")

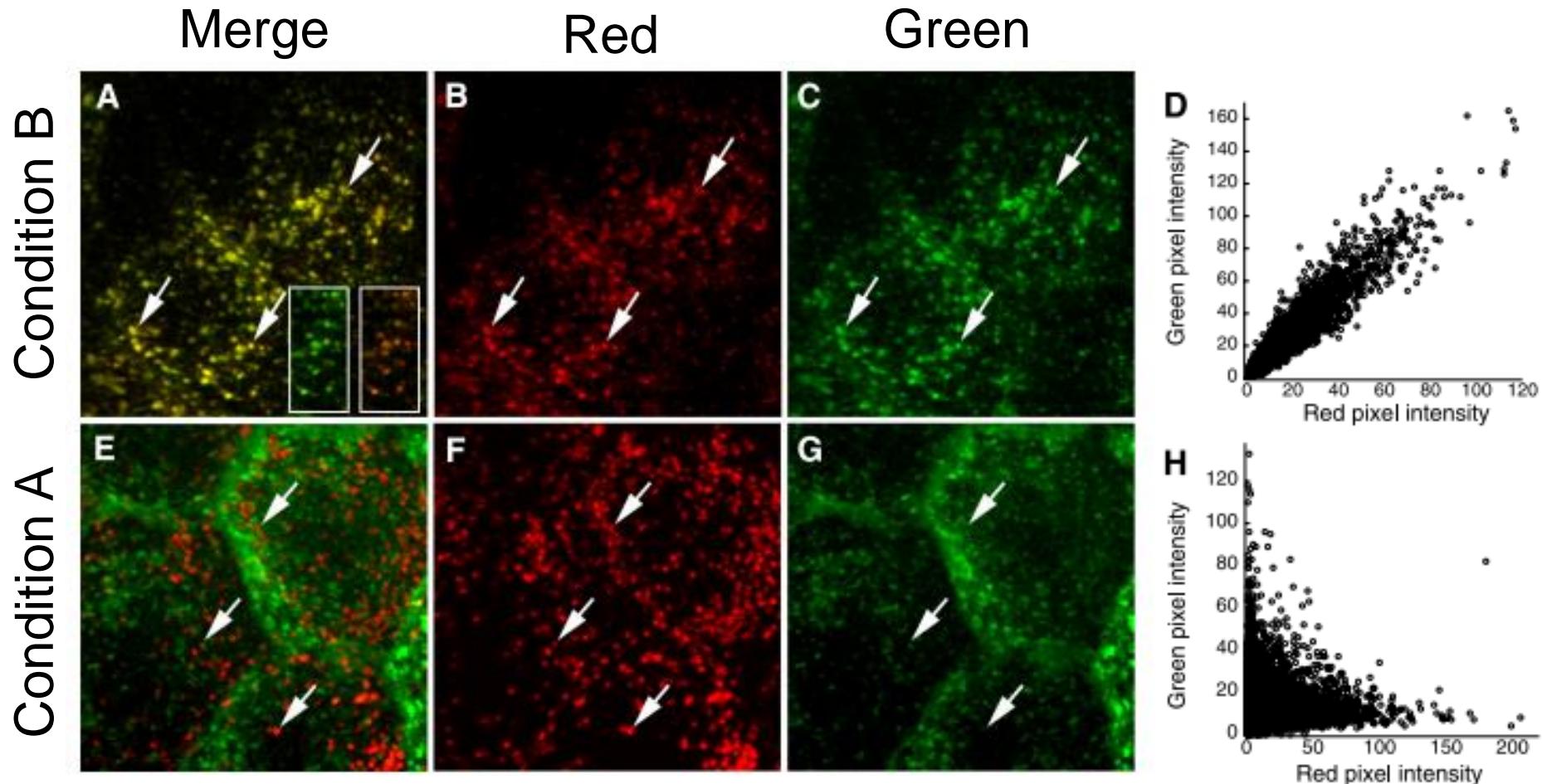




Decoupling global biases and local interactions between cell biological variables



Quantifying protein-protein co-localization



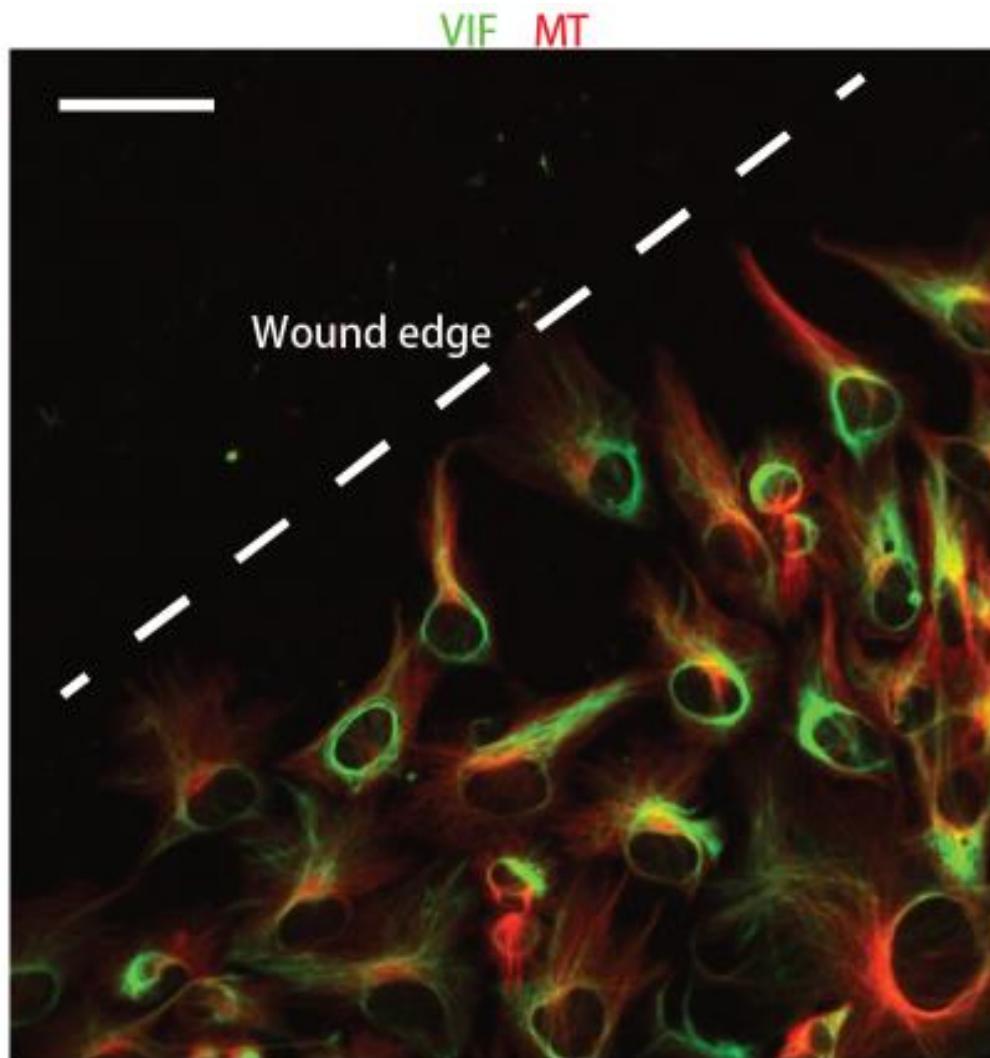
Dunn et al. (2011)

What additional information is hidden in co-localization data?

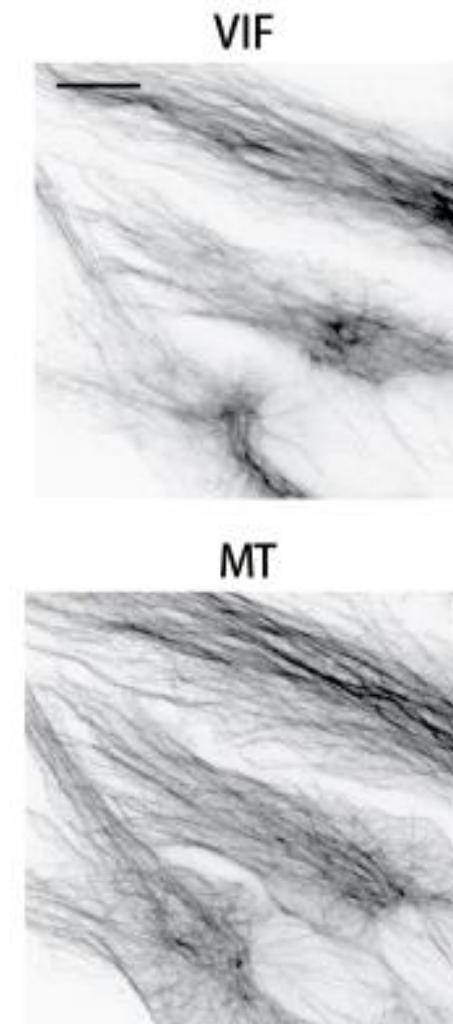


Co-orientation of intracellular cytoskeletal networks in migrating cells

Vimentin provides a structural template for microtubule growth



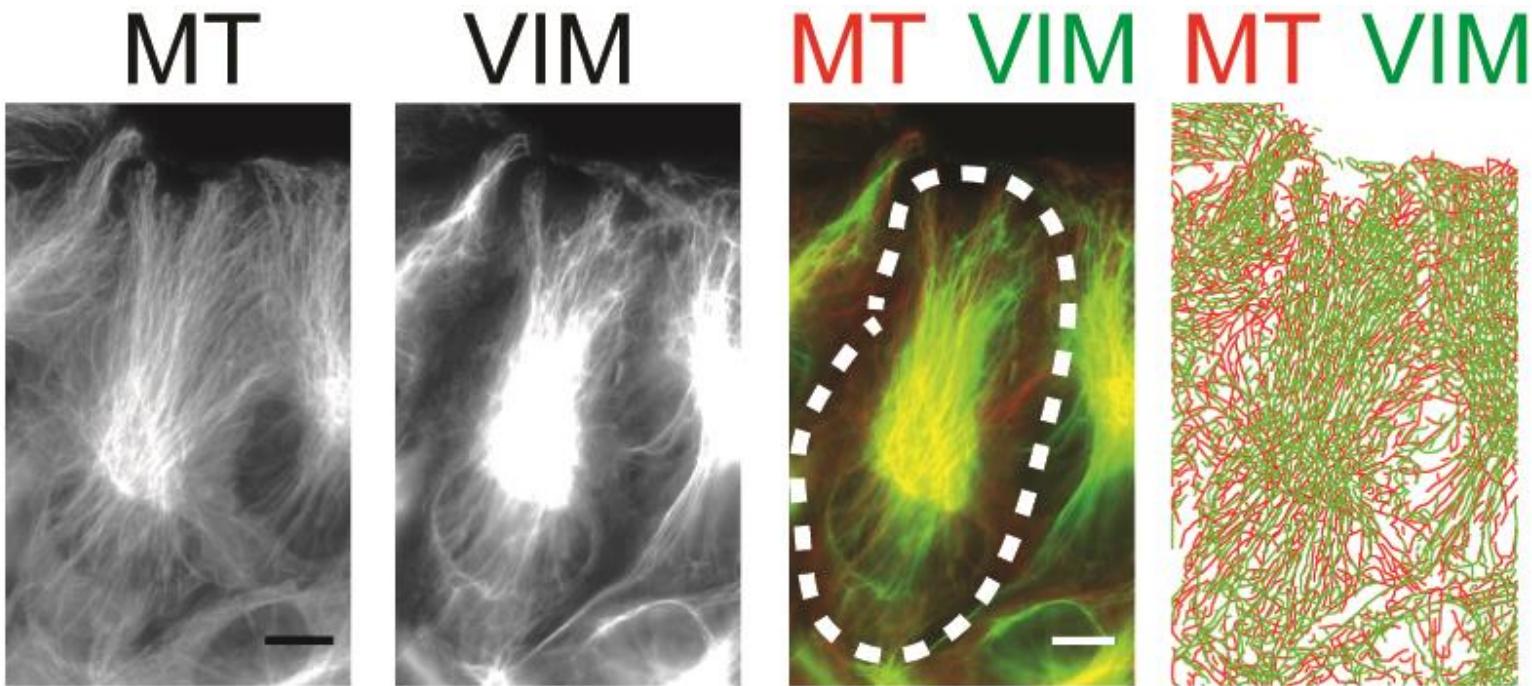
Genome-edited Retinal Pigment
Epithelial (RPE) cells



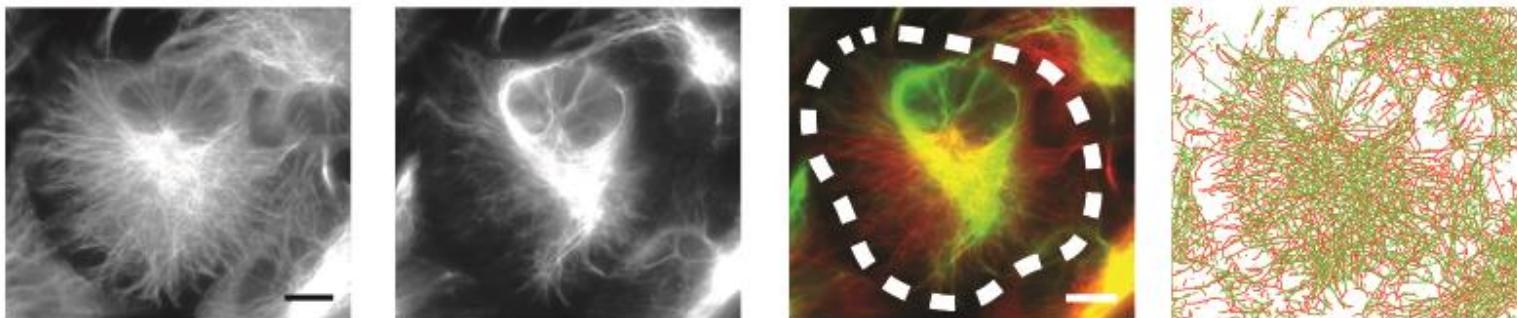
Gan, Ding and Burckhardt et al. (2016)

A relation between cell polarity and vimentin-microtubule interaction?

Front

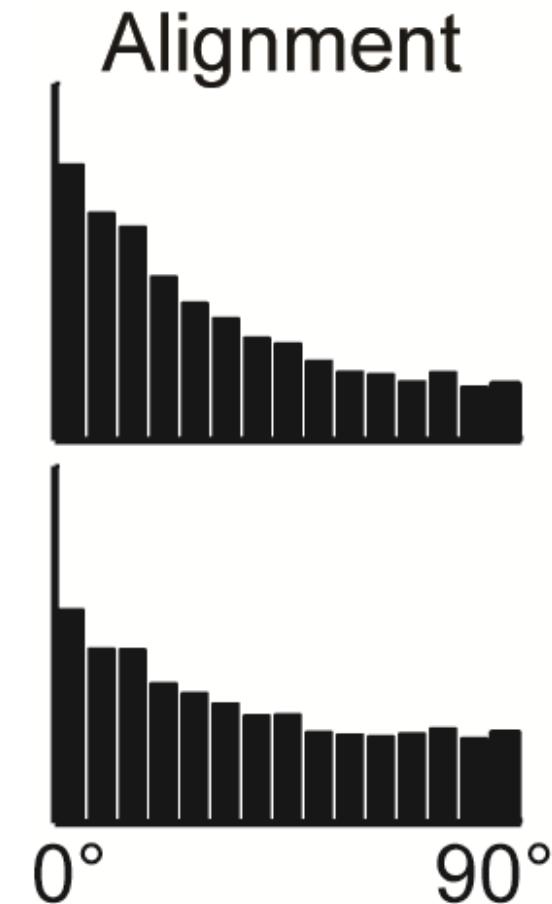
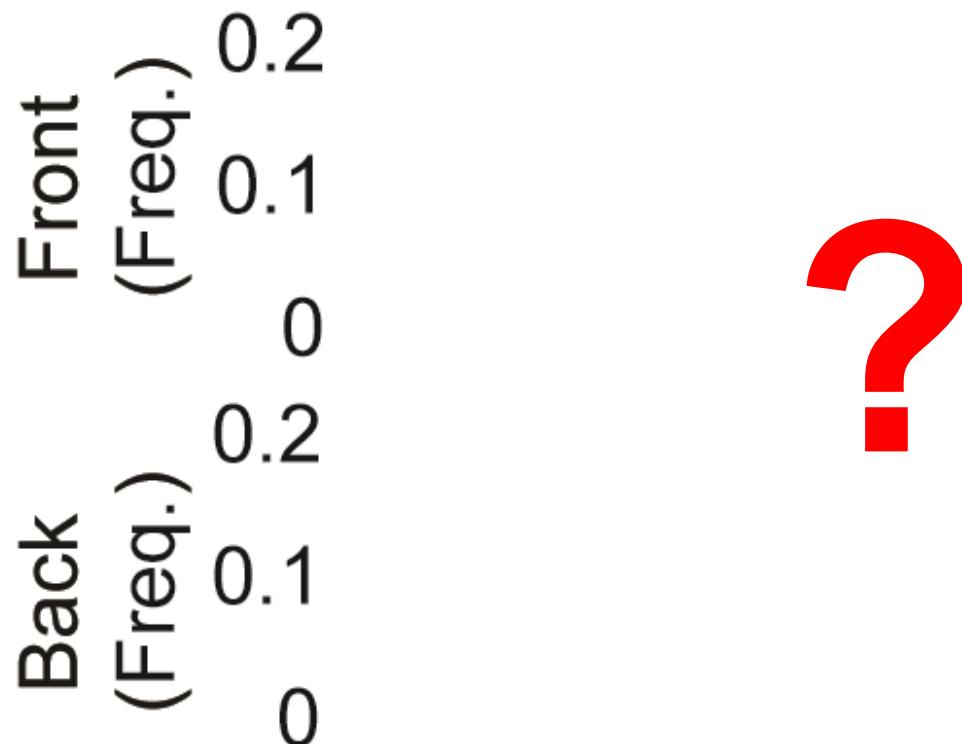


Back

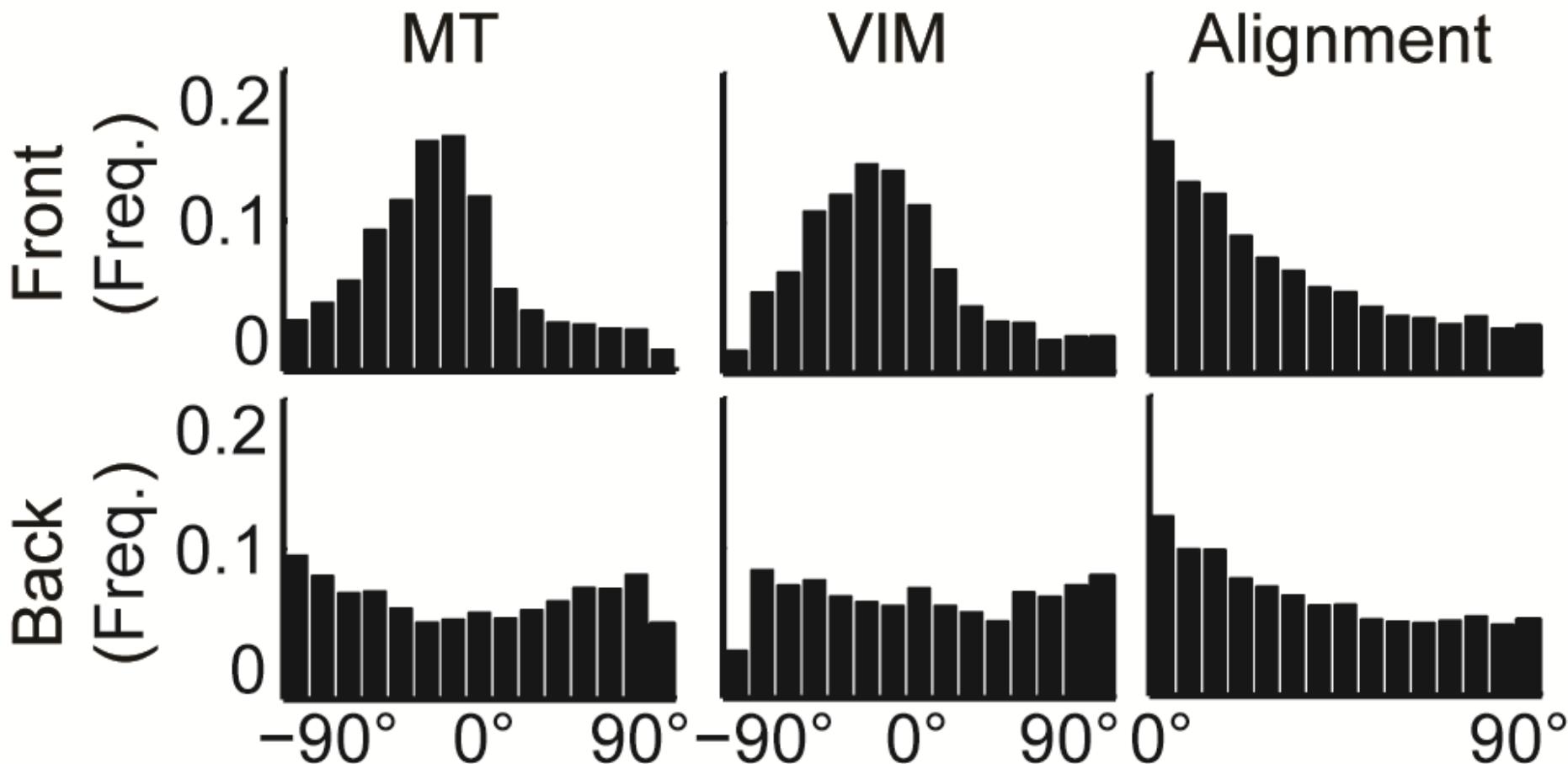


Zhuo Gan

Polarity-independent interaction of vimentin and microtubules



Polarity-independent interaction of vimentin and microtubules



What do we want to achieve?

- Simultaneous investigation of mechanisms that drive global bias and local interactions

How?

- By modeling the observed agreement between matched variables as the cumulative global and local components

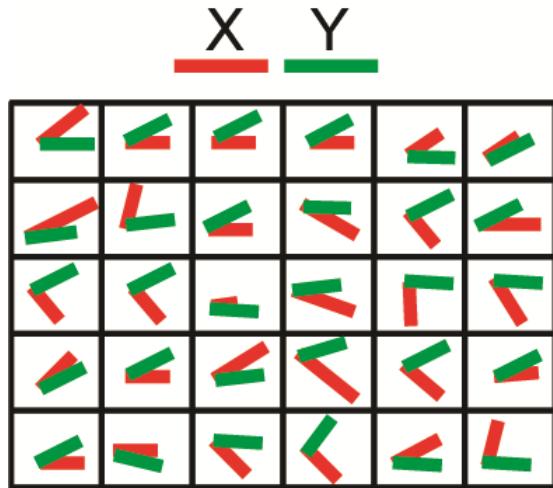
Observed
colocalization

$$= \text{Global bias} +$$

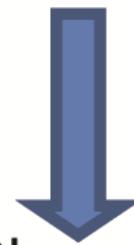
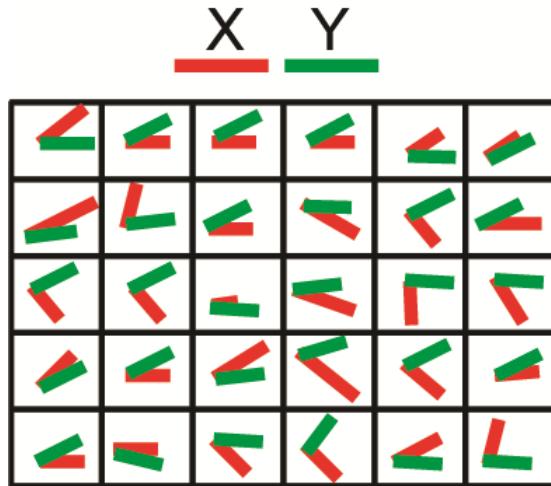
Local
interaction



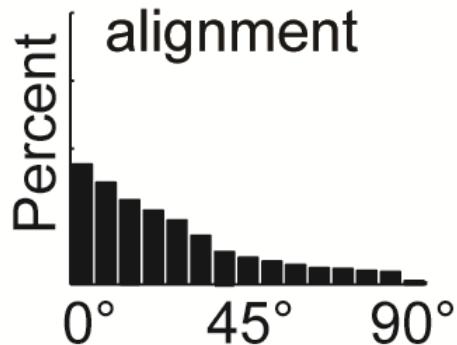
DeBias



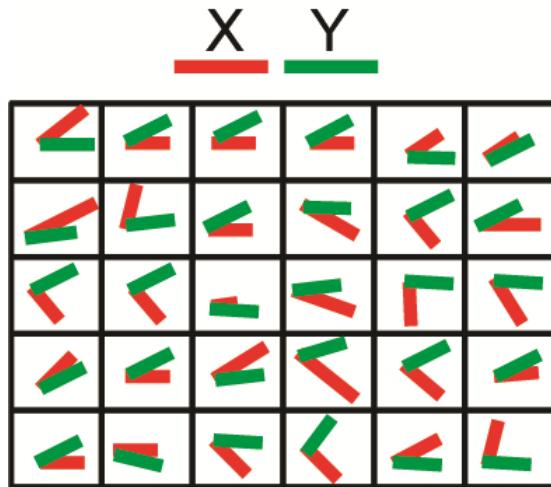
DeBias



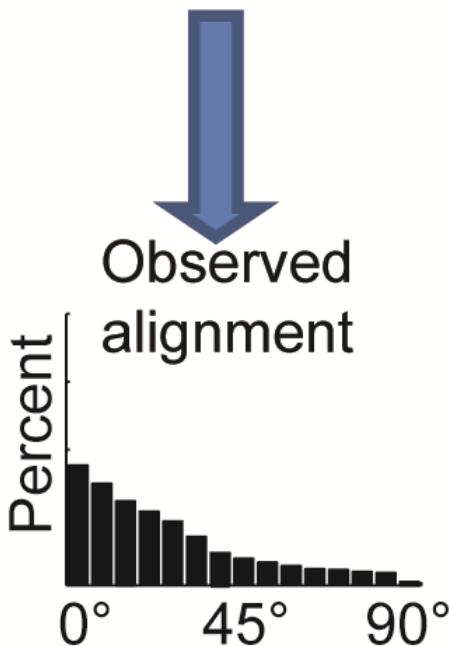
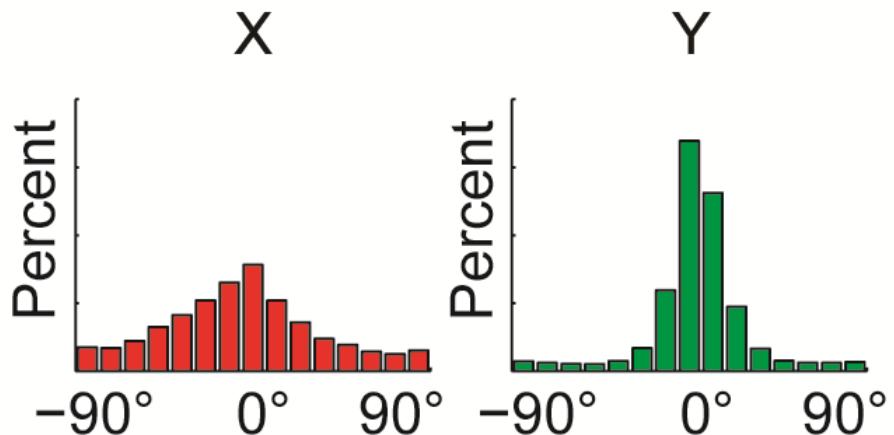
Observed
alignment



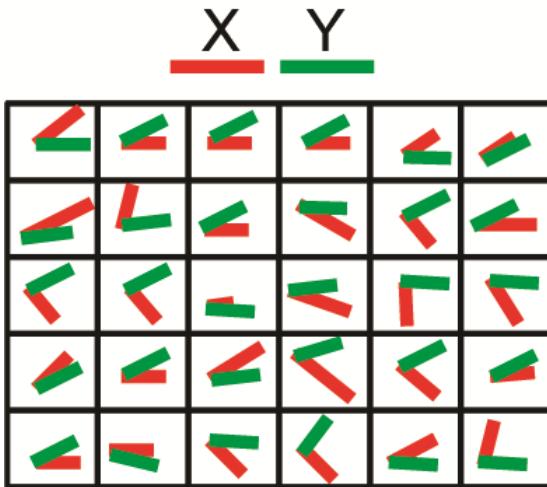
DeBias



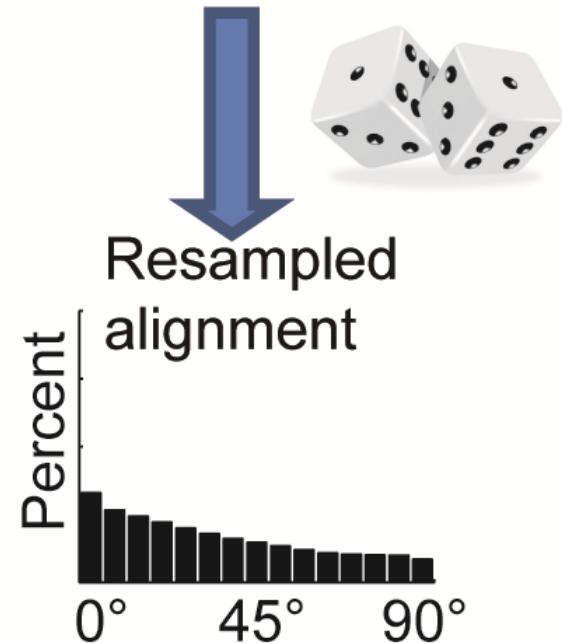
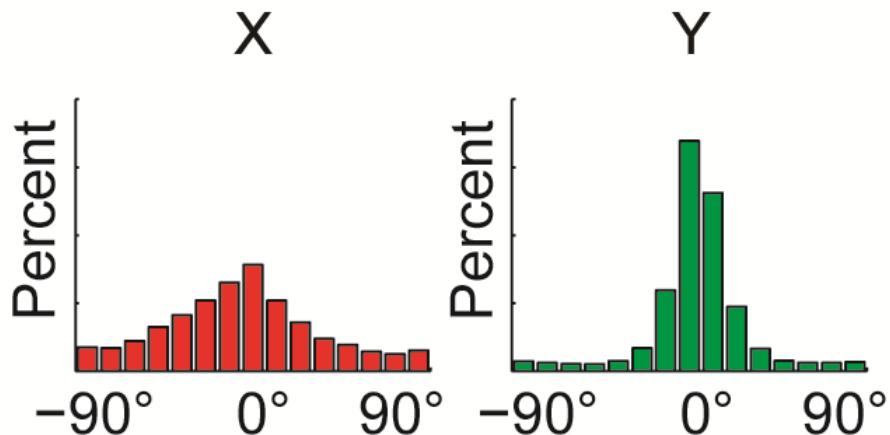
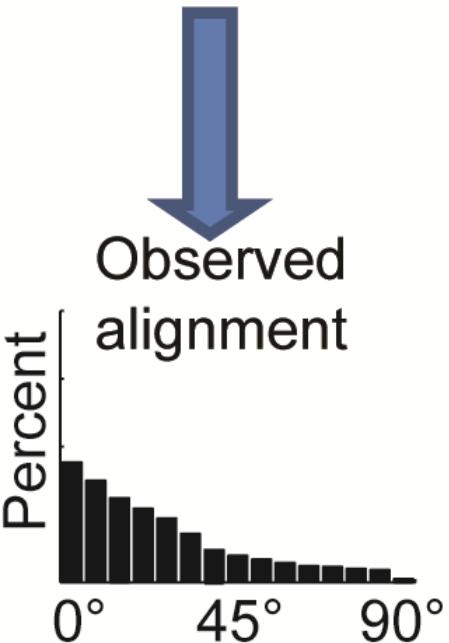
Decouple
pairs



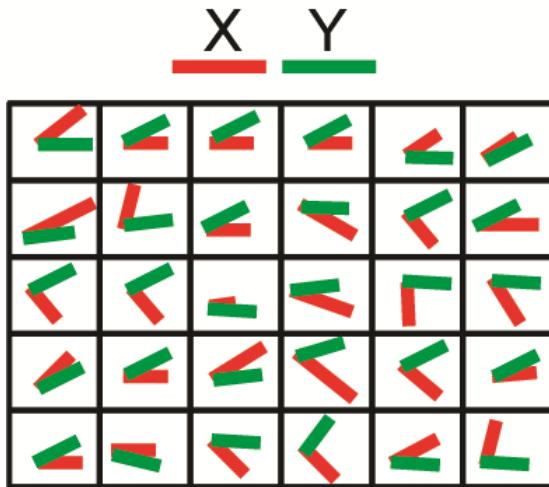
DeBias



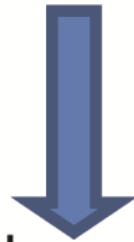
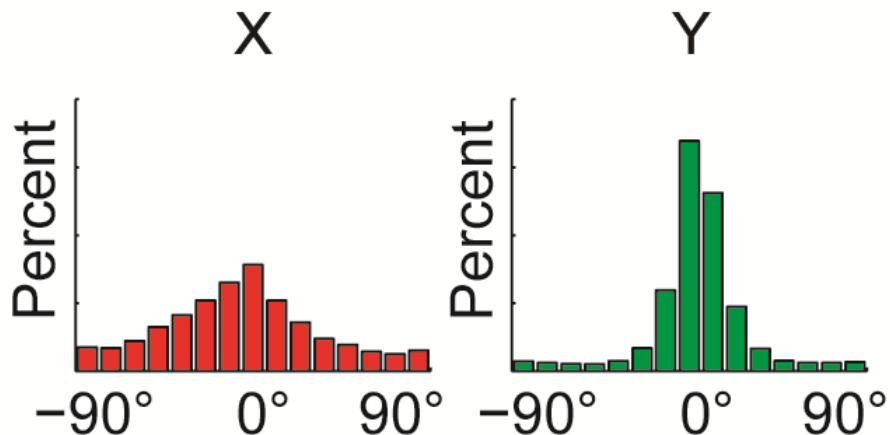
Decouple
pairs



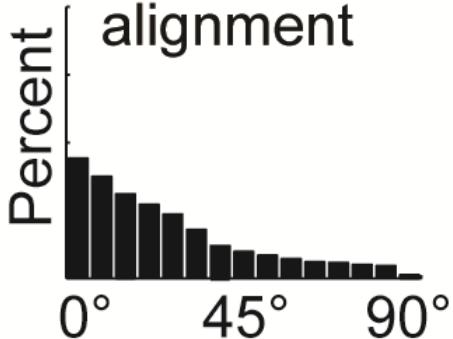
DeBias



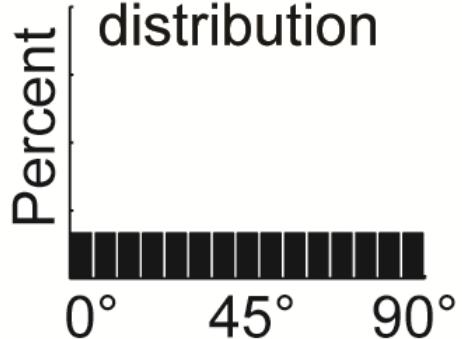
Decouple
pairs



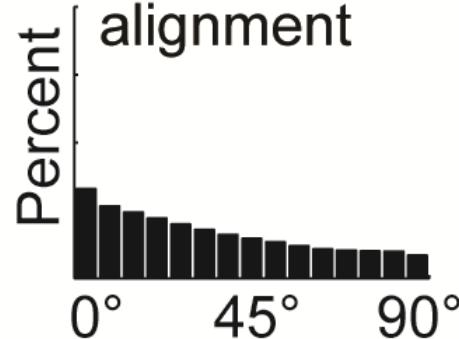
Observed
alignment



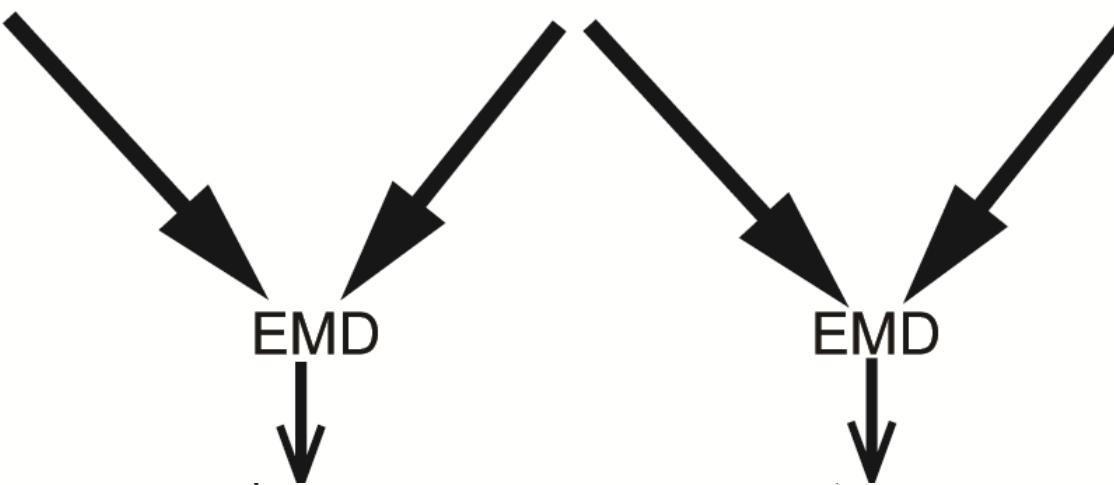
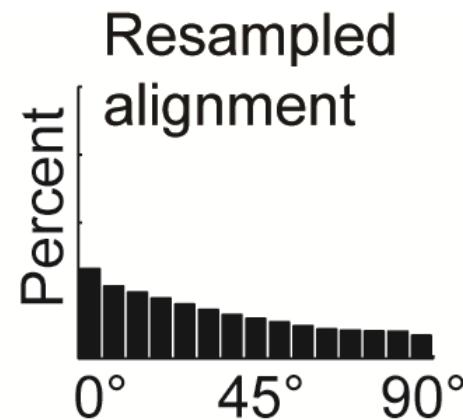
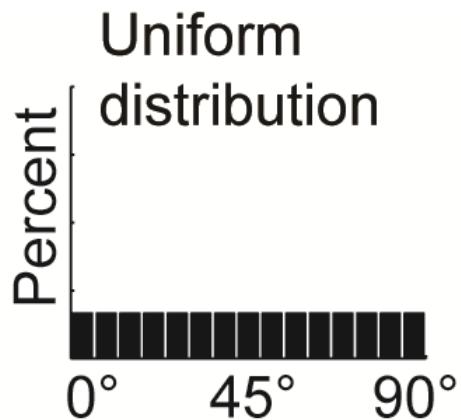
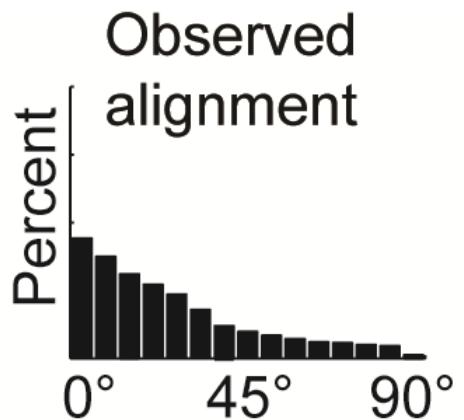
Uniform
distribution



Resampled
alignment



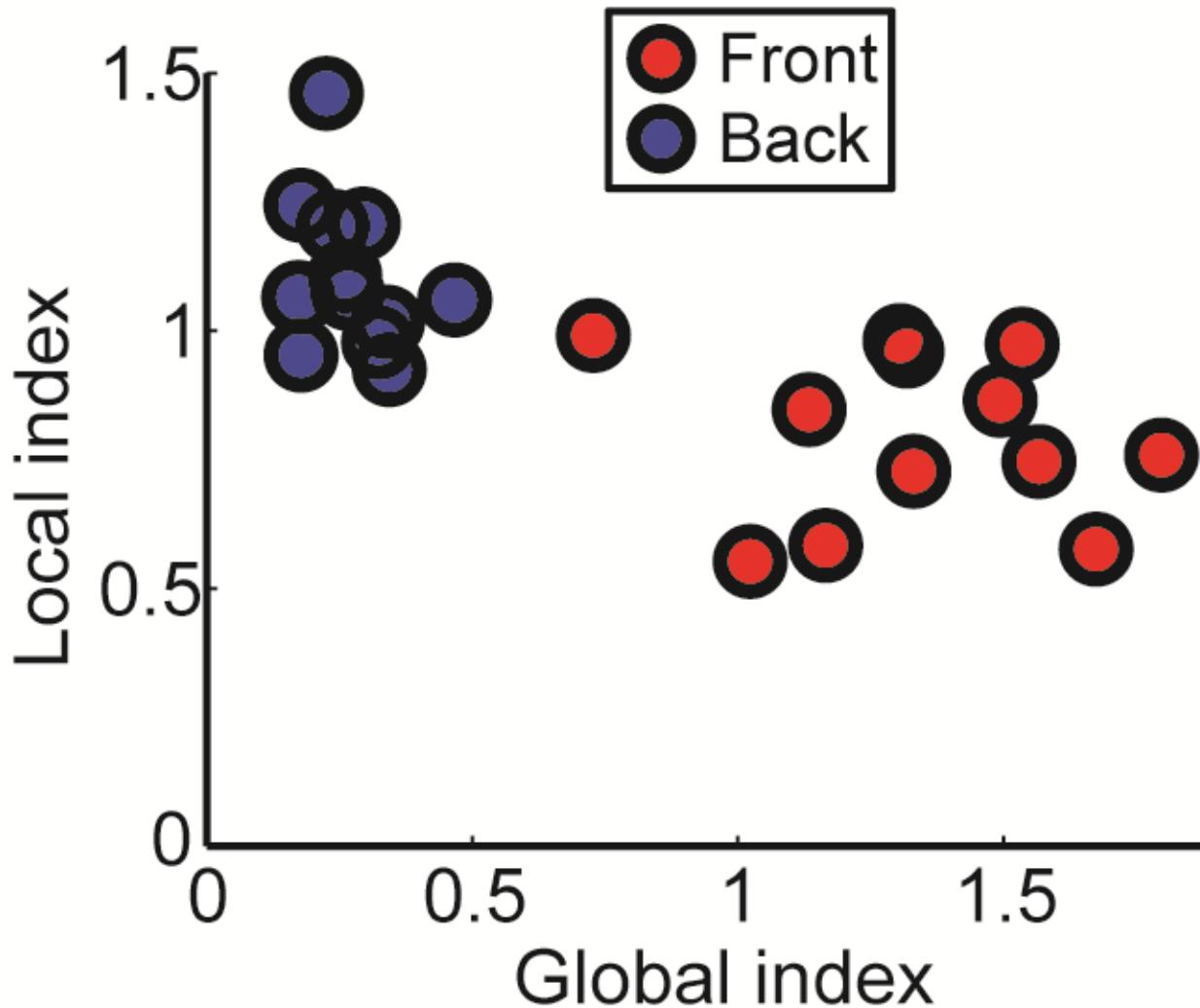
DeBias



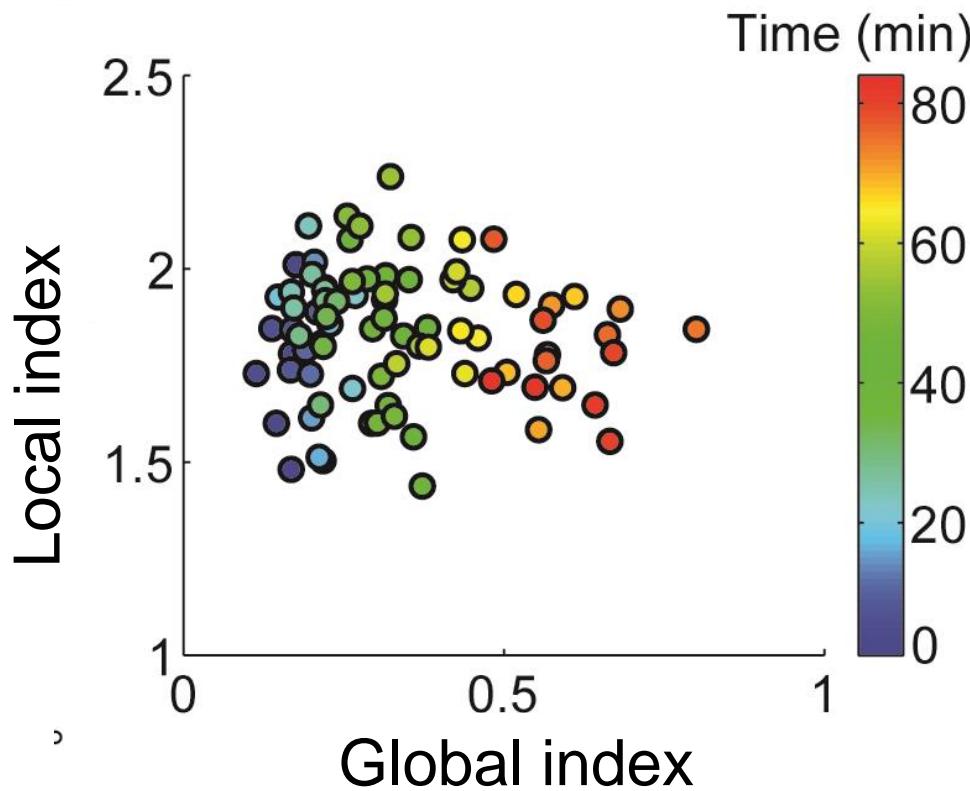
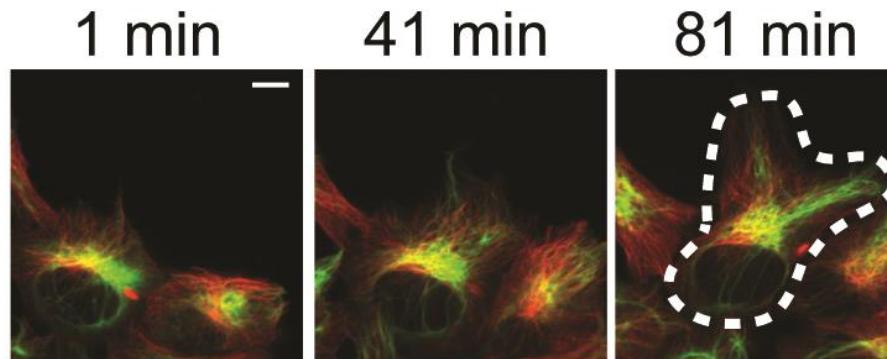
$$\text{EMD}(\text{observed}, \text{uniform}) - \text{EMD}(\text{resampled}, \text{uniform}) = \text{local index}$$

global index

Polarity-independent interaction of vimentin and microtubules



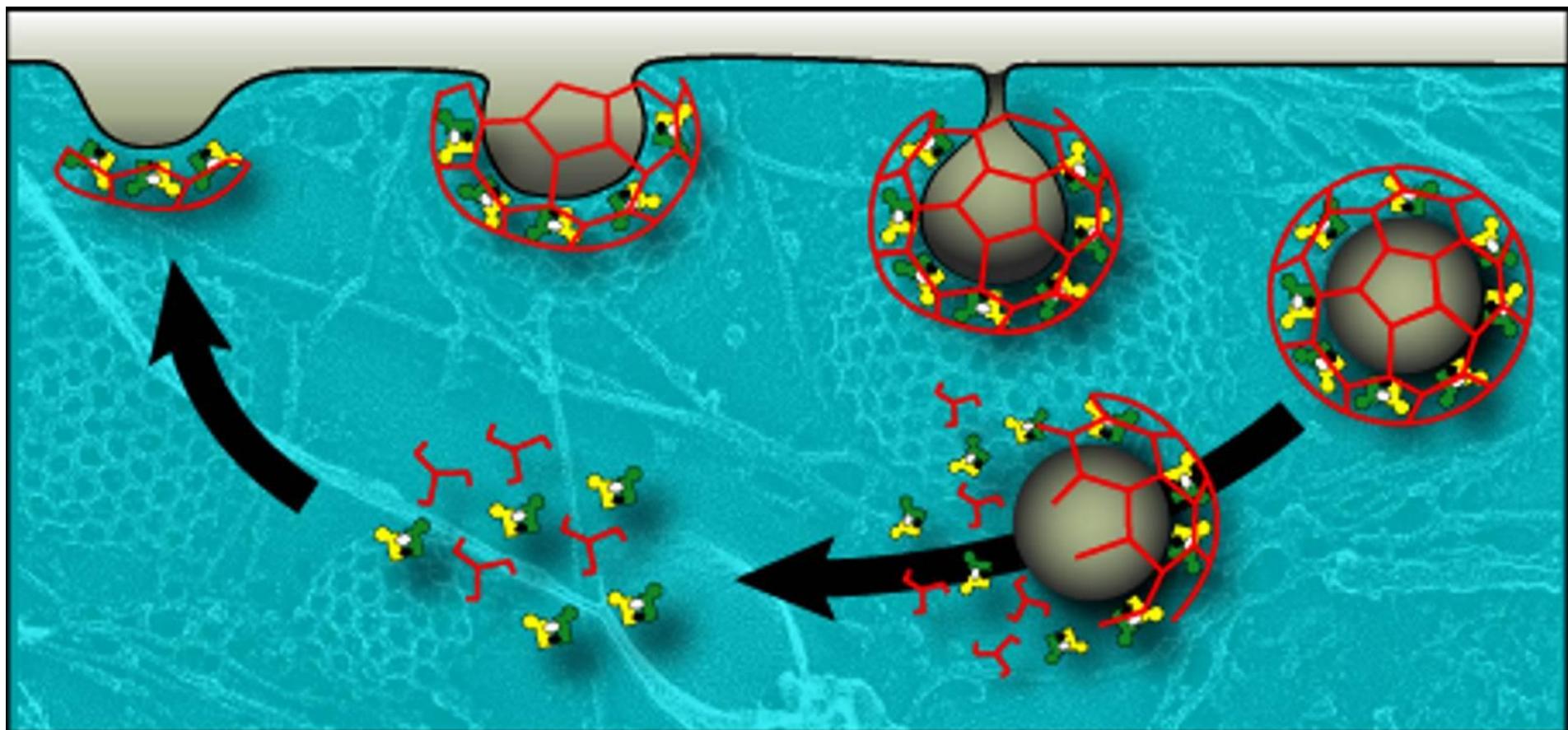
Polarity-independent interaction of vimentin and microtubules



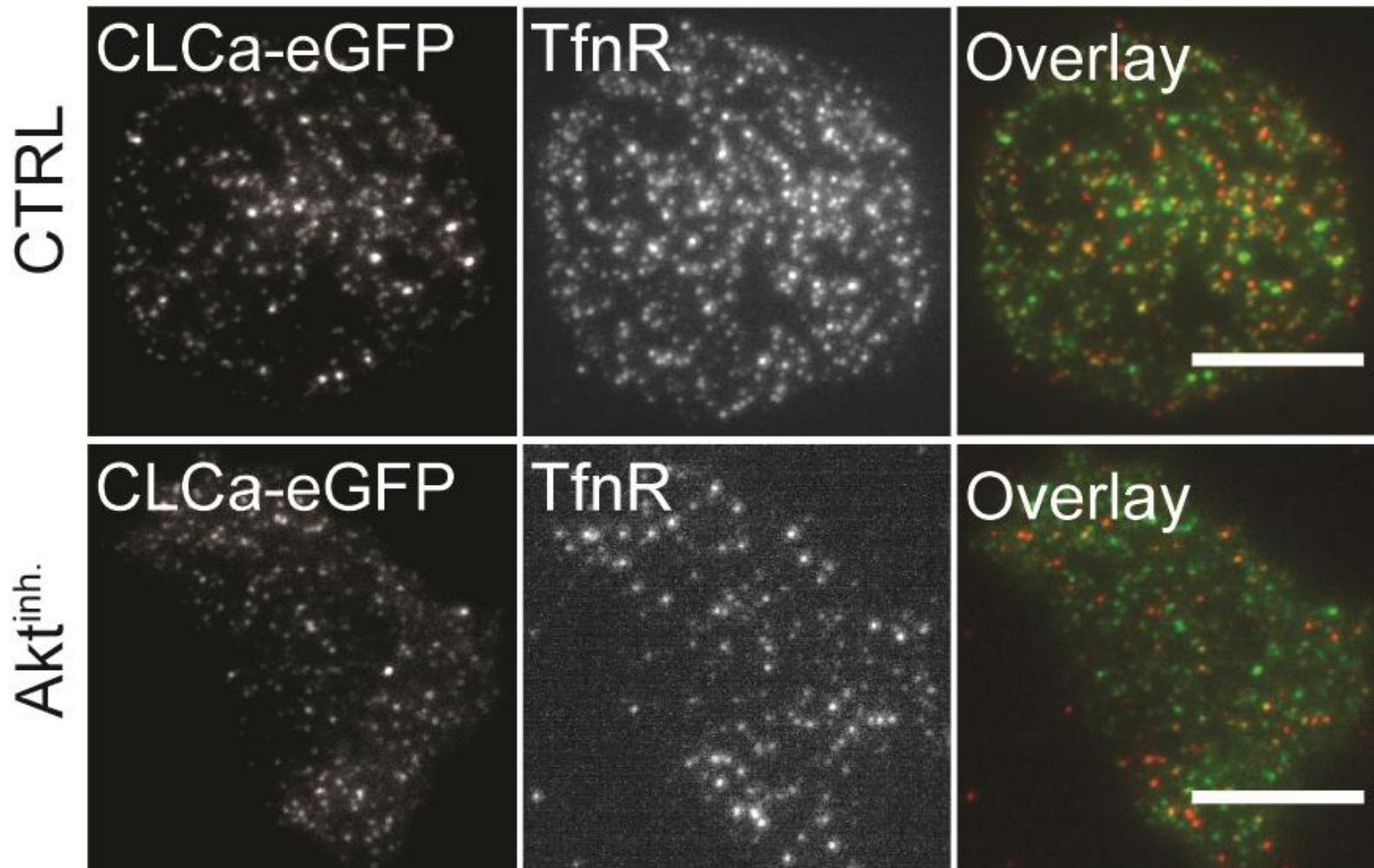
Global bias: cell polarity

Inferring co-localization and
predicting dynamics from fixed
cells during clathrin-mediated
endocytosis (CME)

Maturation of a clathrin coated pit

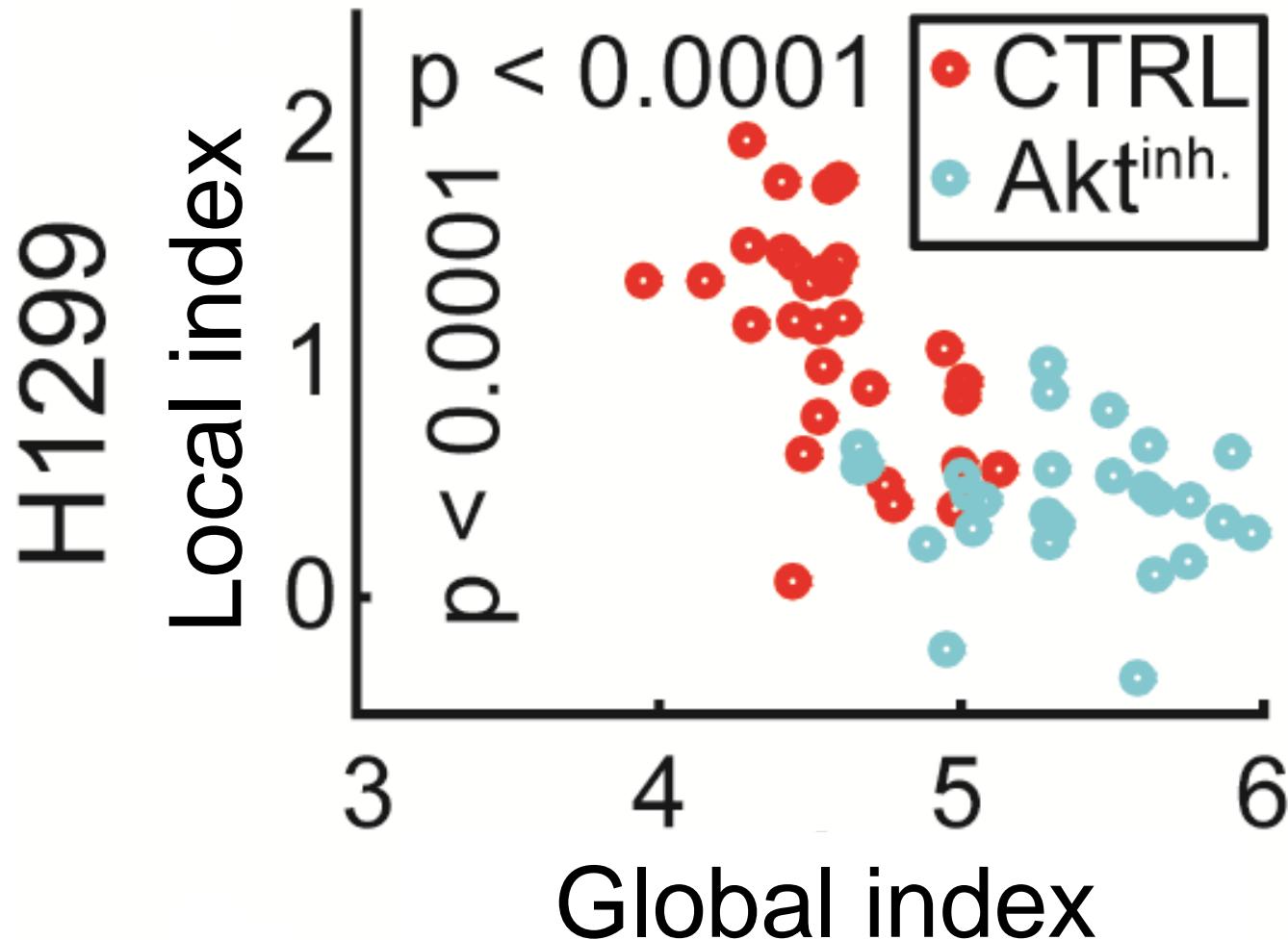


Cross-talk between signaling receptors (AKT) and components of the endocytic machinery

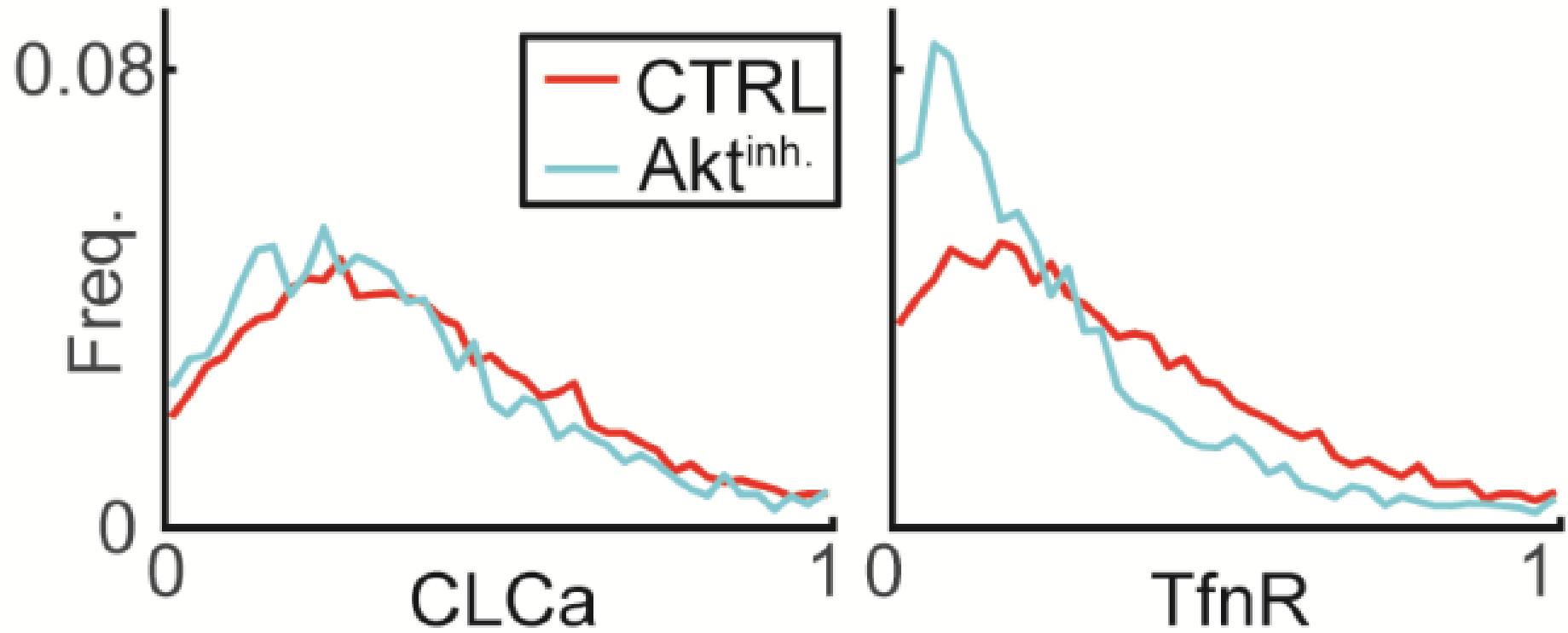


Carlos Reis, H1299 cells

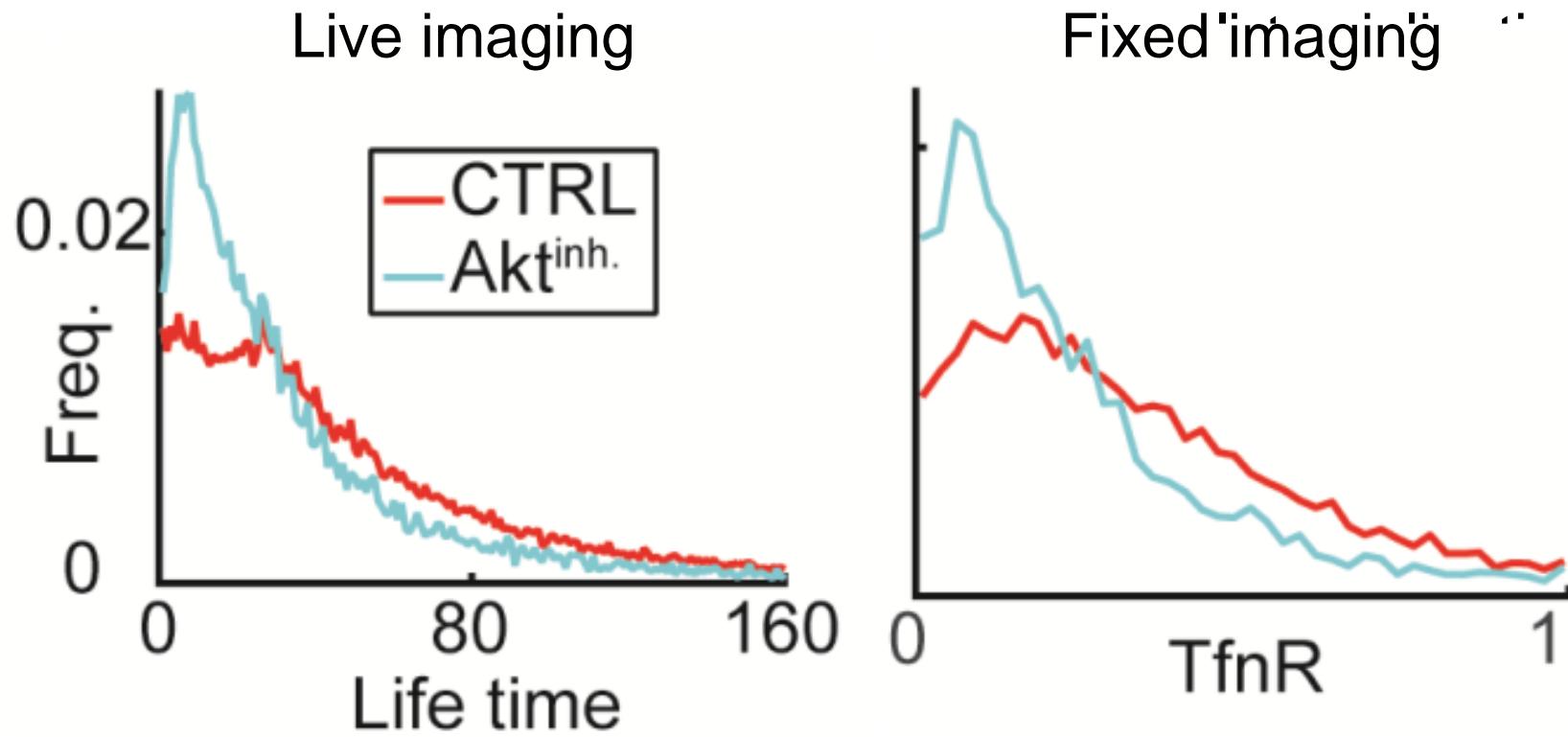
Inferring co-localization and predicting dynamics from fixed cells during clathrin-mediated endocytosis (CME)



Global bias: reduced TfnR in CCPs upon Akt inhibition

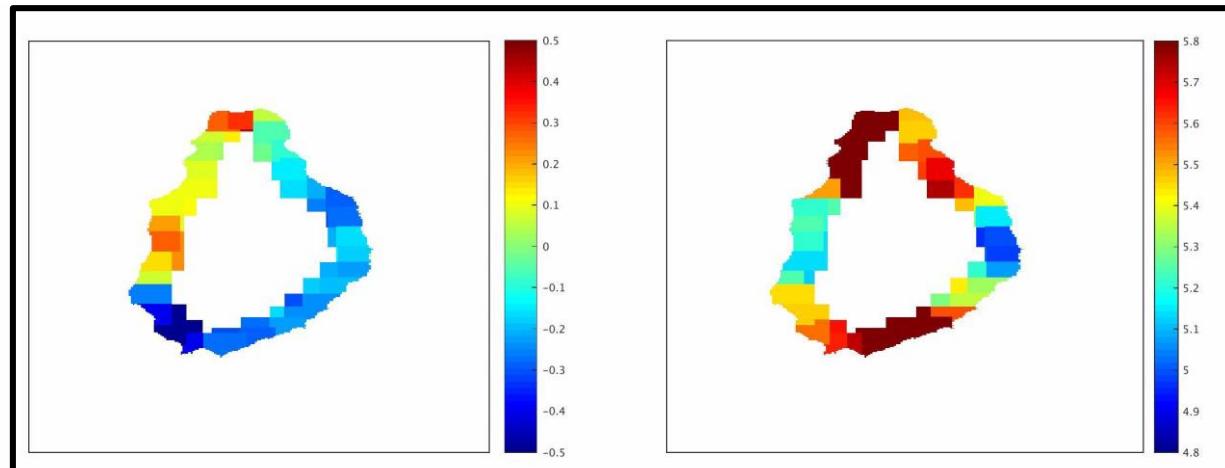
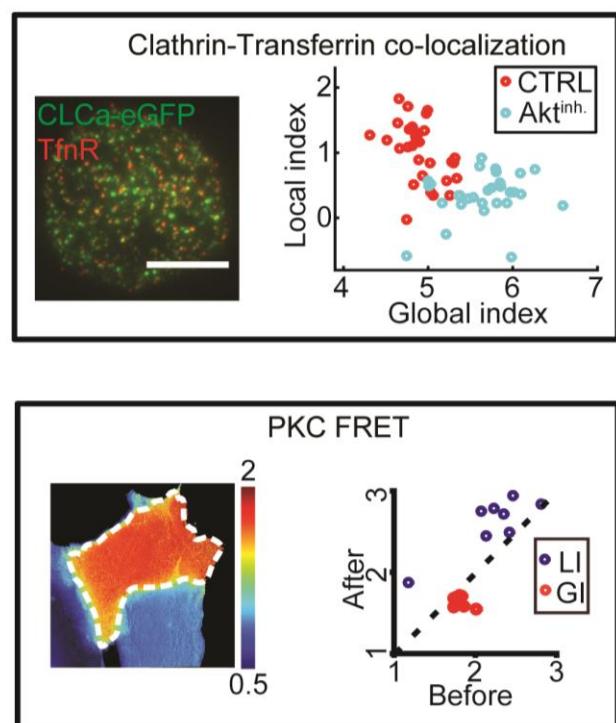
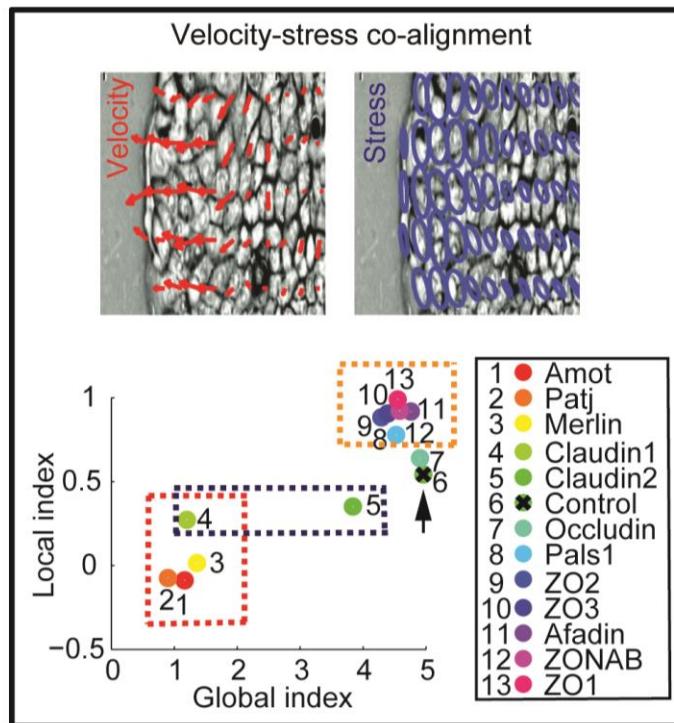
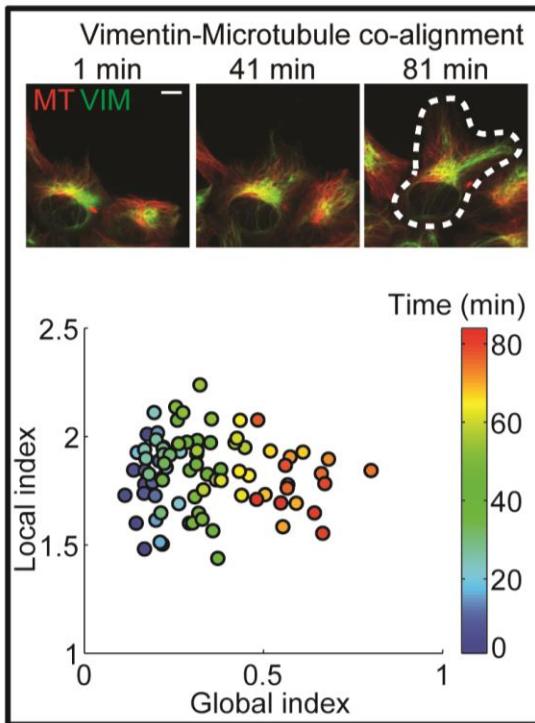


More CCPs containing less TfnR alter CCPs dynamics upon AKT inhibition



Reduced TfnR in CCPs upon Akt inhibition increased short-lived, (most likely) abortive events → decrease in CME efficiency

Global bias: more CCPs with less
TfnR upon Akt inhibition



Spatio-temporal co-localization of Rac1 and Vav1 activity in a migrating cell (With Dan Marston, UNC)

References, resources

References:

- Zaritsky et al. Decoupling global biases and local interactions between cell biological variables (2017)
<https://elifesciences.org/content/6/e22323>

Webserver:

- <https://debias.biohpc.swmed.edu/>

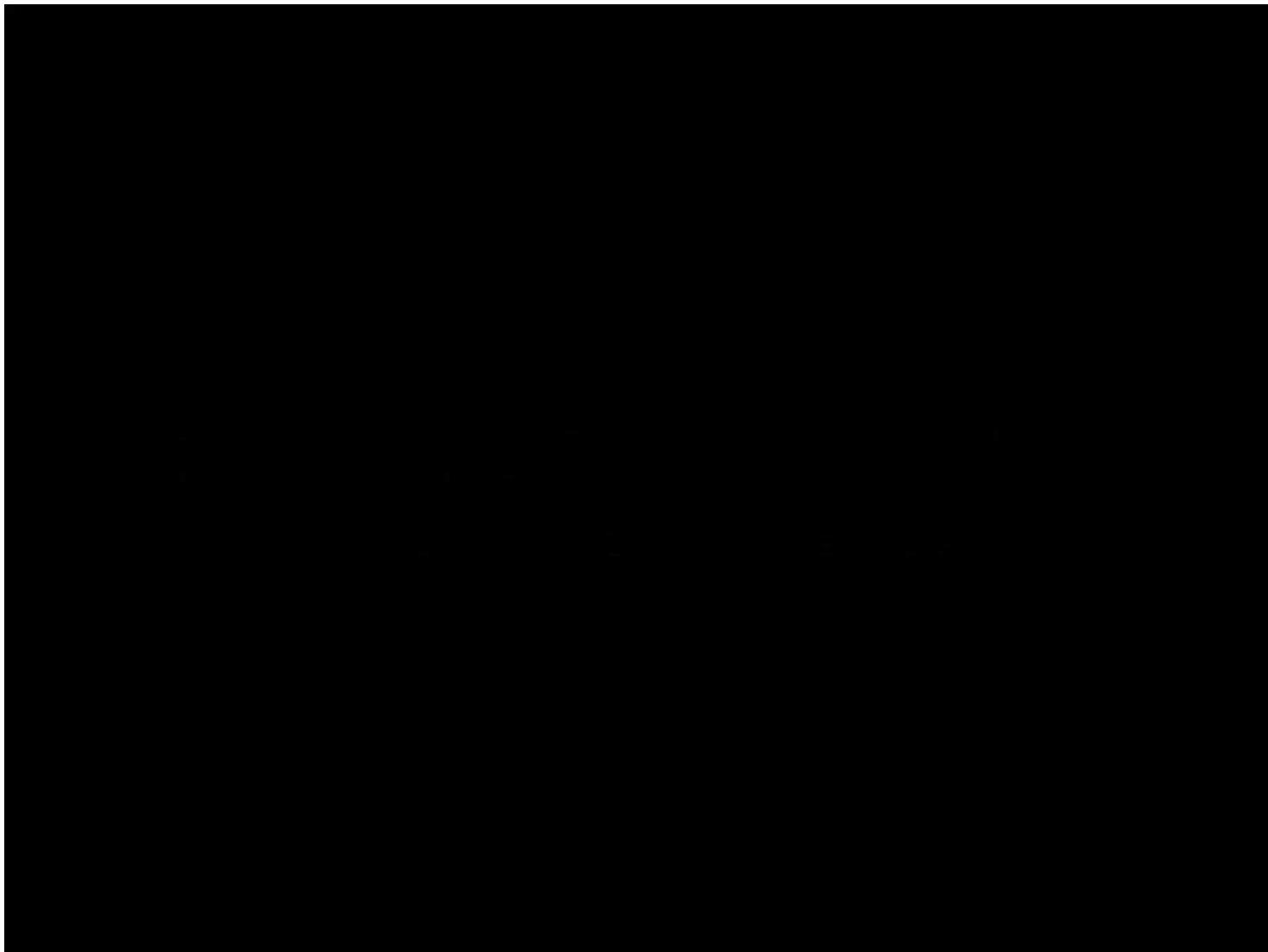
Source code:

- <https://github.com/DanuserLab/DeBias>

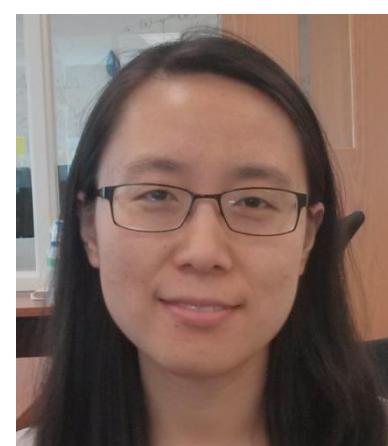


Take home message

DeBias enables identifying the gorilla



Acknowledgments



 **Uri
Obolski,
(Theory)**

**Carlos
Reis
(Endocytosis)**

**Zhuo
Gan,
(Vimentin, PKC)**

**Yi
Du
(Webserver)**

Tamal Das Joachim Spatz



Liqiang Wang



Liya Ding



Christoph Burckhardt



**Sandy
Schmid**



**Gaudenz
Danuser**

Thank you!



DeBias

<https://elifesciences.org/content/6/e22323>