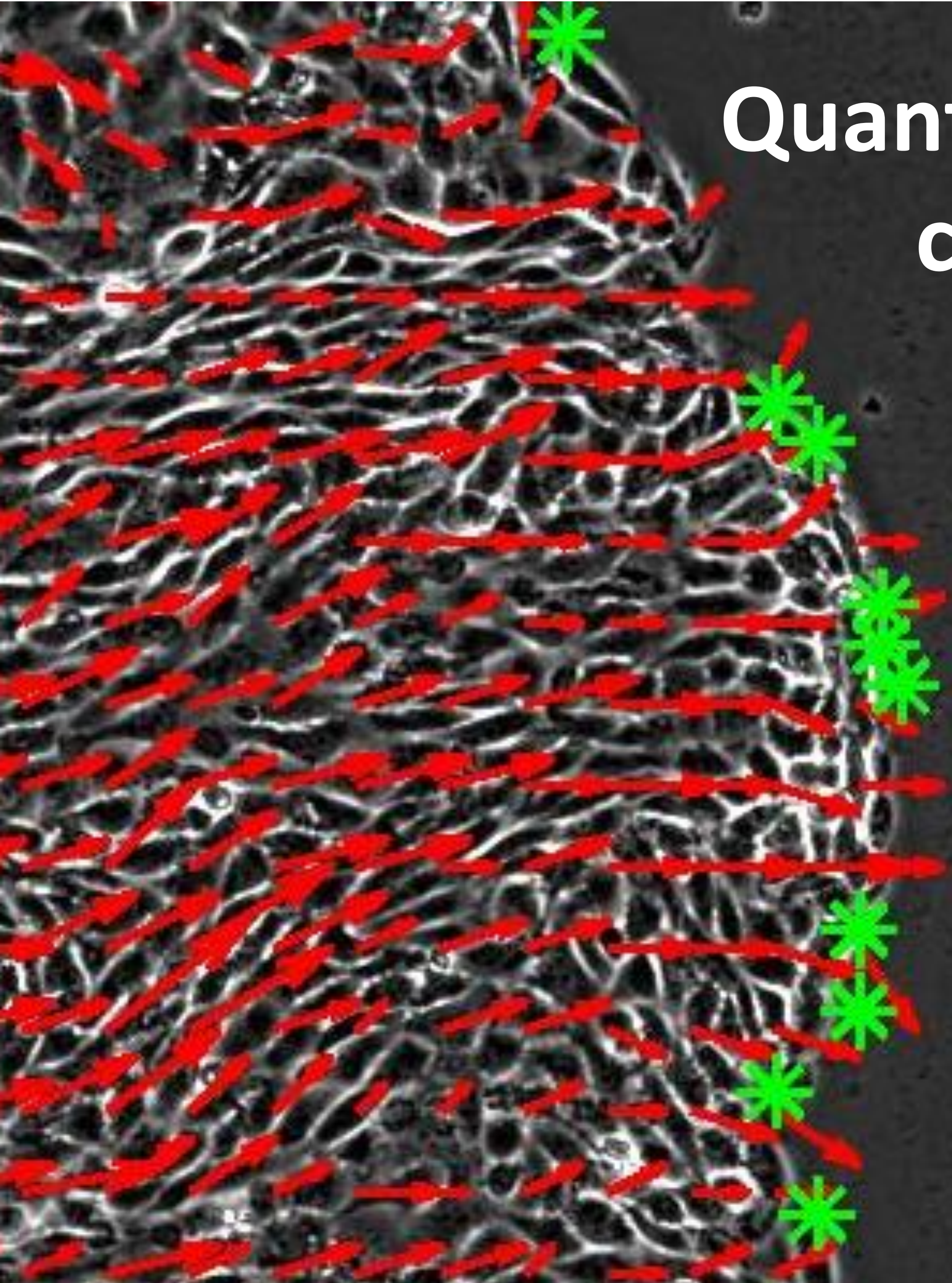


Quantifying monolayer cell migration

Assaf Zaritsky

UTSW, WIS

January 29th, 2018

Training school for Bioimage Analysts

Szeged, Hungary

Today’s goals

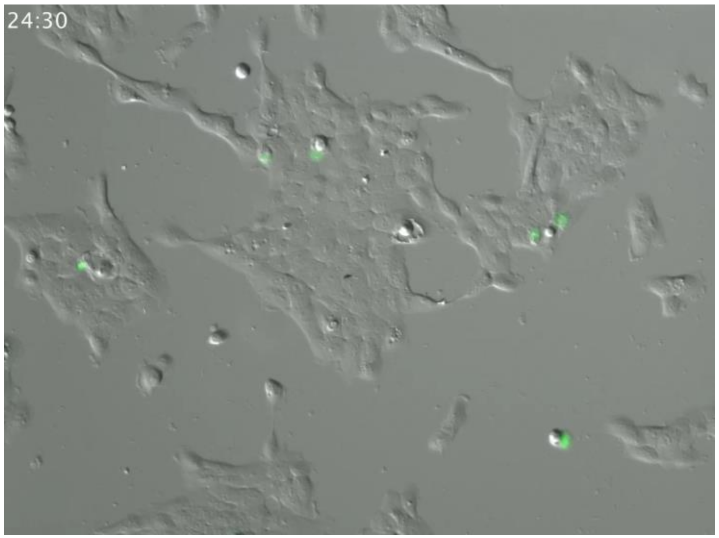
- Tools:
  - Identifying clusters of coordinated motion in flow-fields
  - Quantifying spatiotemporal dynamics of wound healing (“scratch” assay)
- Ideas on:
  - Phenotypic screening
  - Extracting new information from “old” data

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)

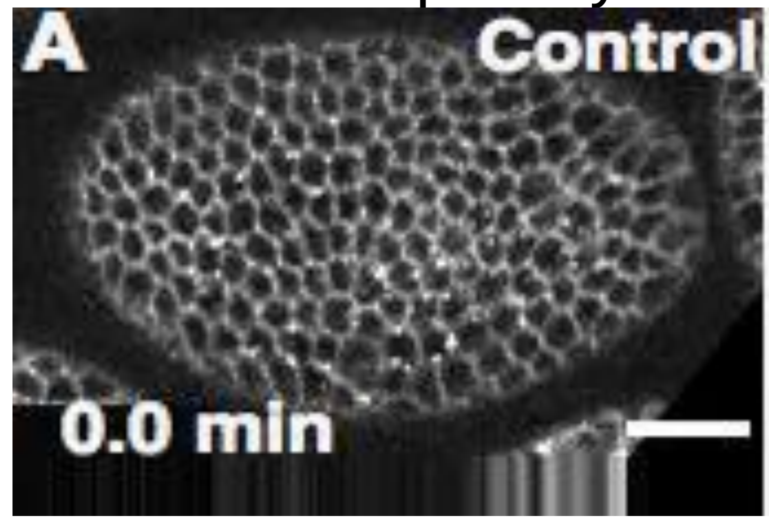
Emergence of collective cell behavior from single cell action and cell-cell communication

Collective cell death



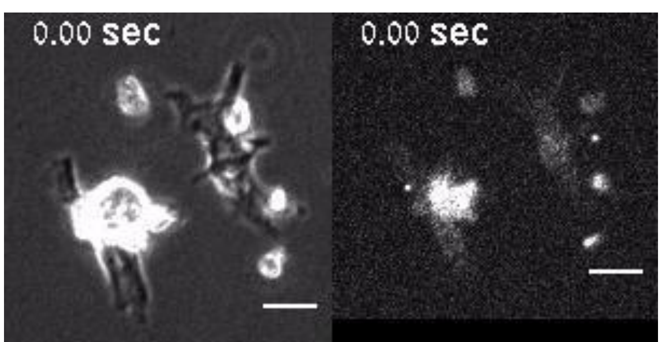
Overholtzer lab

Planar cell polarity



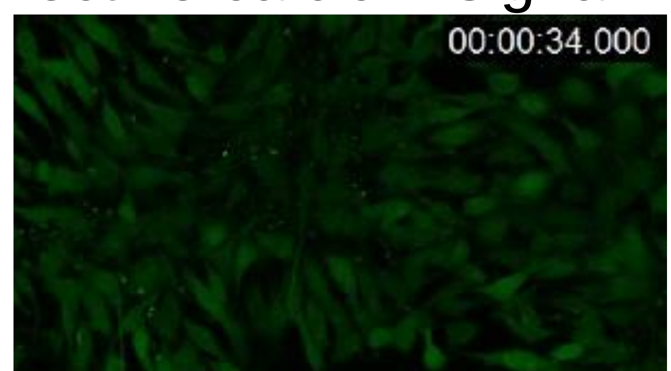
Barlan et al. (2017)

Synchronized cardiac cells



Nitsan et al. (2016)

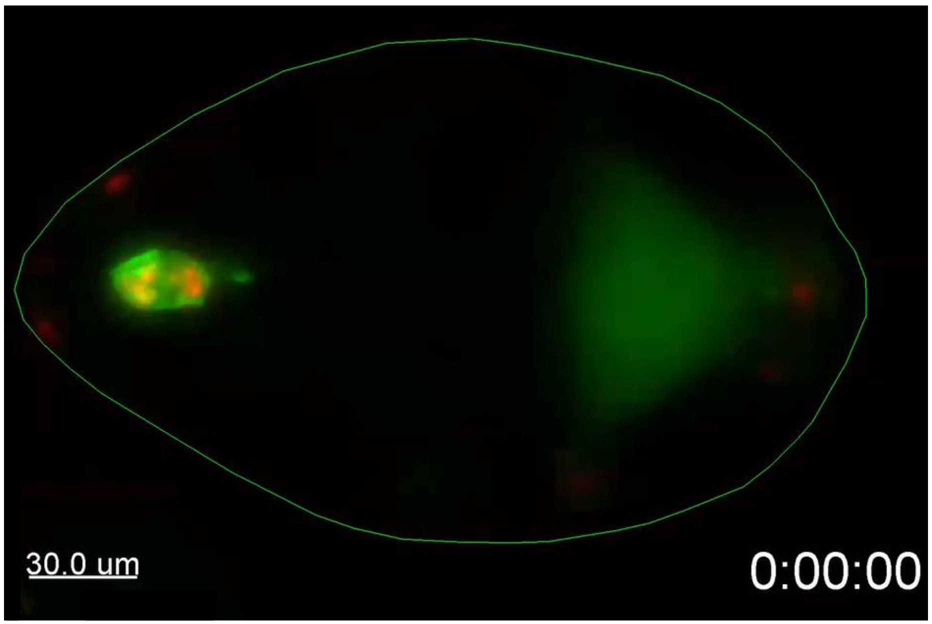
Collective calcium signaling



Sun et al. (2012)

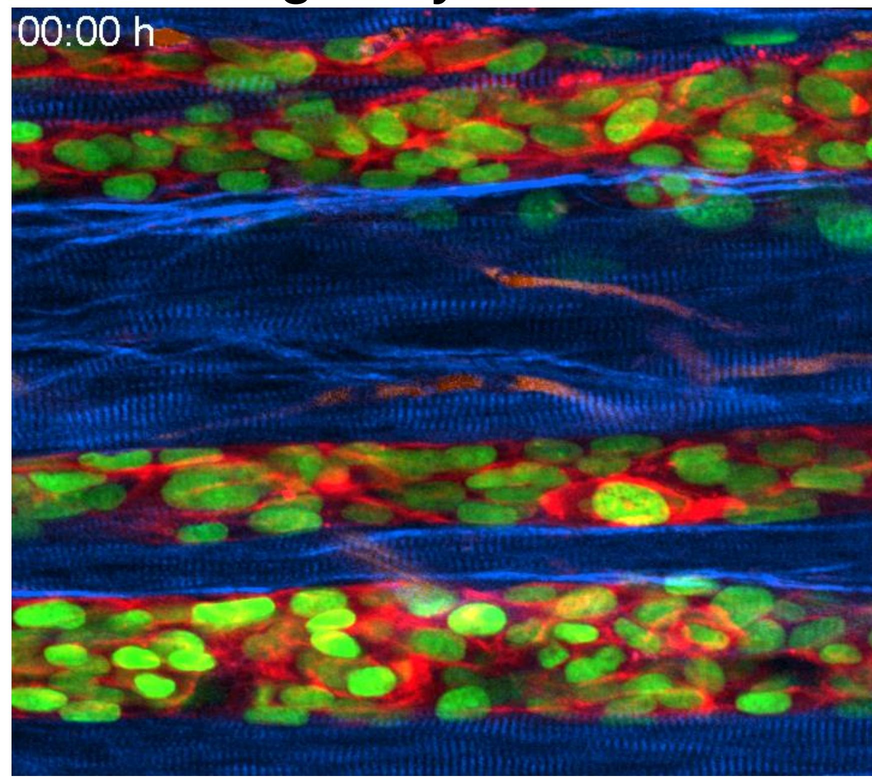
Collective cell migration

Border cells migration,
Drosophila Oogenesis



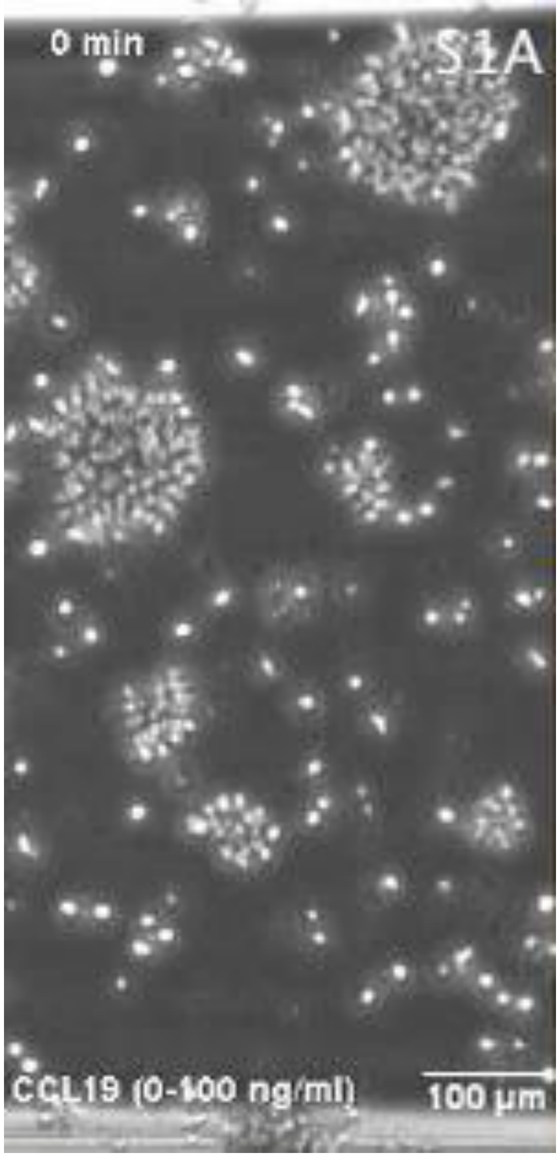
Cai et al. (2014)

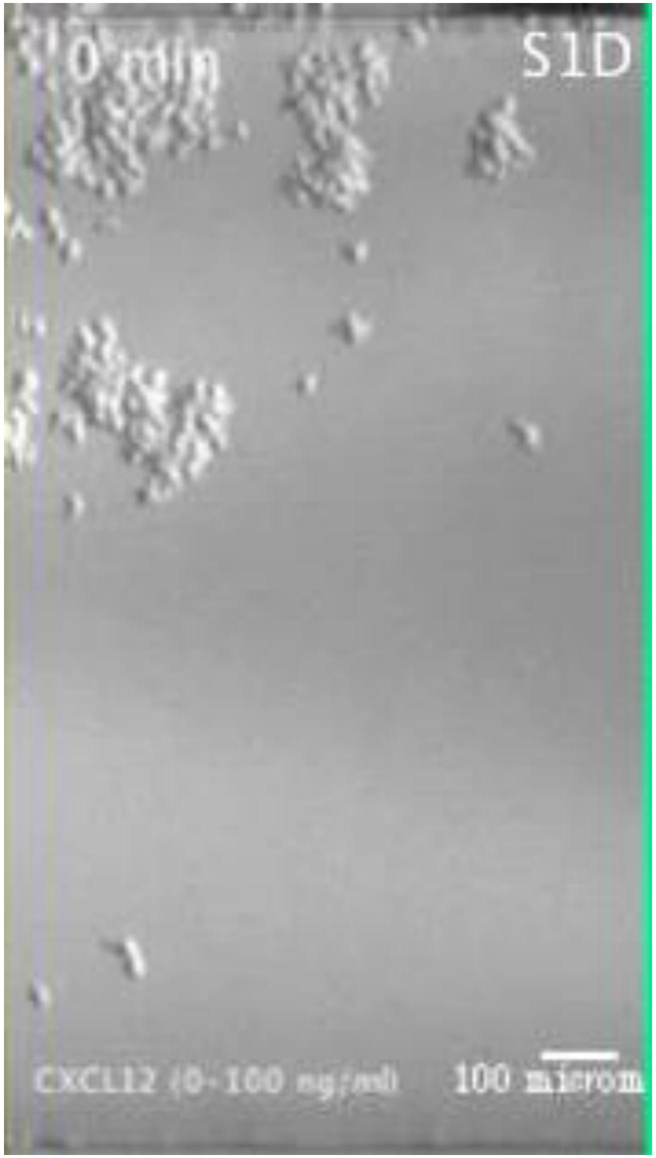
Collective tumor migration
on “highways” in vivo



Bettina Weigelin, Peter Friedl

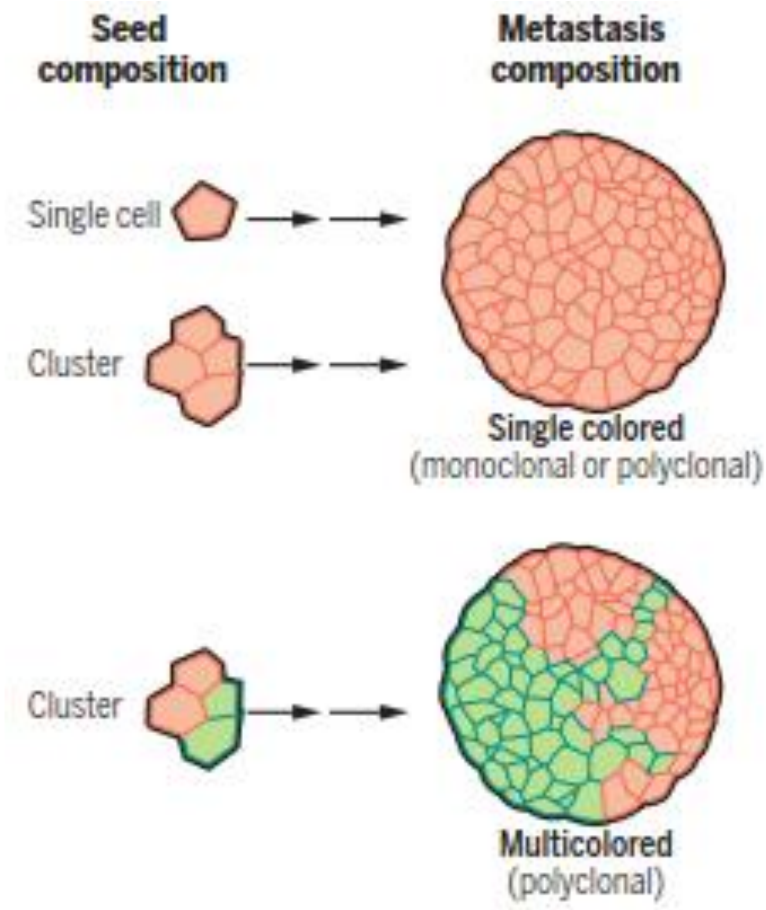
Cells migrate more efficiently in groups than individually

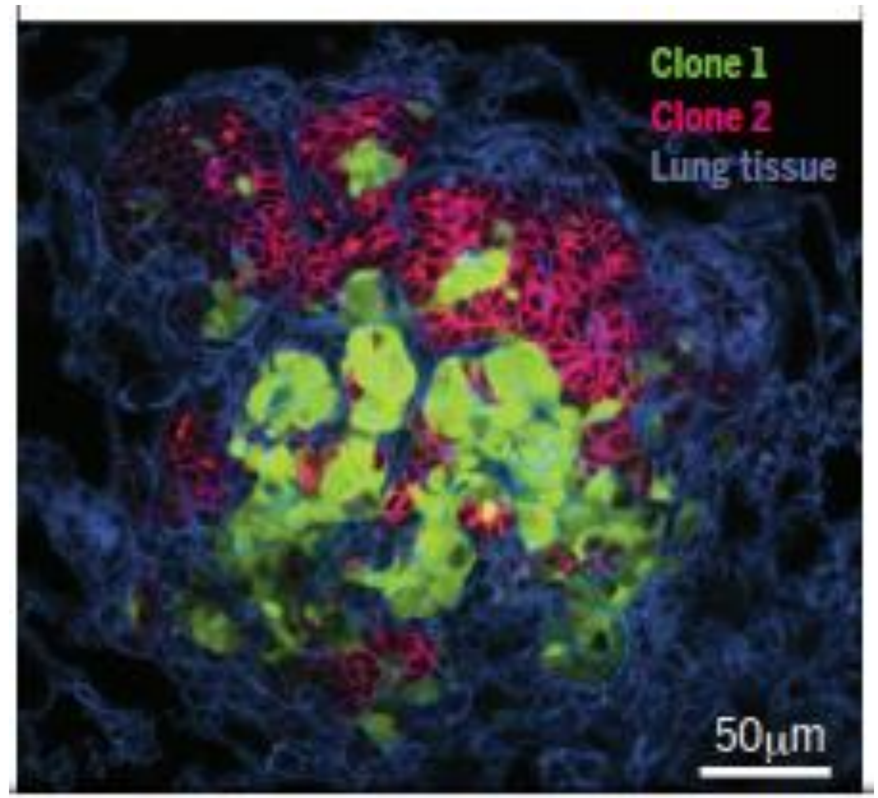




Malet-Engra et al. (2015)

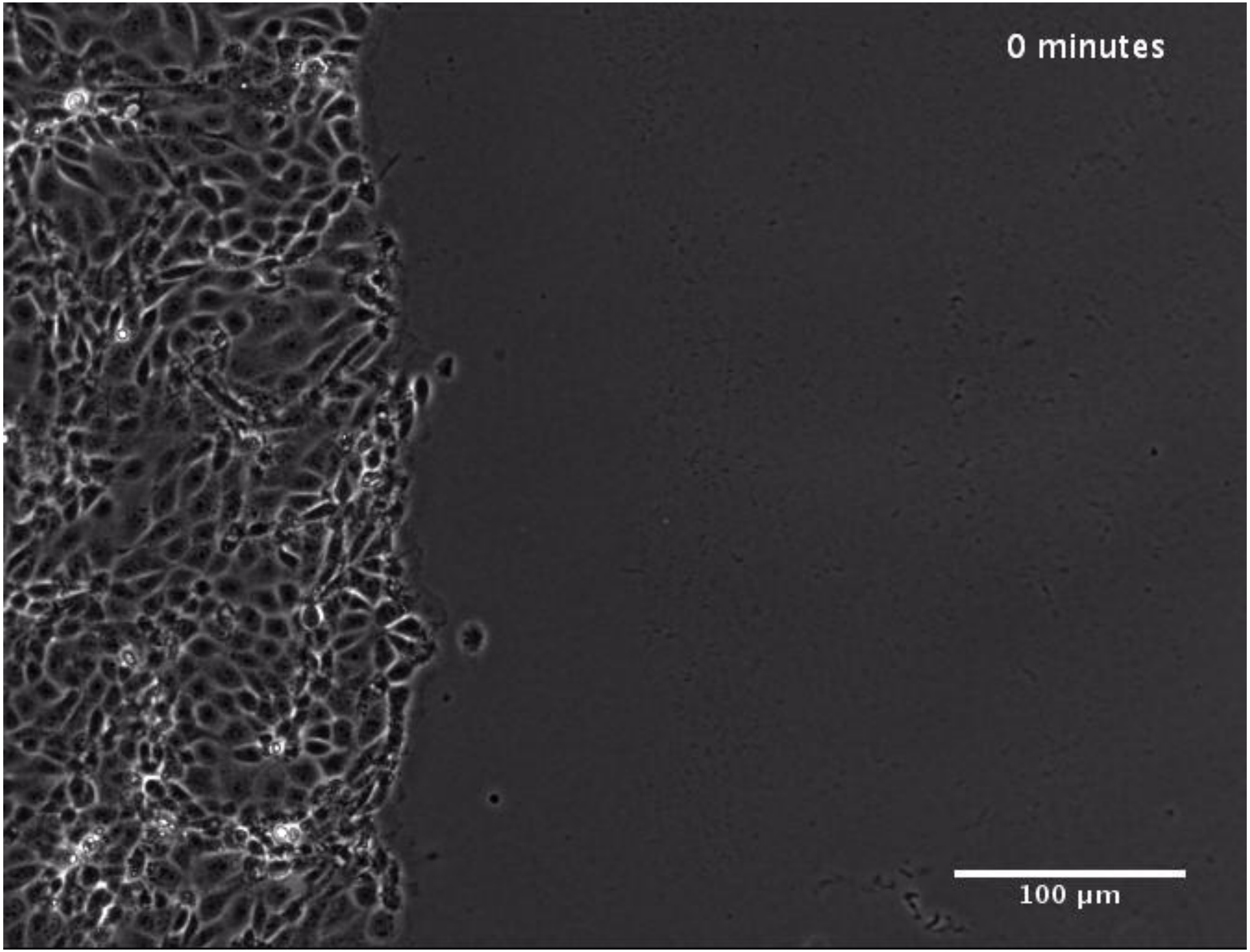
Tumor cell clusters as precursors of metastasis



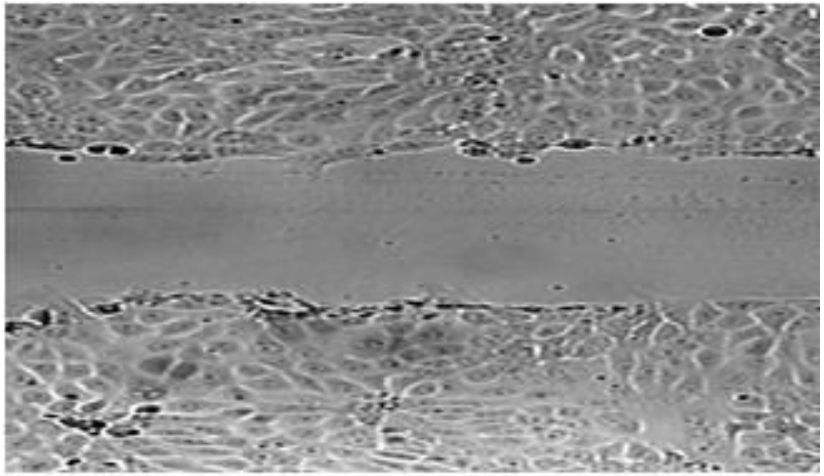


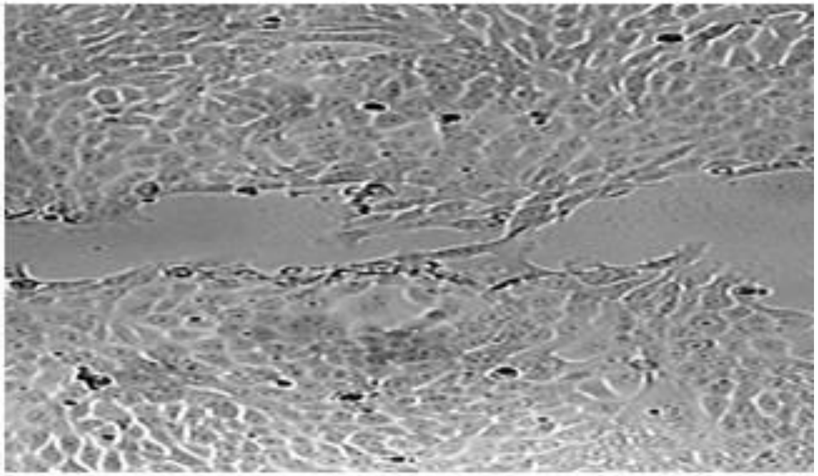
Cheung et al. (2016)
Aceto et al. (2014)

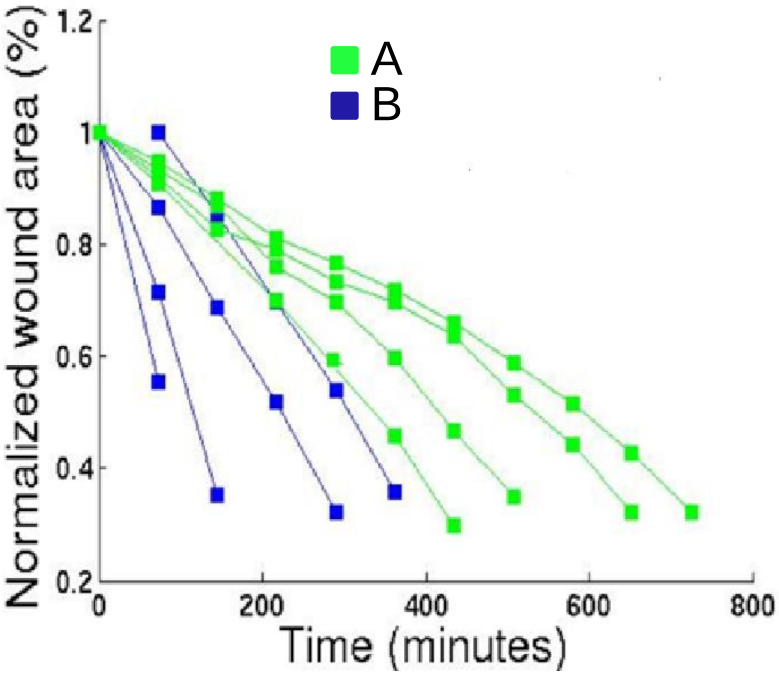
A simple model system to study collective cell migration

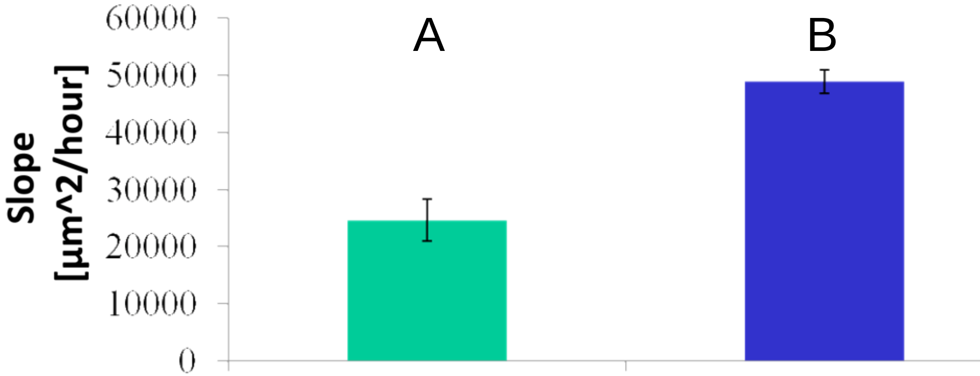


Standard quantification: monolayer migration rate









Tools available

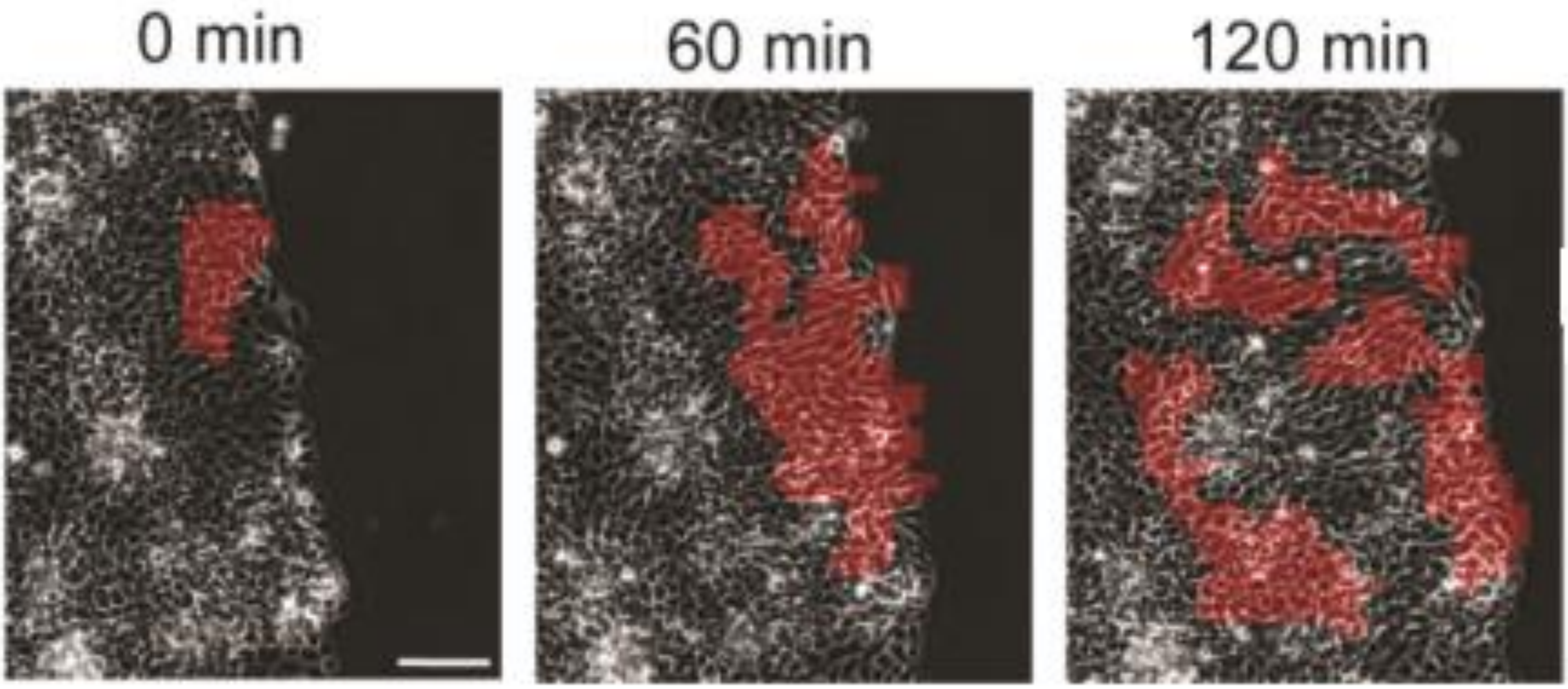
OpenLab	PerkinElmer[6]	Mac/A	2D/3D FL	MSExcel, comma-separated values	Wound-healing measurements, percentage of wound closure
AveMap	[102][8]	MATLAB; Windows, Mac/A	2D PC	Tab-delimited text	Wound-healing measurements: local velocities, monolayer edges, wound area, wound shape, productive velocities
Cell 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

Masuzzo et al. (2016)

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)

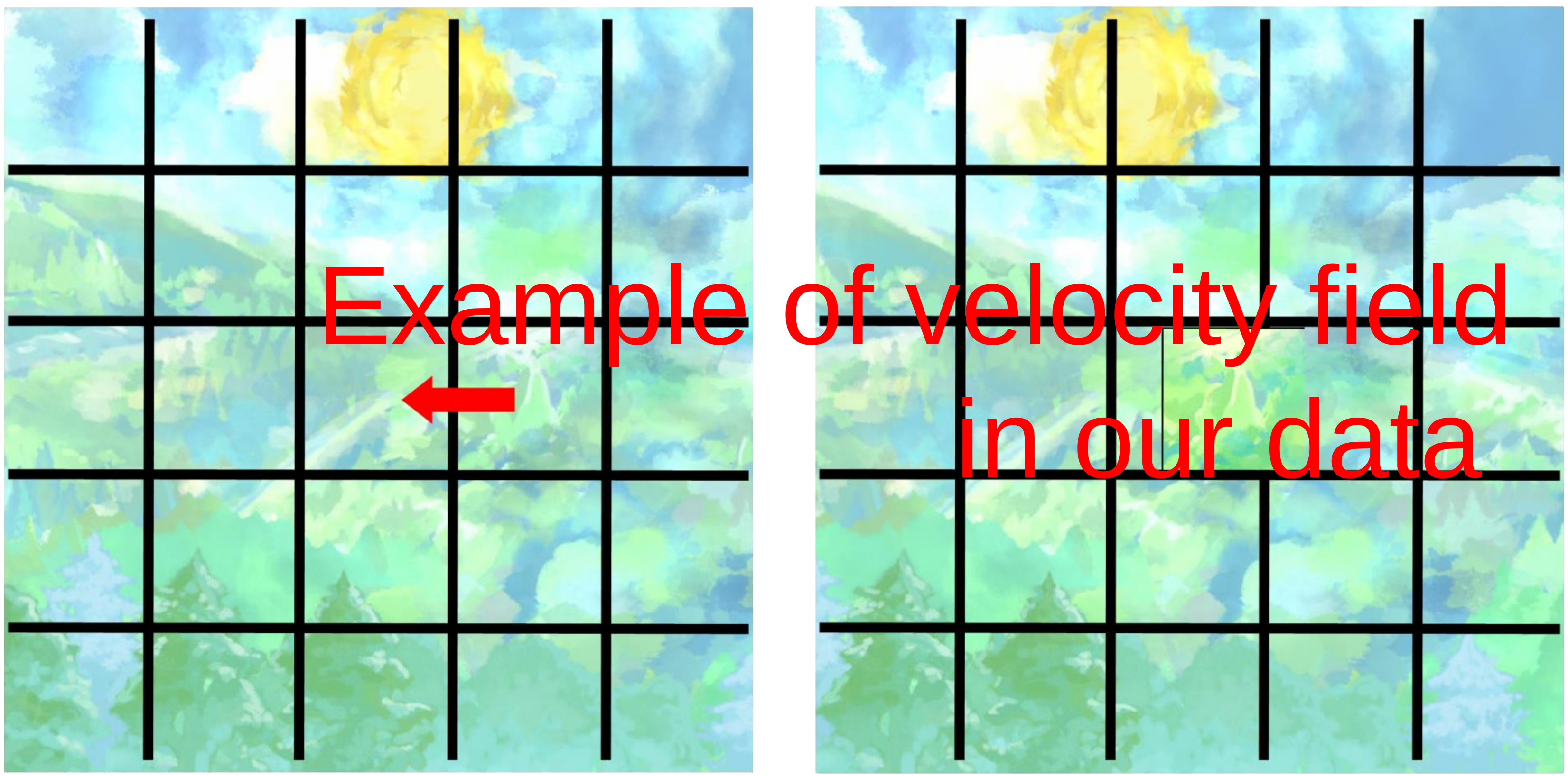
Explicit detection of coordinated cells



Motivation

Exploiting spatial heterogeneity to assess mechanisms of coordinated migration

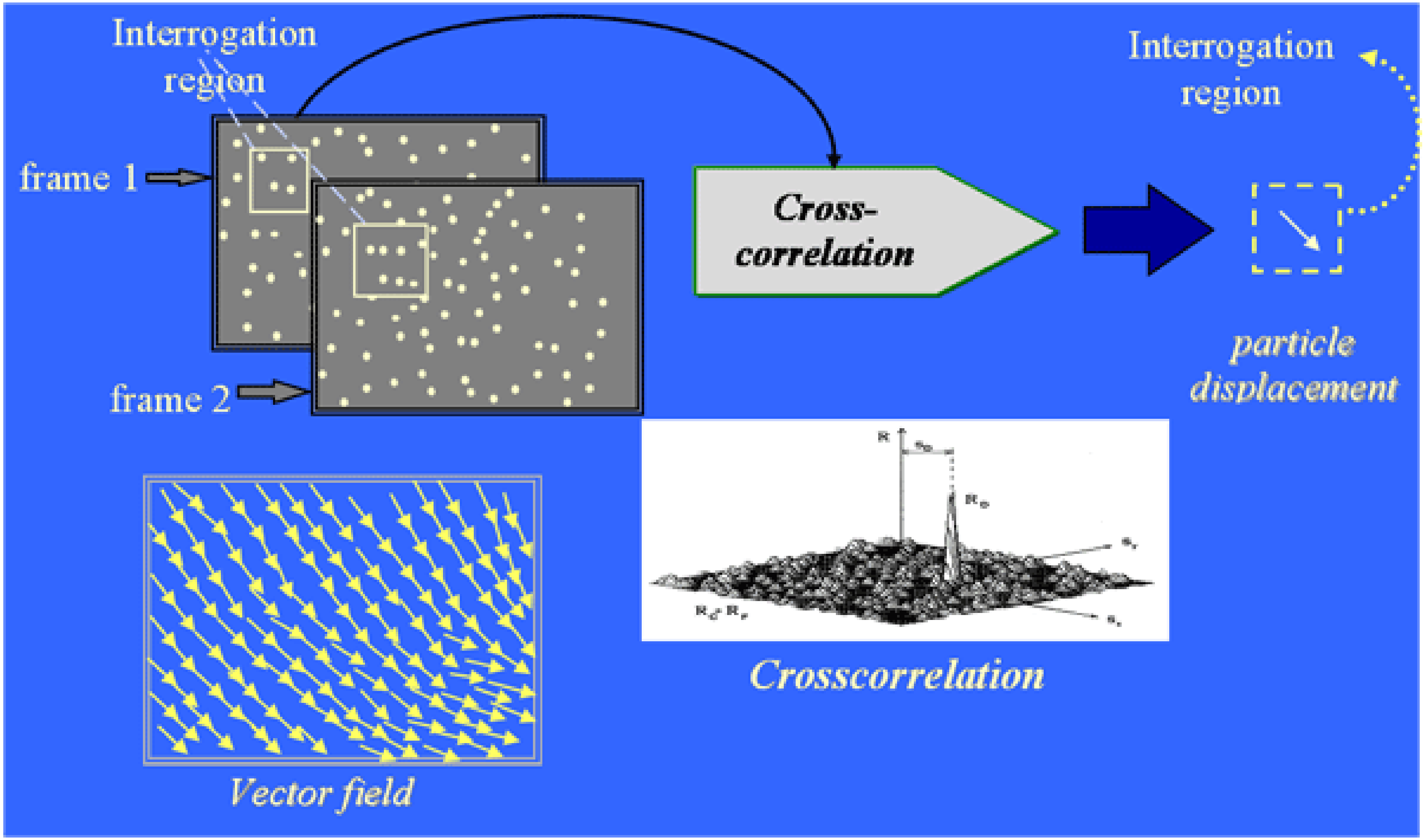
Determining flow fields: Particle Image Velocimetry (PIV)



Time 1 **Time 2**

Artwork: David Hellman (*Braid*); Adapted by Rachel Lee (U. Maryland)

Particle Image Velocimetry (PIV)



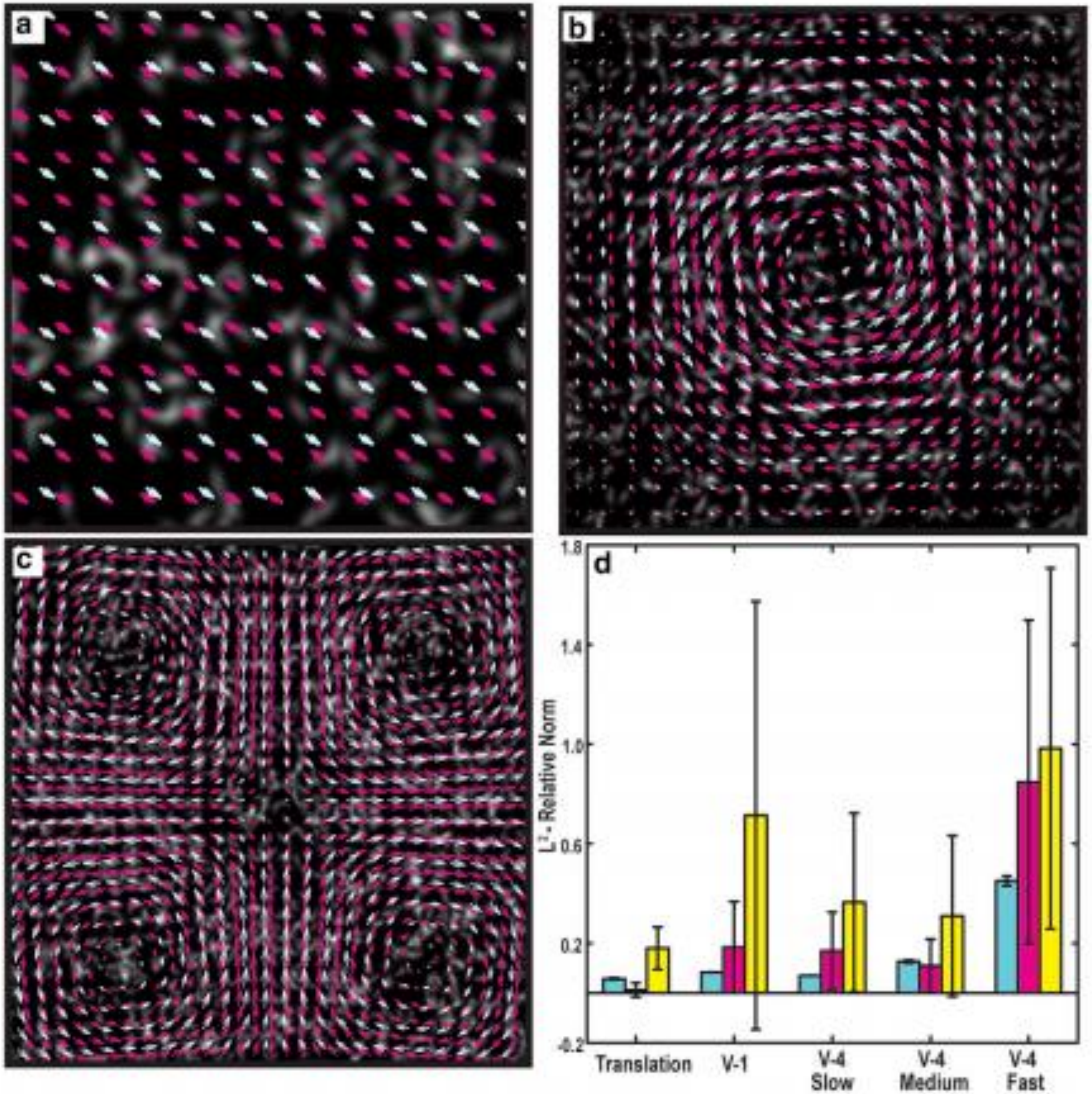
Source: https://www.erc.wisc.edu/piv.php

(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

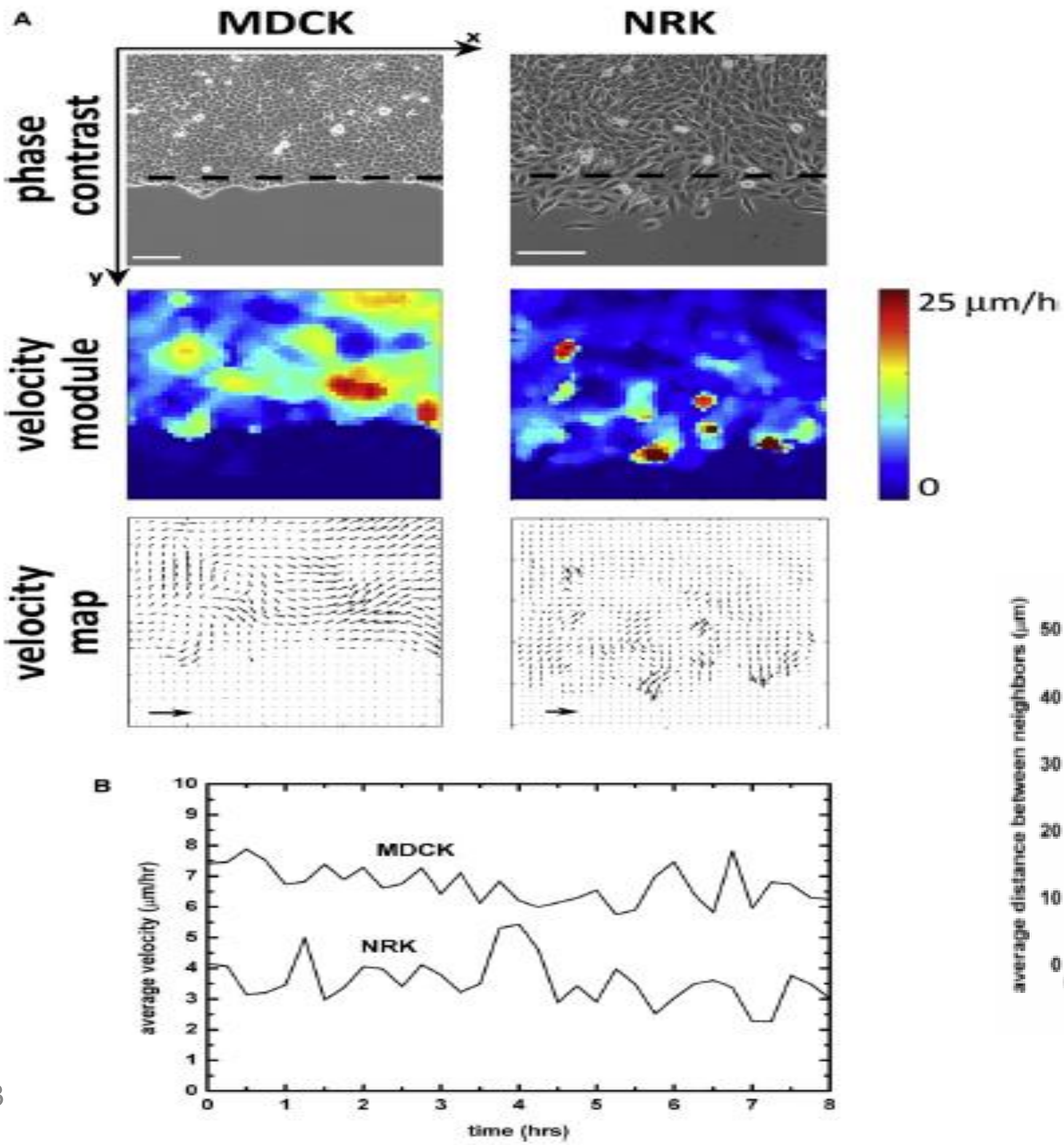
Lucas & Kanade (1981)

Optical flow versus PIV

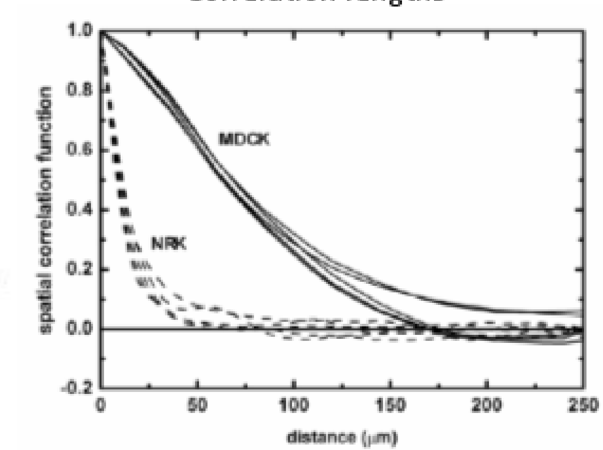


Vig et al. (2016)

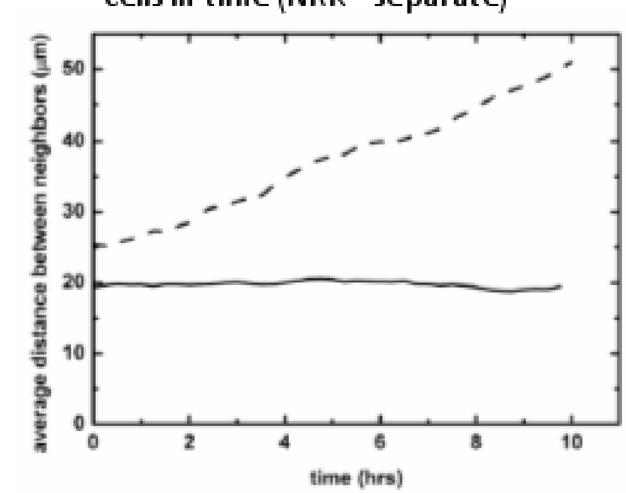
Measuring coordination



Correlation lengths

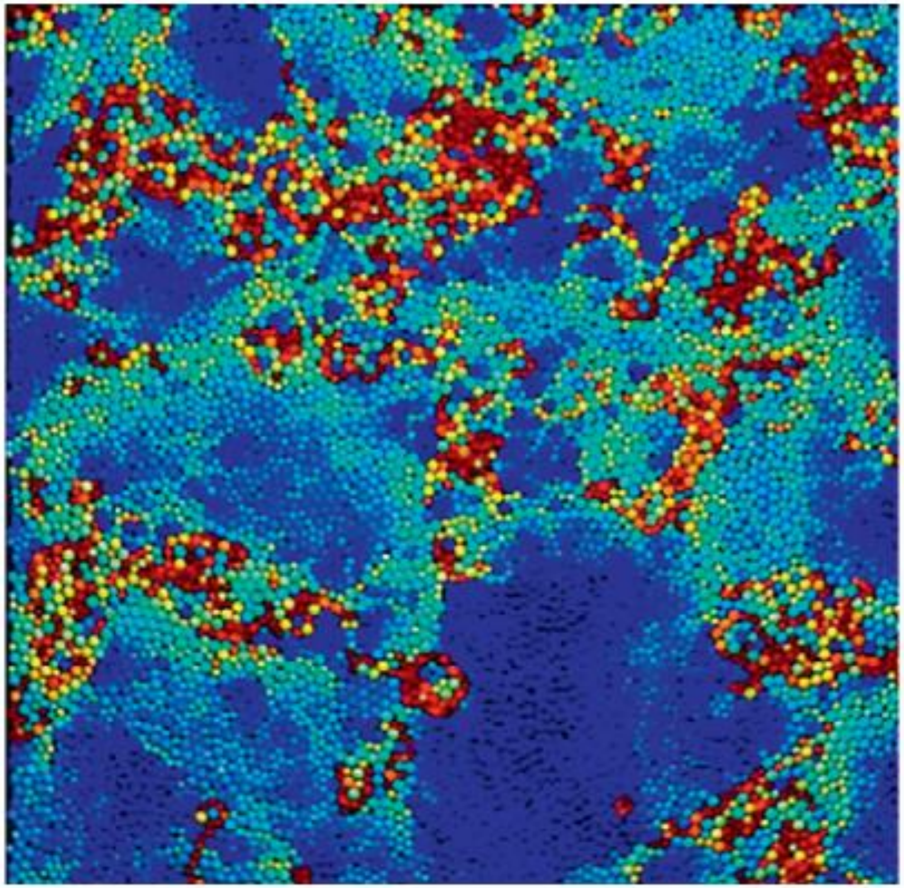
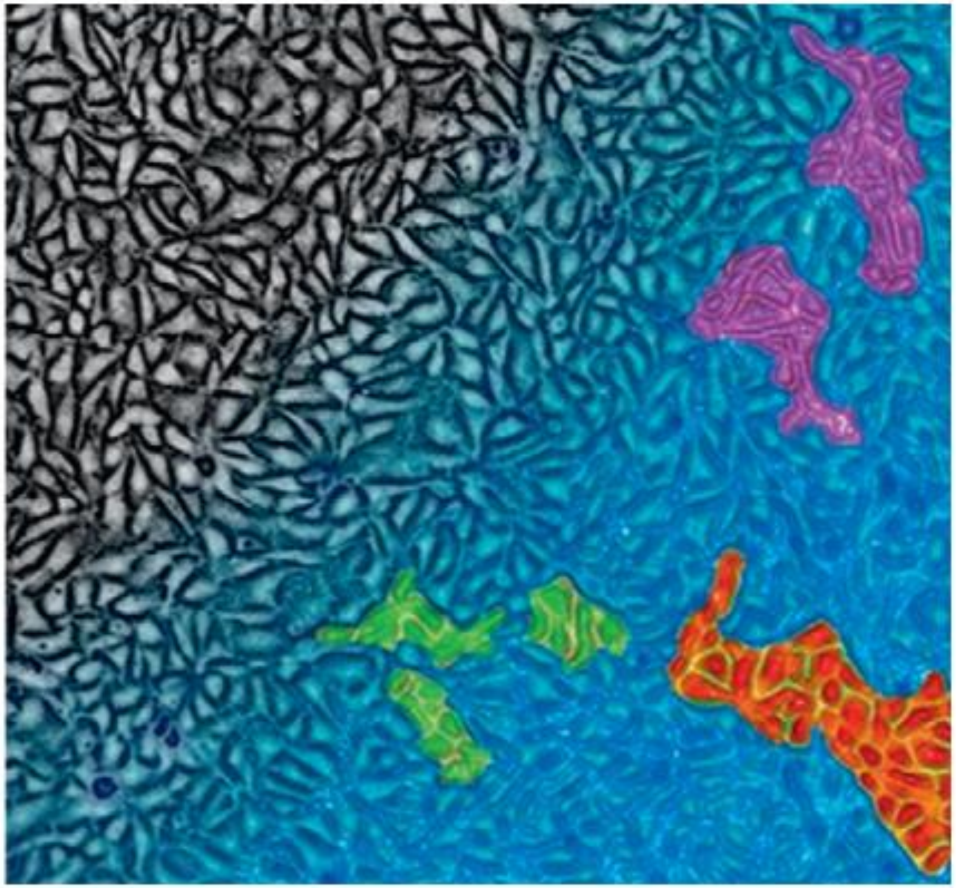


Distance between neighboring cells in time (NRK - separate)



Petitjean *et al.* (2010)

Visualization (of cell speed)

Angelini (2011)
Trepat and Fredberg (2011)

Statistical Region Merging

Nock and Nielsen (2004)

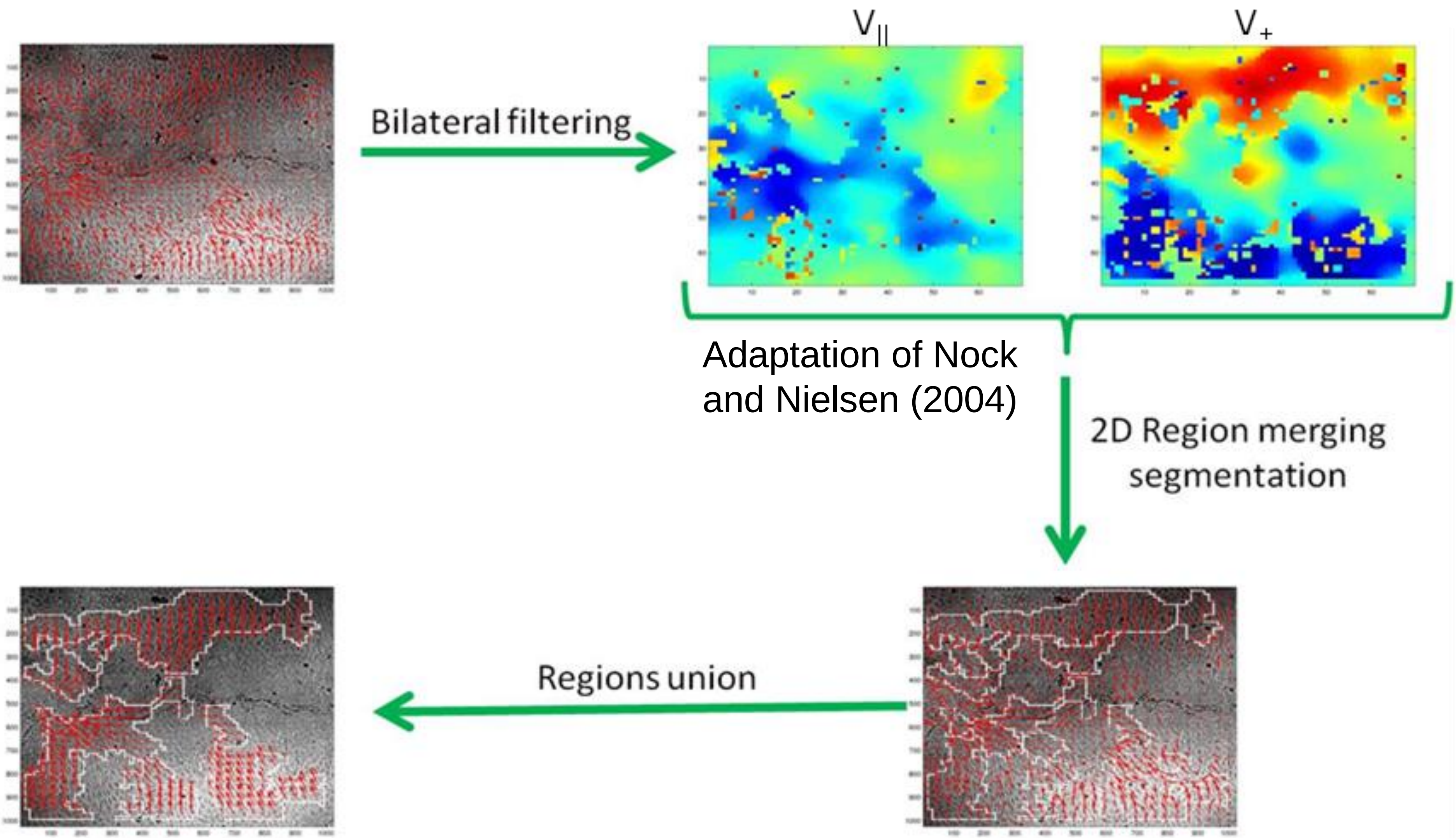
Region growing / merging

- 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

Algorithmic components

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

Explicit detection of coordinated cells



Zaritsky *et al.* (2014)

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 is 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, $sim(r_1,r_2)$
    c. Calculate the threshold for merging two regions $TH = b(r_1) + b(r_2)$, whereas $b(r) = \log(size(r)) * Q$
    d. Merge r1 and r2 if and only if $sim(r_1,r_2) < TH$
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

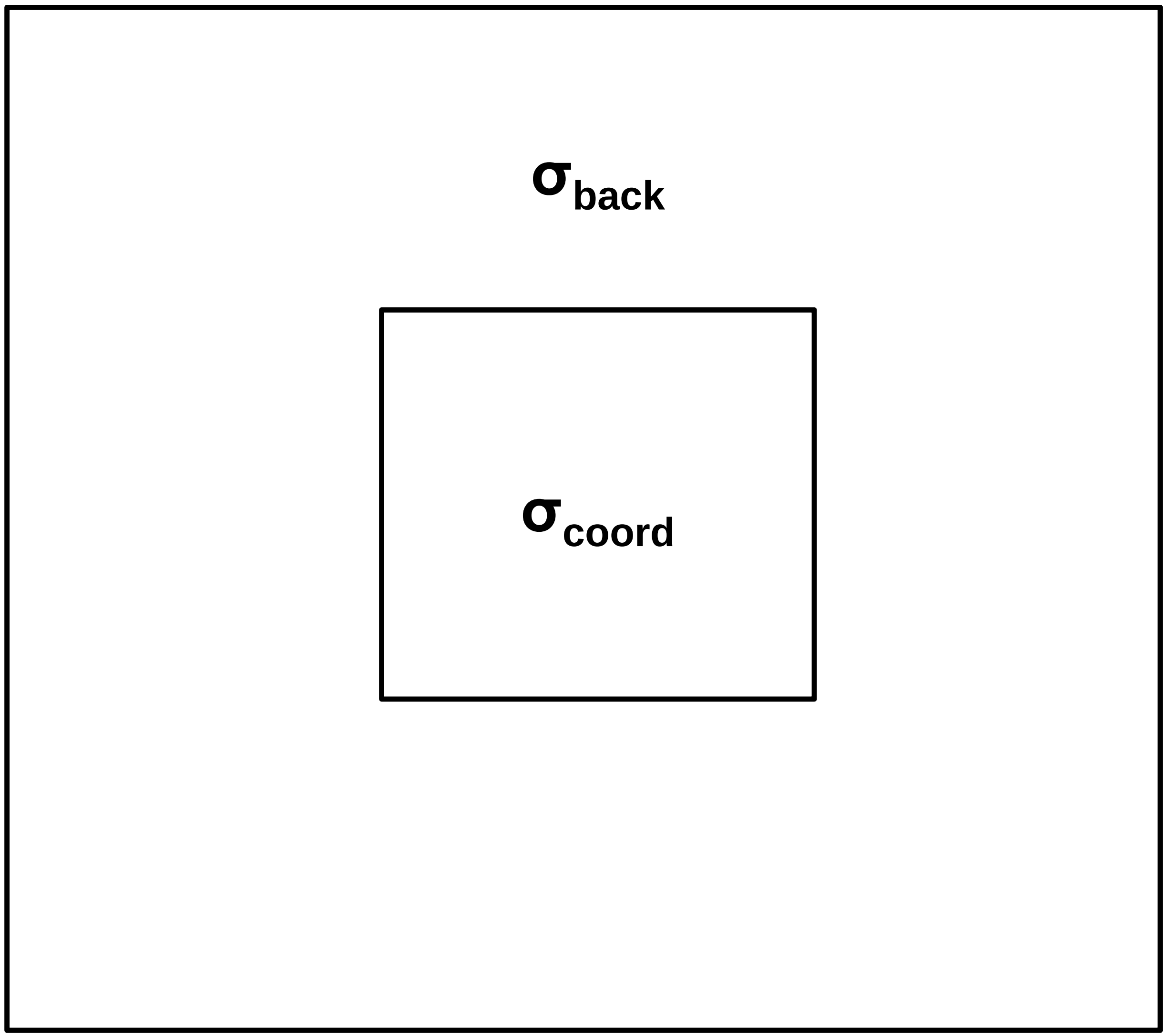
- Pros:
  - Fast (not in my implementations..) and easily implementable
  - Can handle noise and occlusions
- Cons:
  - Does not capture “flow” patterns
  - Clusters are not sufficiently stable for tracking
  - Setting parameter/s to optimize similarity measure / merging predicate (for any method that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

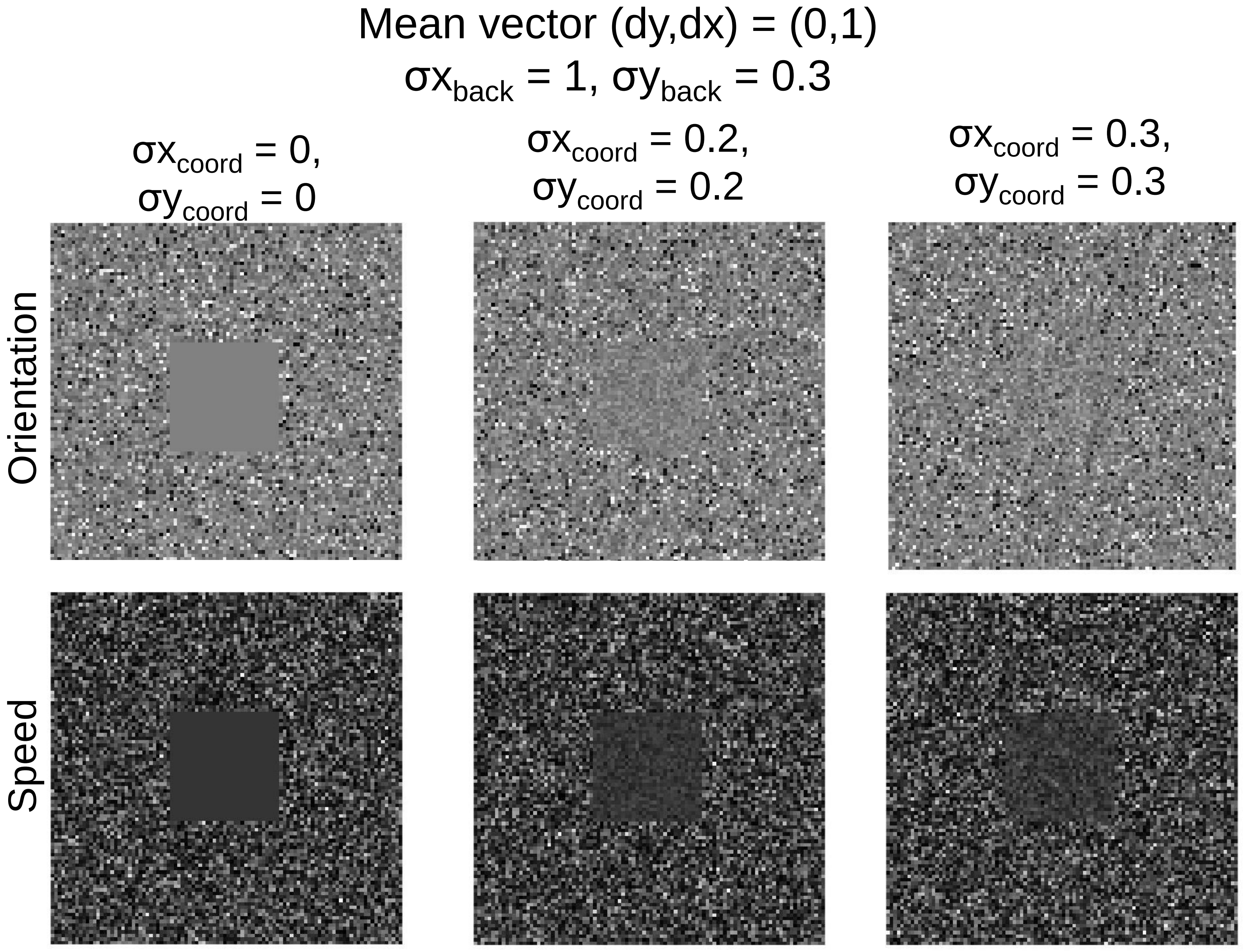
Exercise

1. Download source code, https://github.com/assafzar/MonolayerKymographs
2. Toy simulation
3. Download data, https://cloud.biohpc.swmed.edu/index.php/s/7ks3mjyujpu9pMg
4. Run the code
   - Toy example
   - Change parameter?
5. Use a different clustering approach (e.g., for example, speed thresholding) and visualize? **- clean the code**
6. **Use cosine / speed + kmeans**
7. K-means on speed / orientation?

Toy simulation
simulateCoordination.m

Mean vector (0,1)




Mean vector (dy,dx) = (0,1)
σx_{back} = 1, σy_{back} = 0.3
σx_{coord} = 0, σy_{coord} = 0
σx_{coord} = 0.2, σy_{coord} = 0.2
σx_{coord} = 0.3, σy_{coord} = 0.3
Orientation
Speed

Execute the simulation

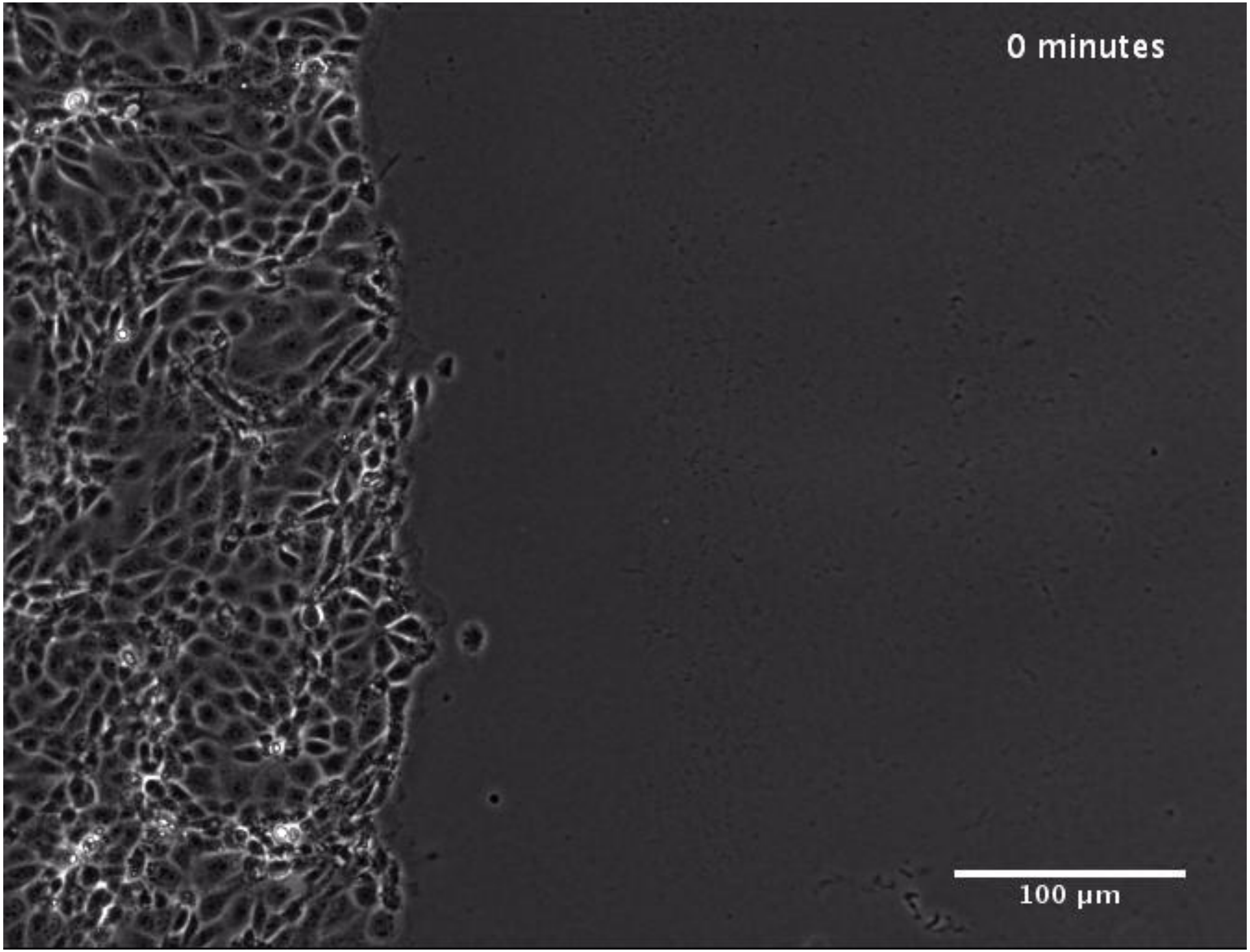
1. Download source code, https://github.com/assafzar/MonolayerKymographs
2. Toy simulation
   - mainCoordination(outSimDname);
   - Explore parameters (params.regionMerginParams.P/Q) to optimally segment

Agenda

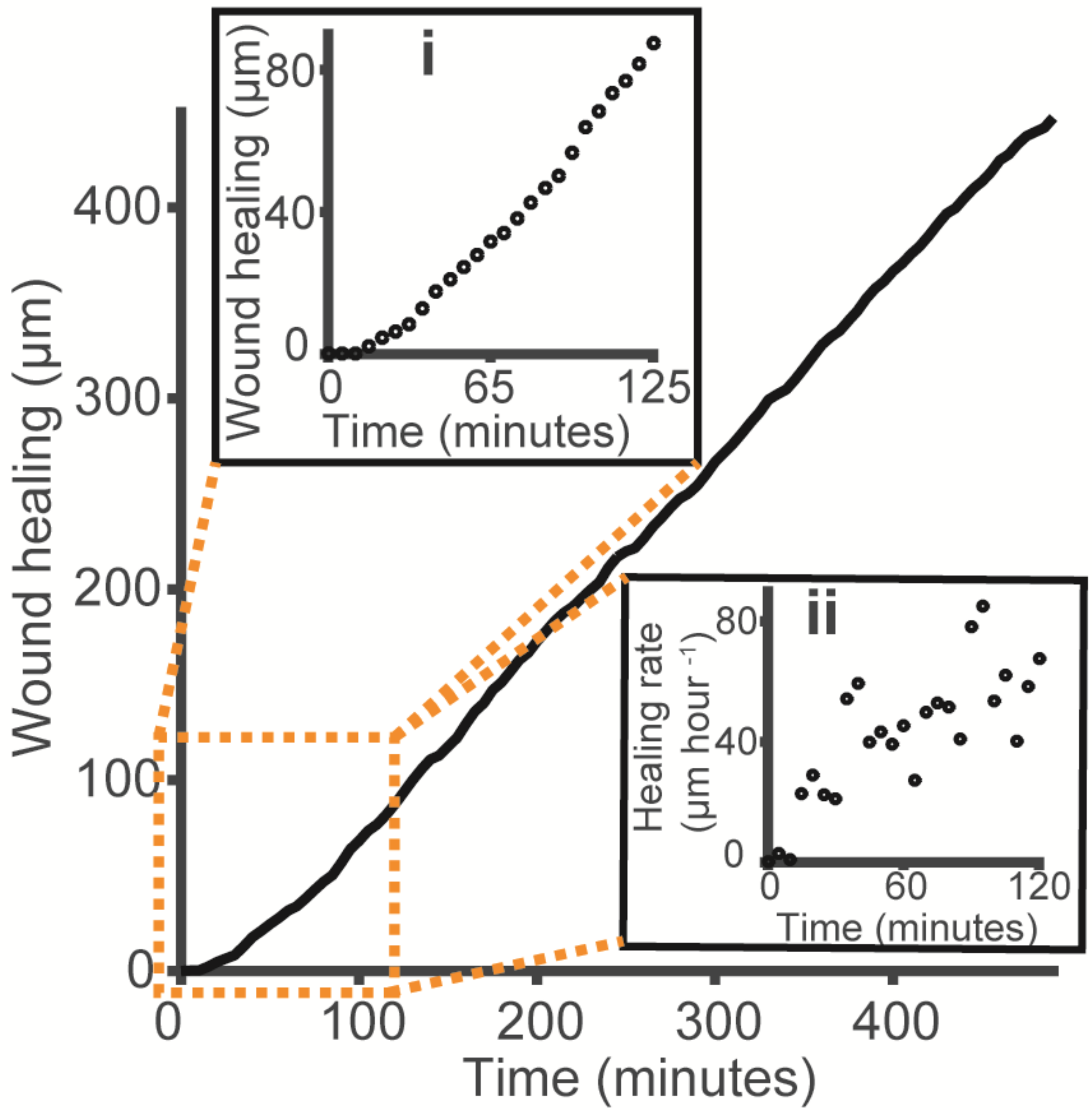
1. Collective cell migration
2. Detection of coordinated clusters (+ exercise)
3. **Example (data reuse)**
4. GEF screen (+ exercise)
5. DeBias – if times allow (co-localization)


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

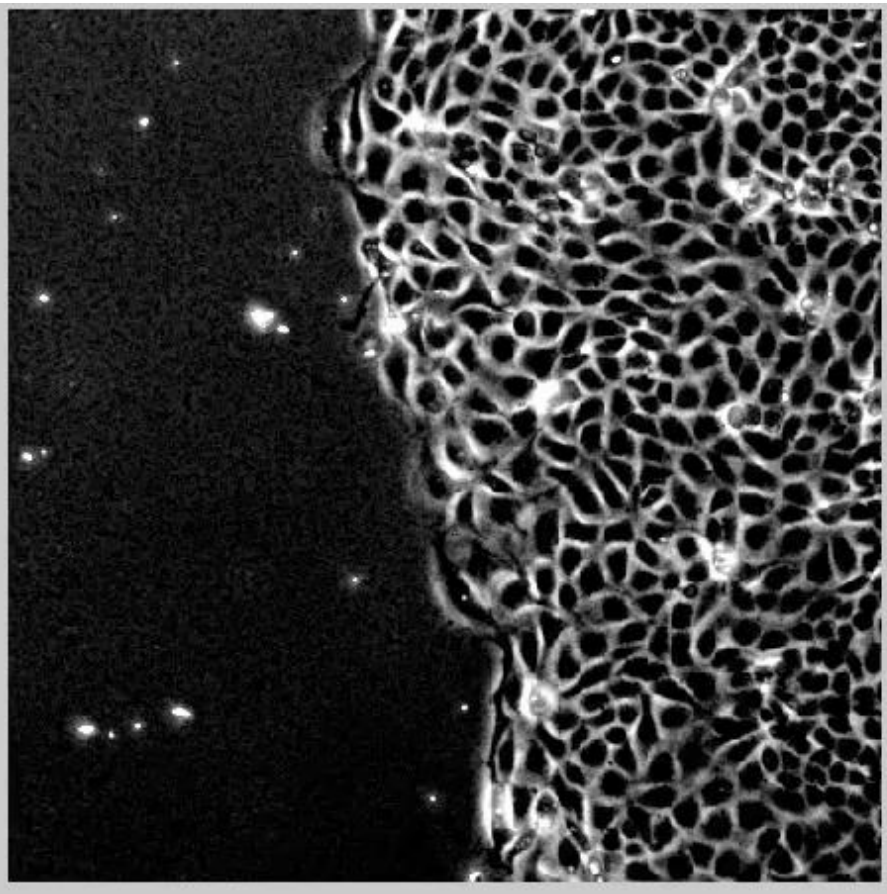
(Zaritsky et al. 2015)

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

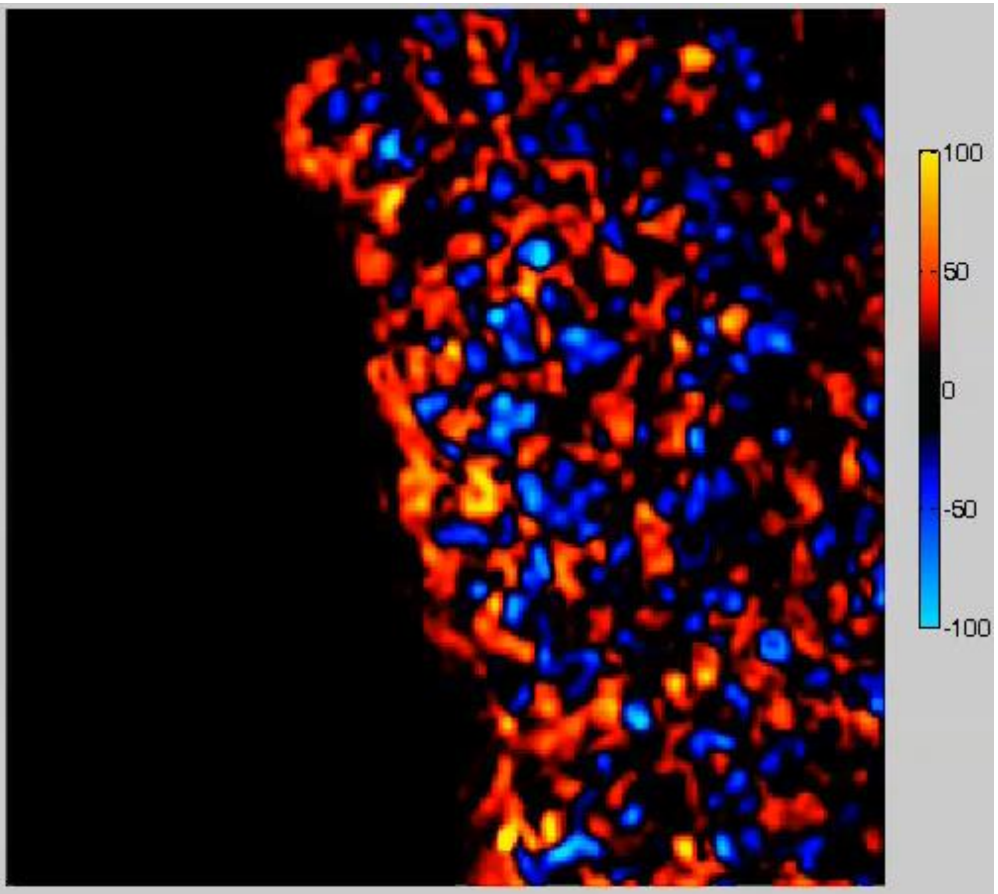
Zaritsky and Tseng et al. (2017)

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

Phase Contrast

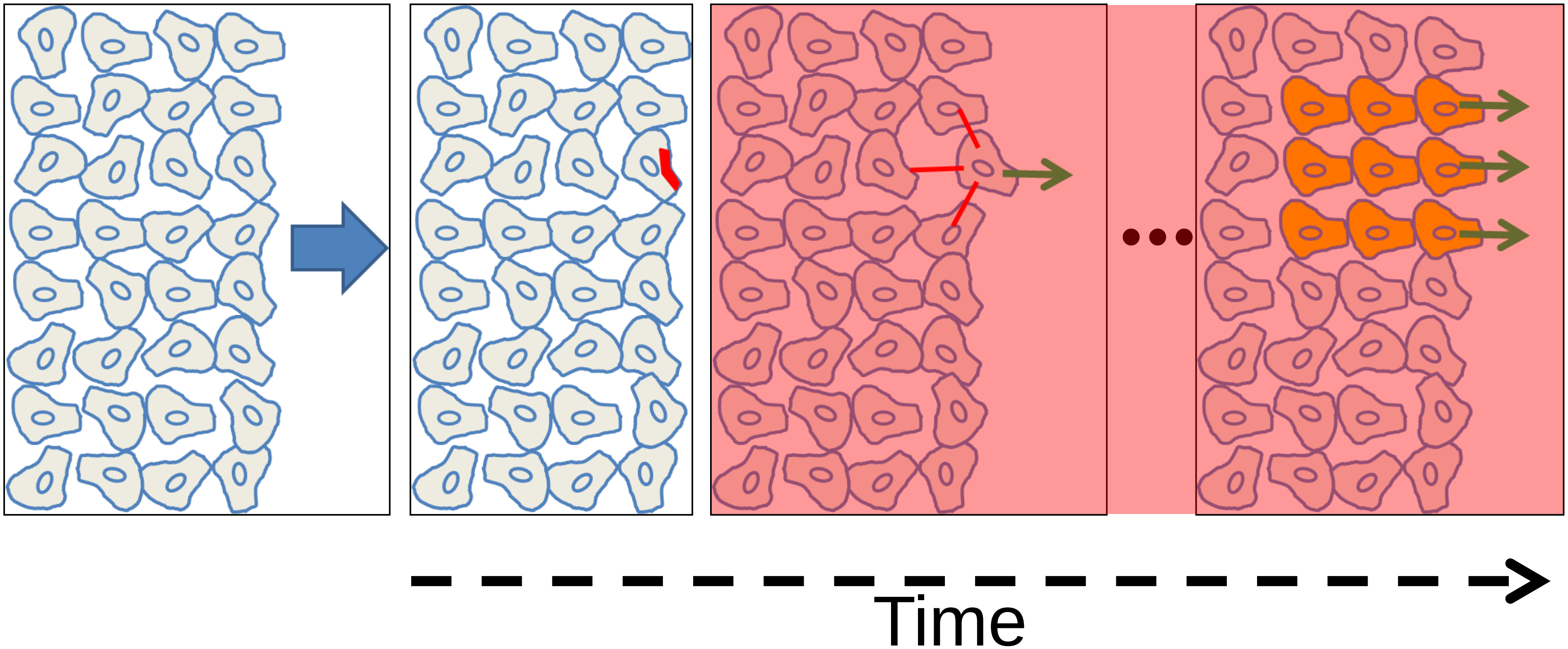


Traction T_x (P_a)



Trepat et al. (2009)

Suggested model

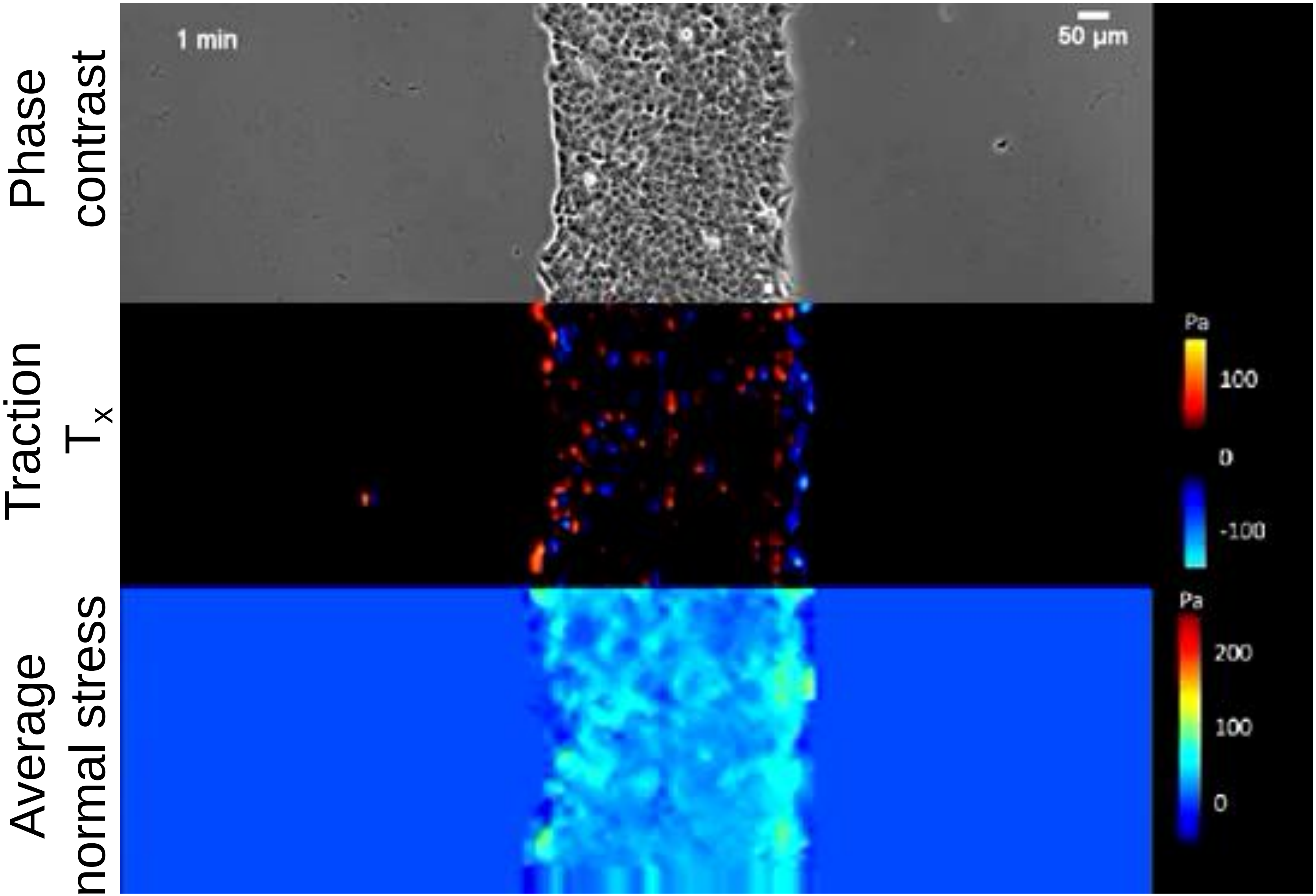


Stochastic force exertion transform to directional migration

Strain on neighbors coordinate their movement

Propagation in time and space to guide groups of cells

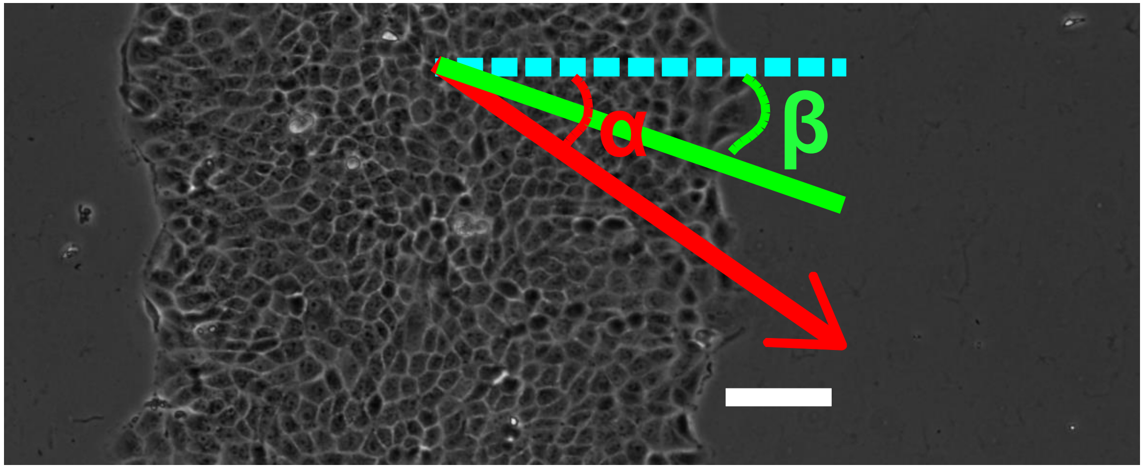
Measuring traction force, stress and velocity



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

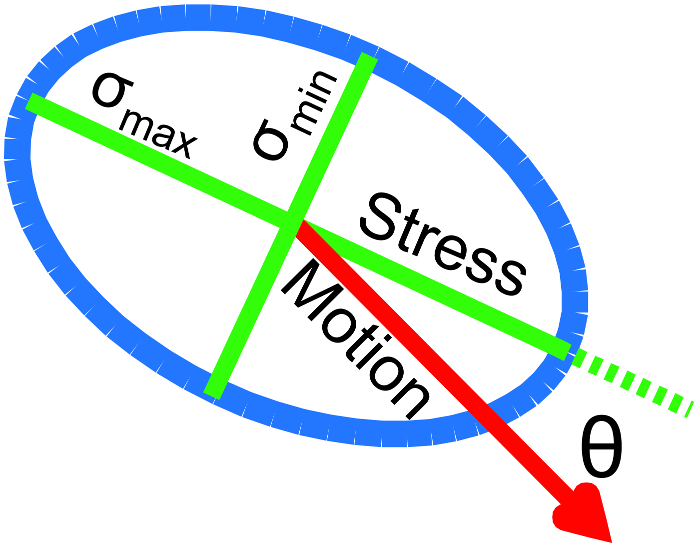
Motion-stress alignment

Velocity angle,
stress orientation



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

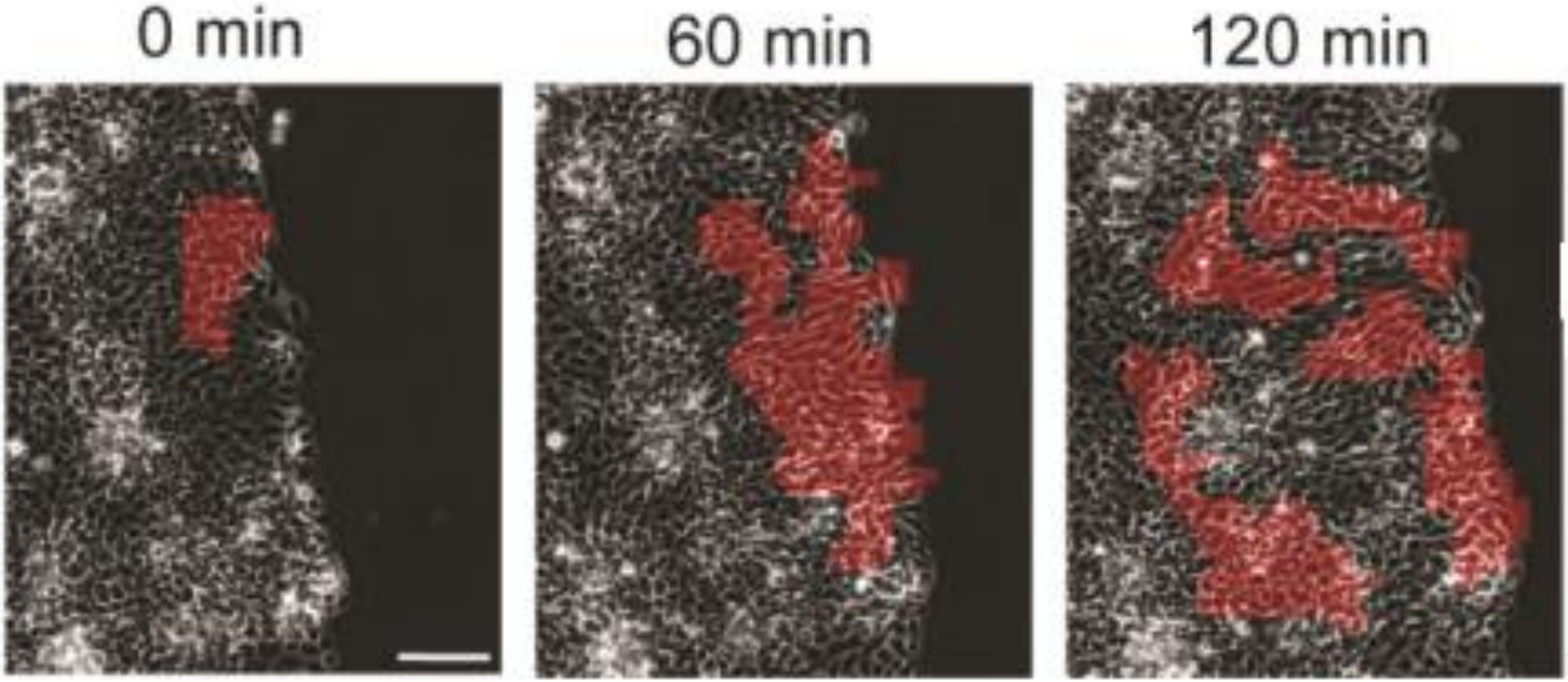
Motion-stress
alignment



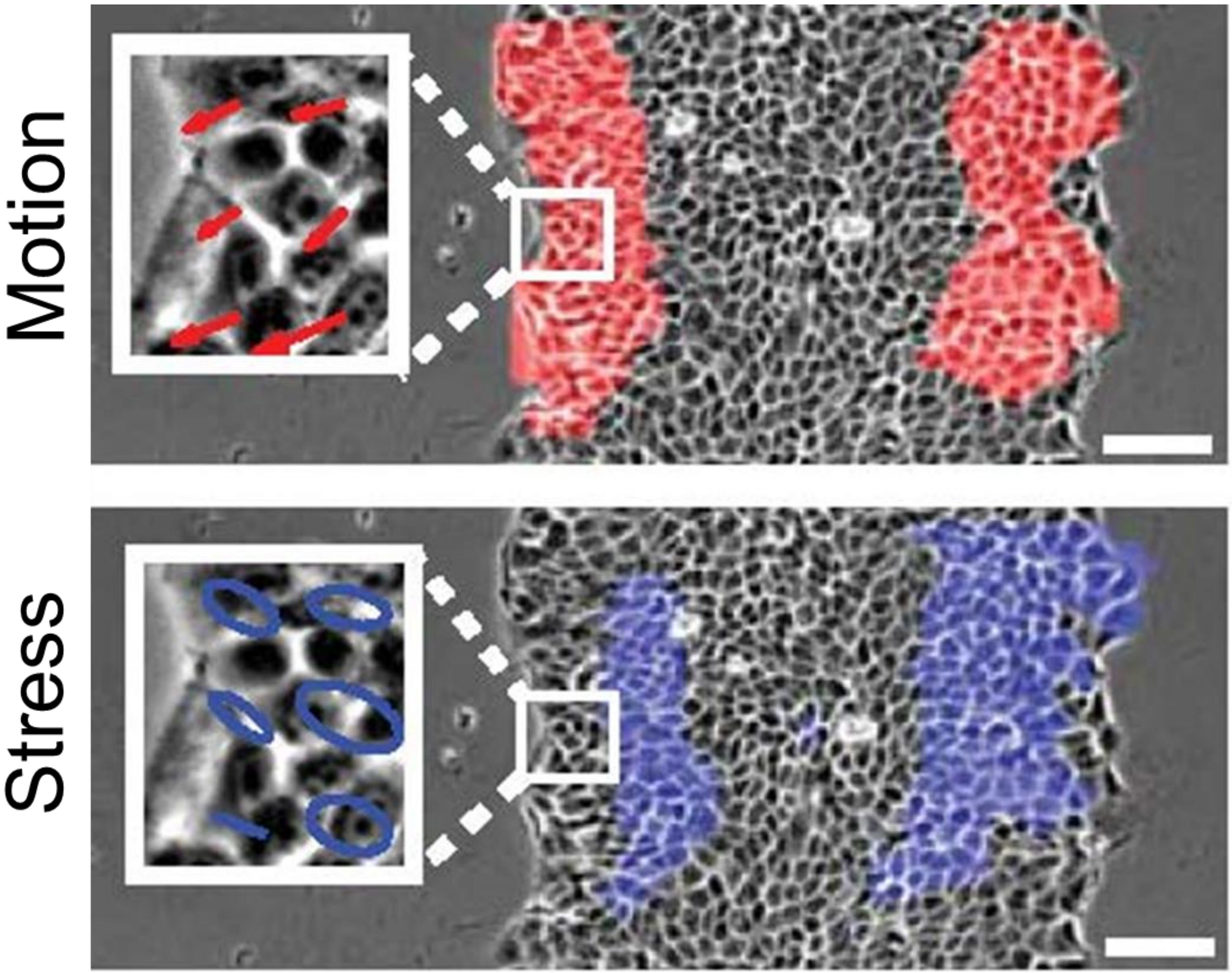
$0 \leq \theta \leq 90$

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

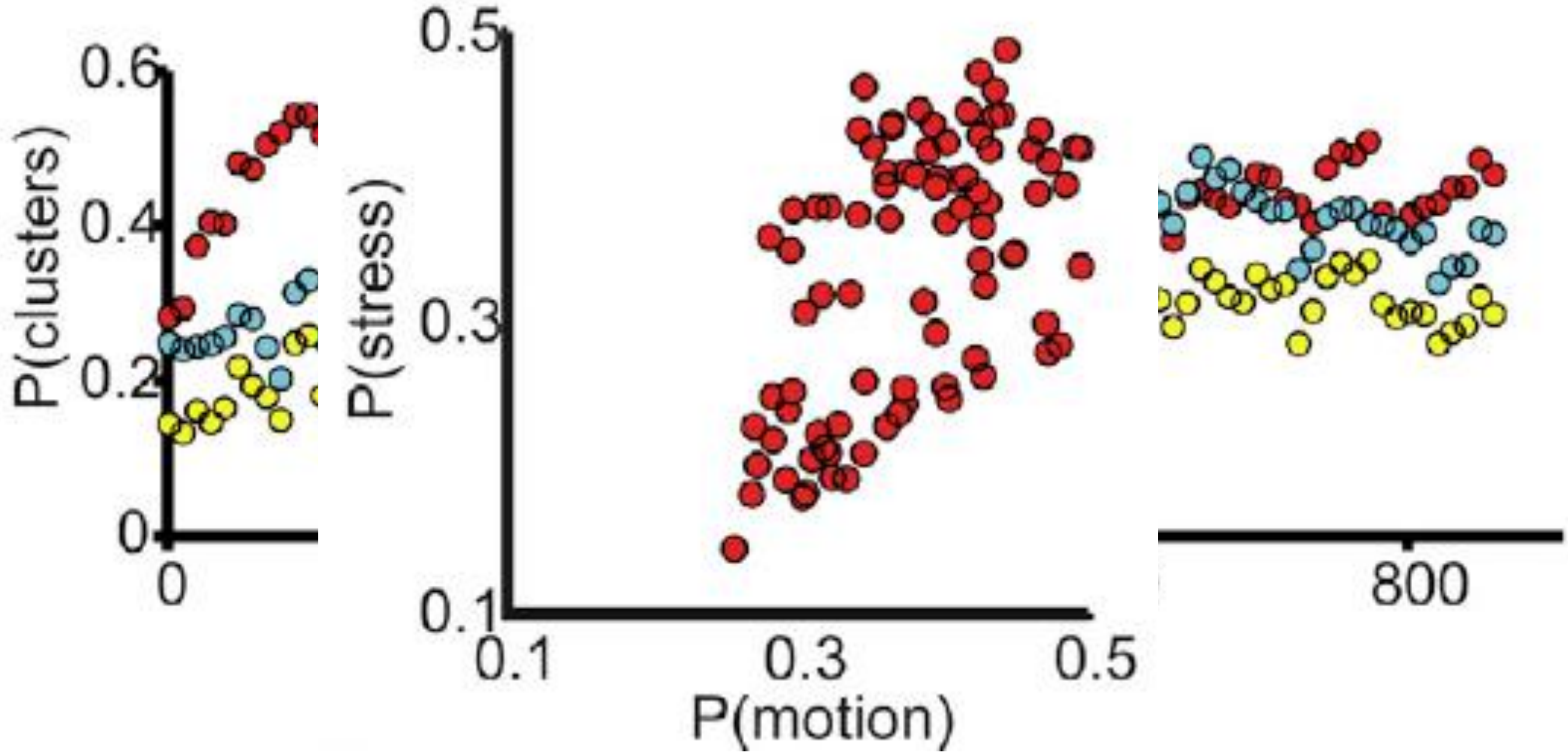
Region-growing segmentation for ***explicit*** detection of coordinated migration clusters



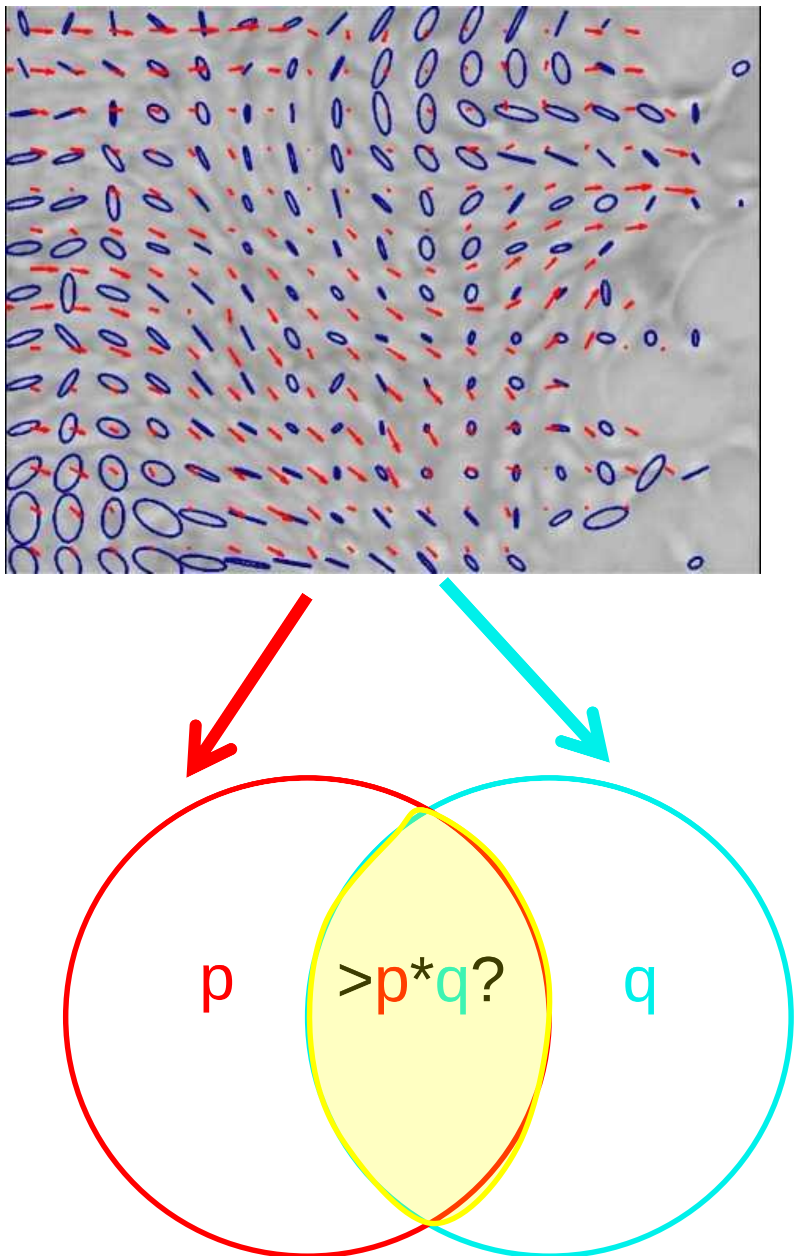
Associating coordinated stress and motion



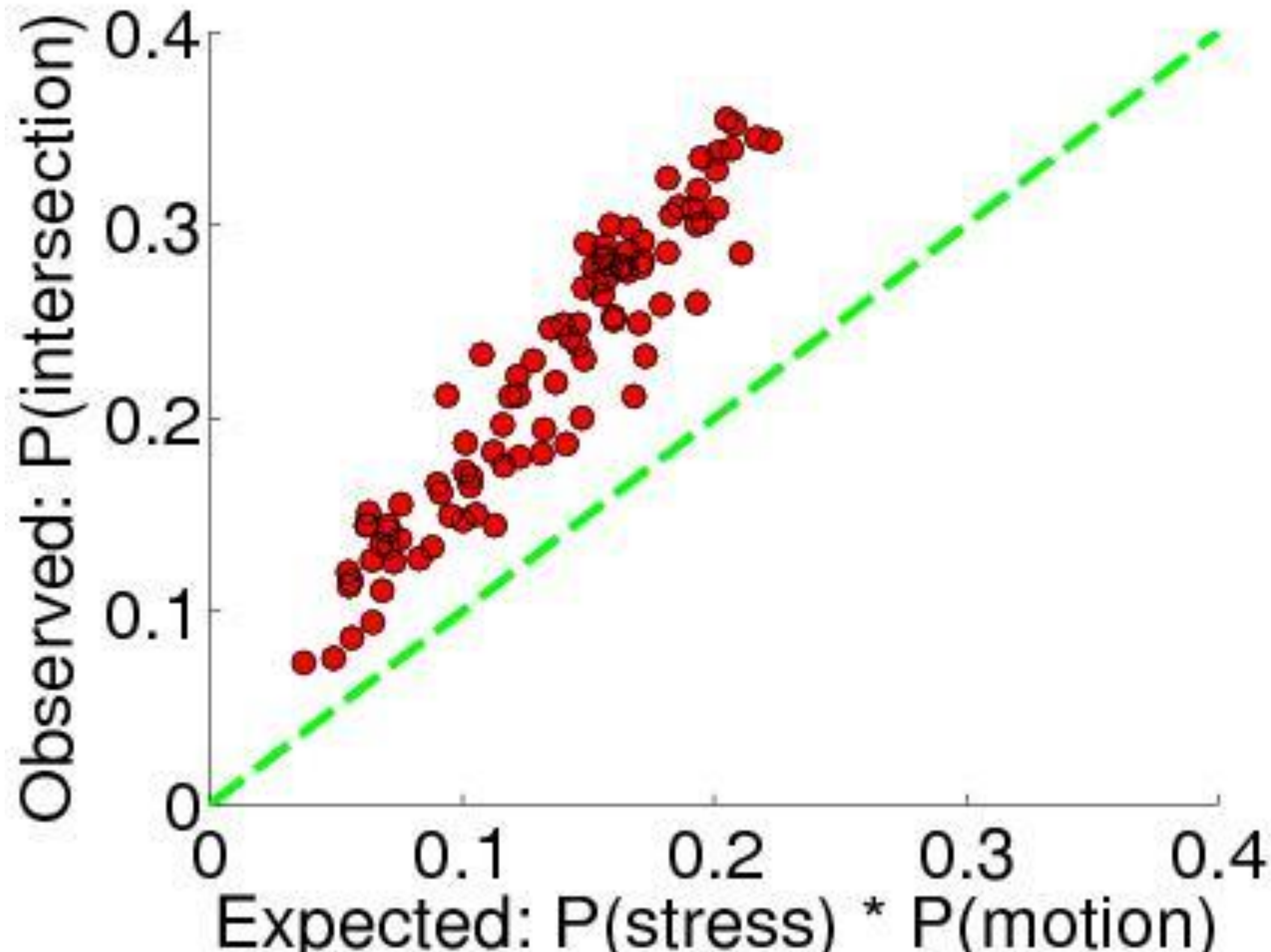
Coordinated motion is correlated to coordinated stress



Associating coordinated stress and motion

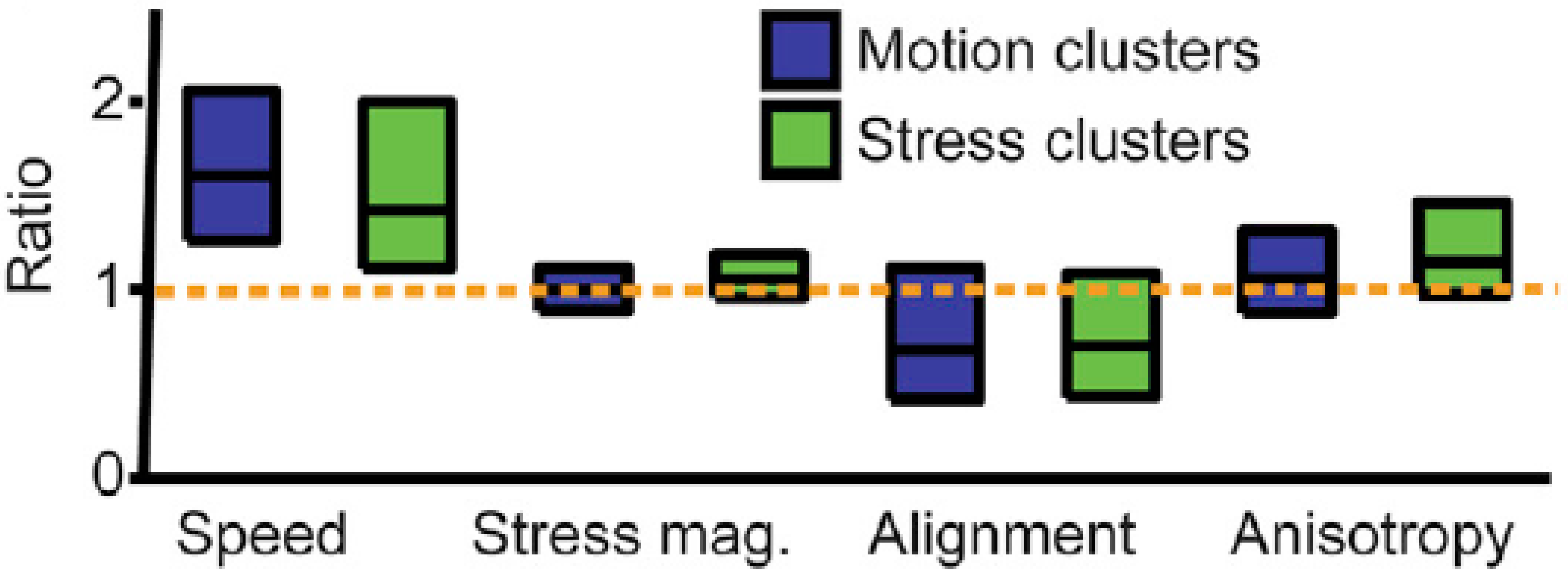


Motion- and stress- coordinated clusters are interlinked

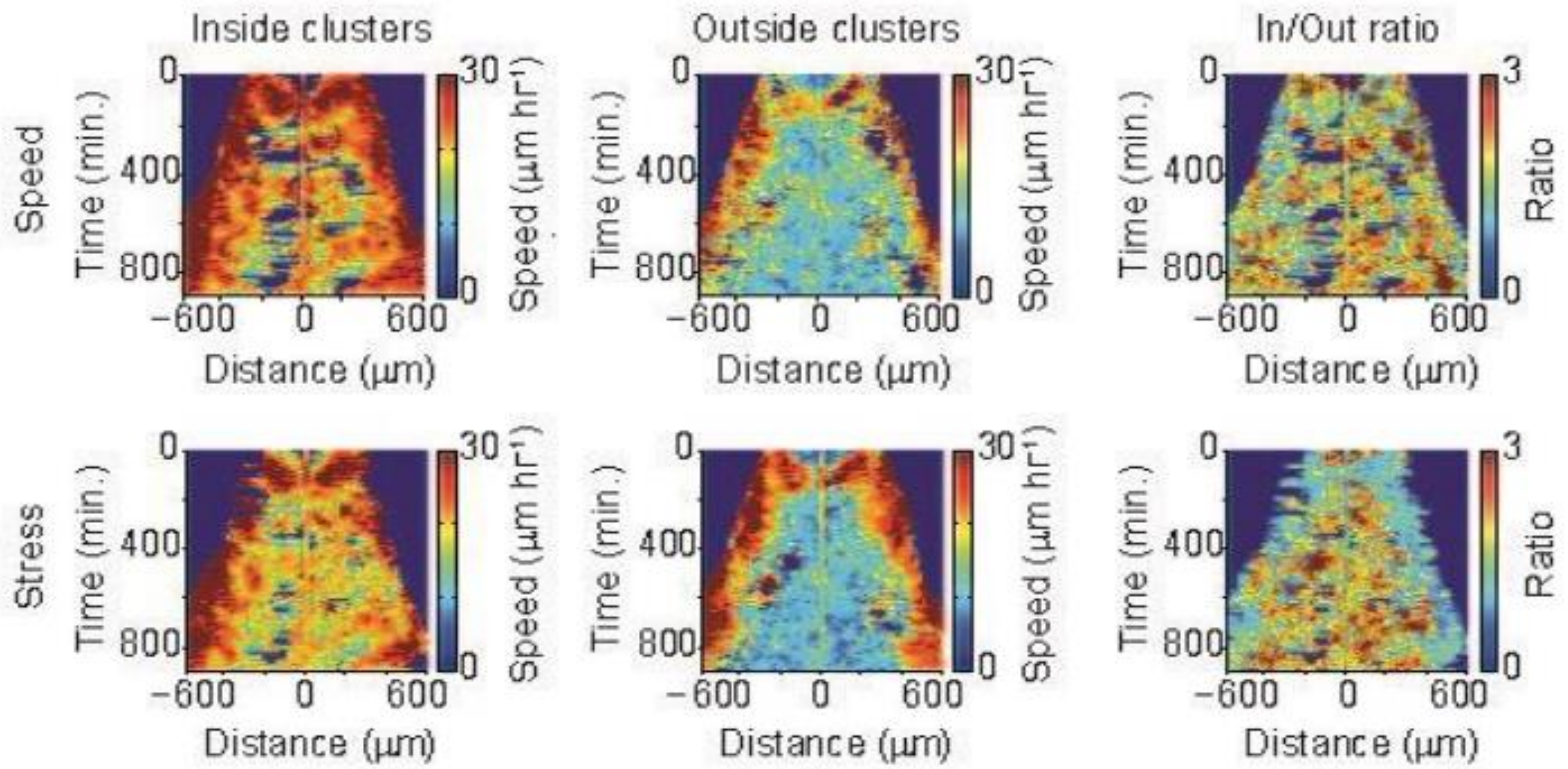


Cells in coordinated clusters move faster, with enhanced motion-stress alignment

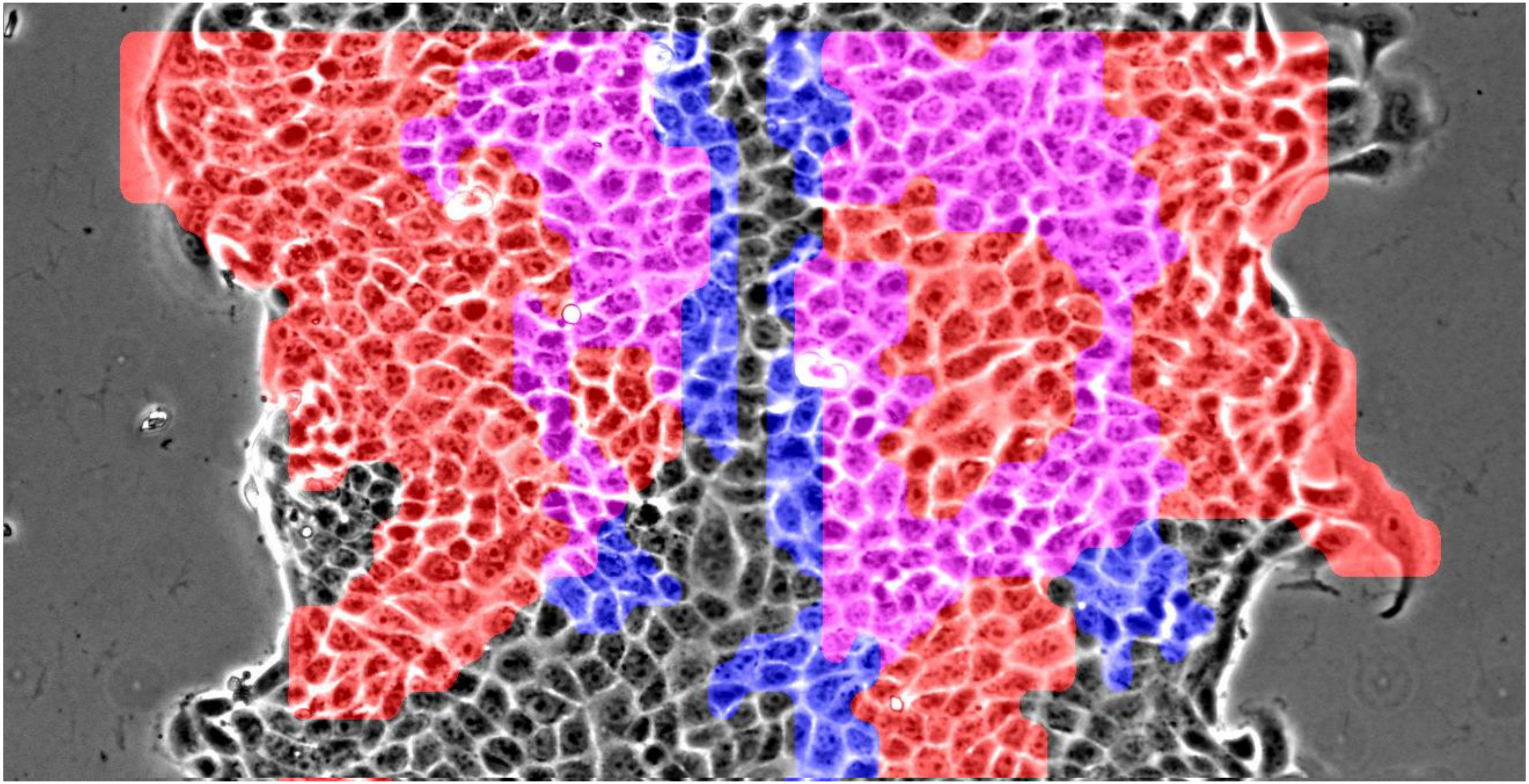
$$\mathrm{Ratio}_{\mathrm{property}} = \frac{\mathrm{property}_{\mathrm{in}}}{\mathrm{property}_{\mathrm{out}}}$$



Spatiotemporal analysis

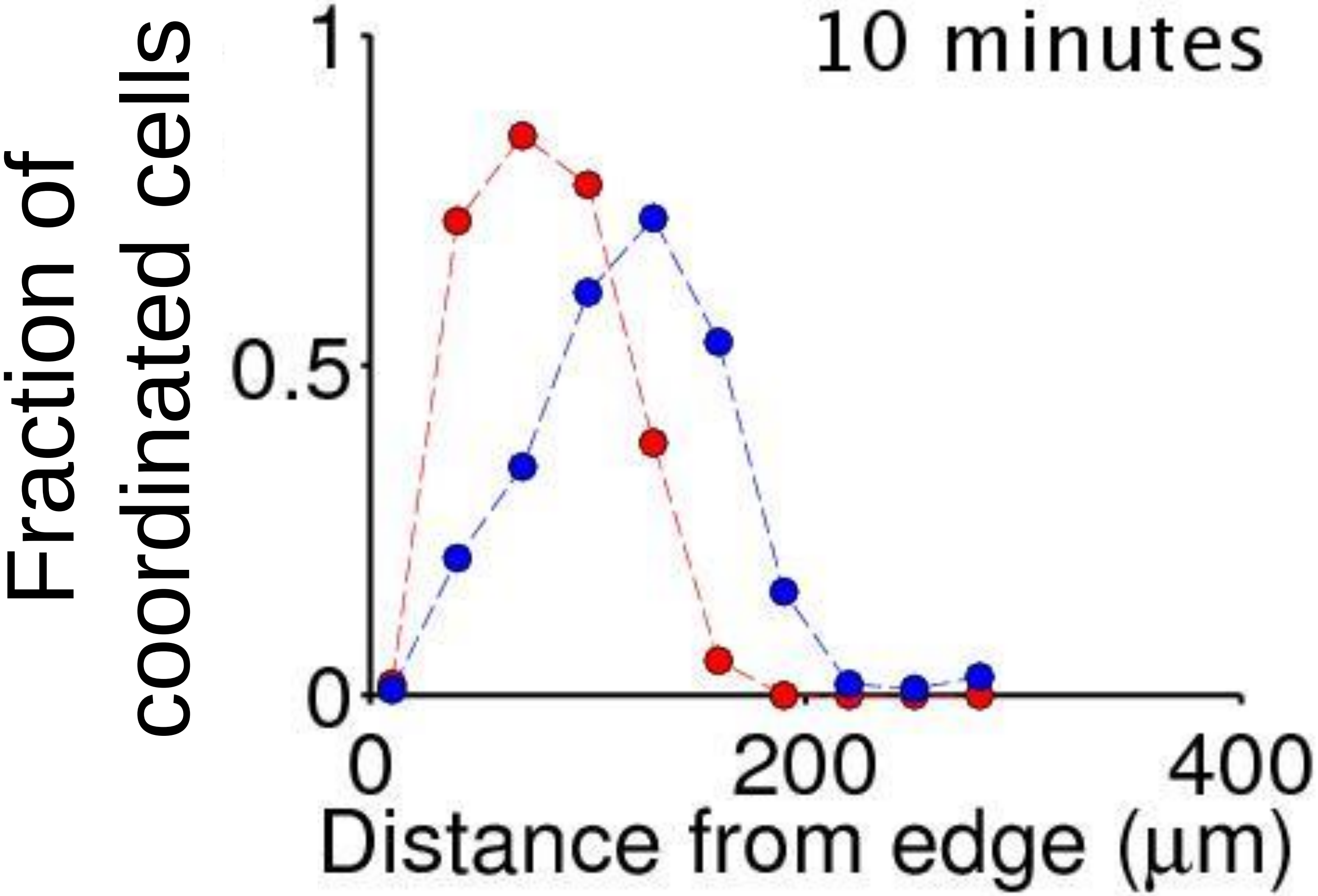


Strain-induced motion coordinates cluster's motility



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

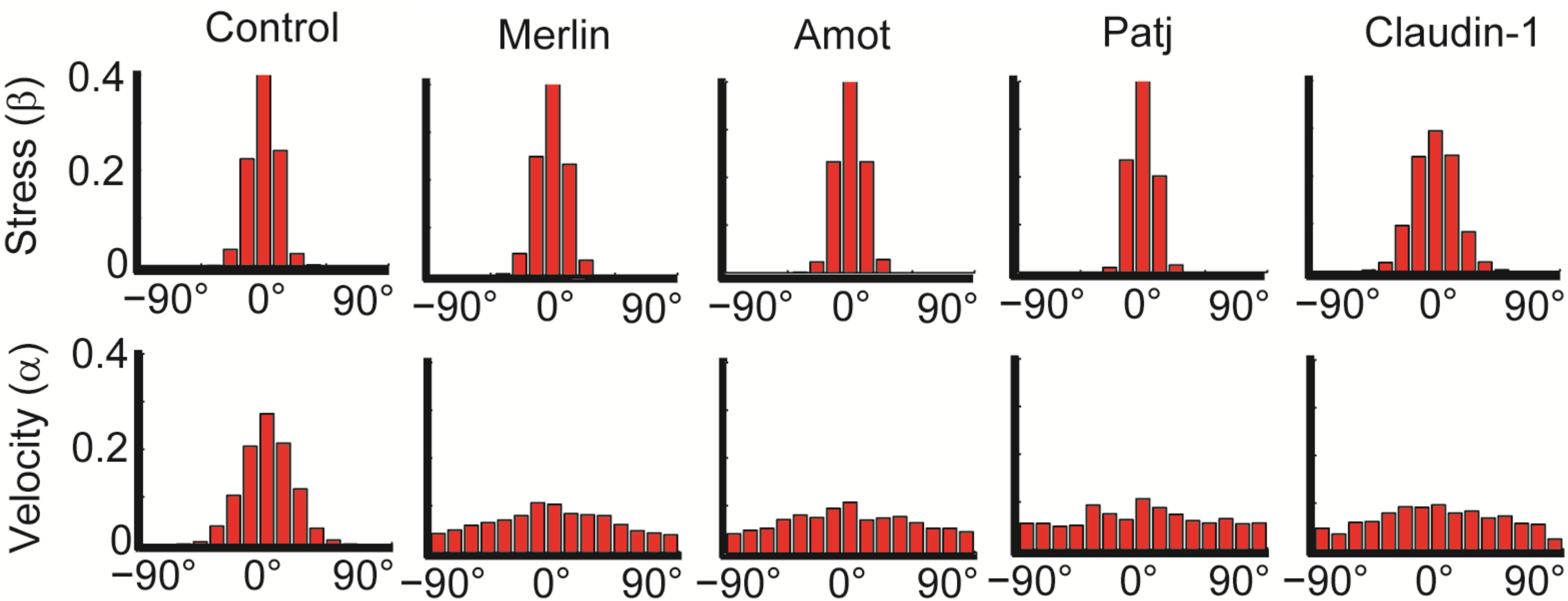
Strain-induced motion coordinates cluster's motility



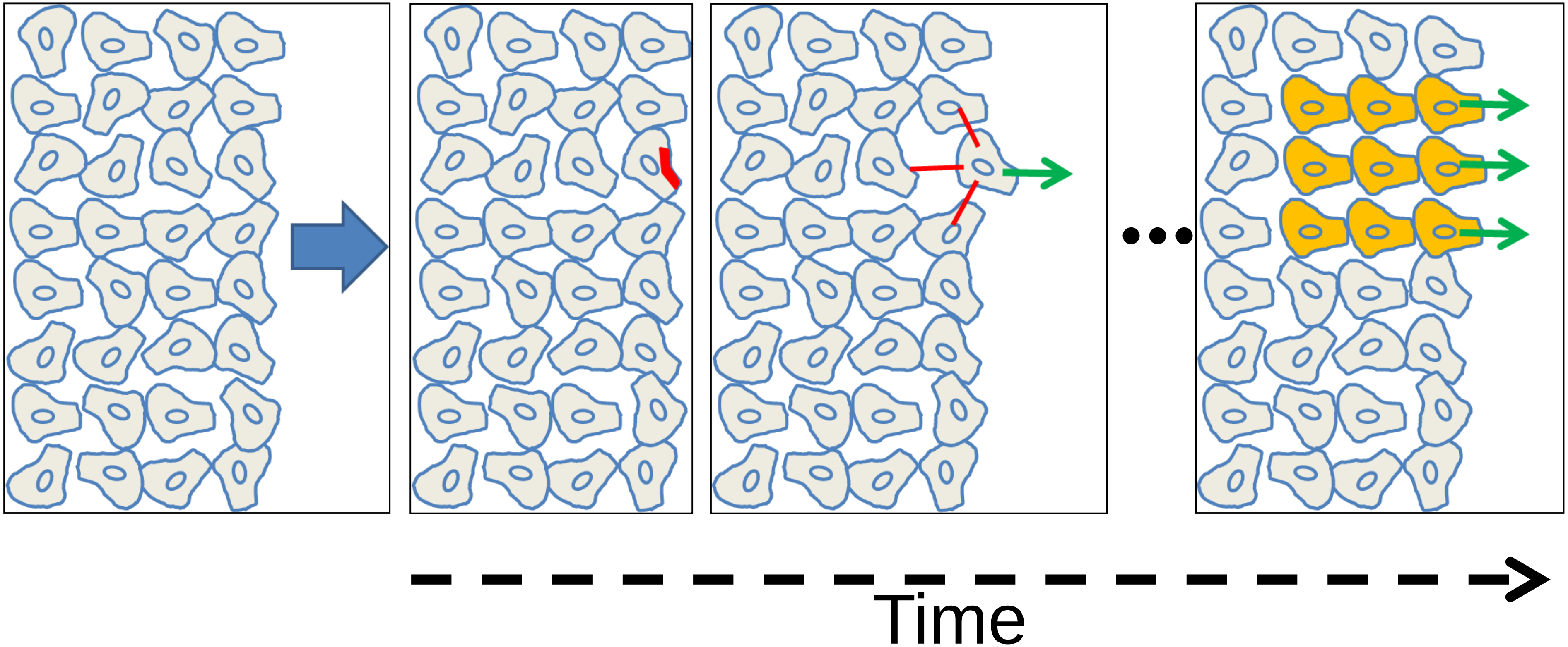
Red: motion clusters, Blue: stress

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)

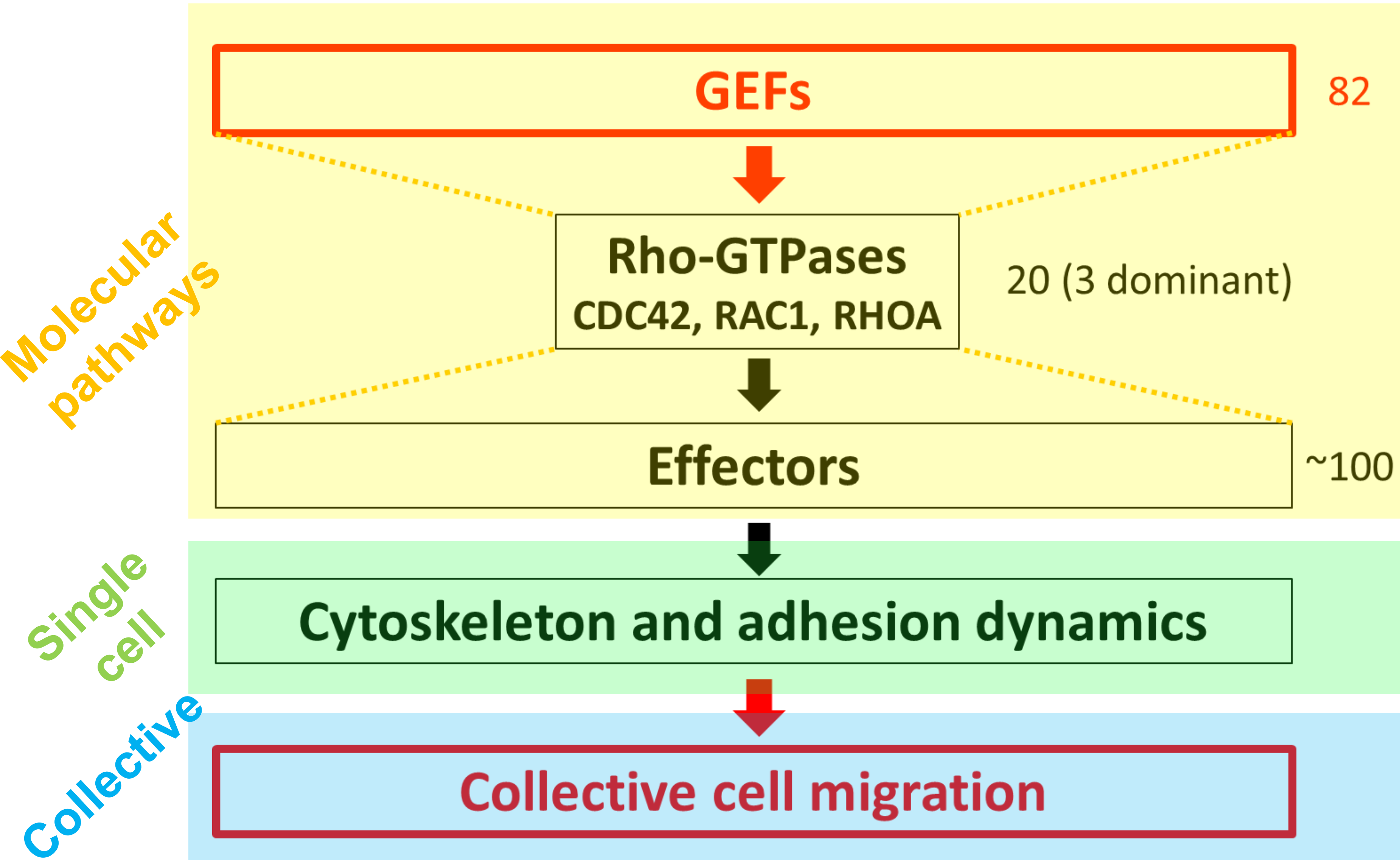
Break (20 minutes)

3. GEF screen (+ exercise)

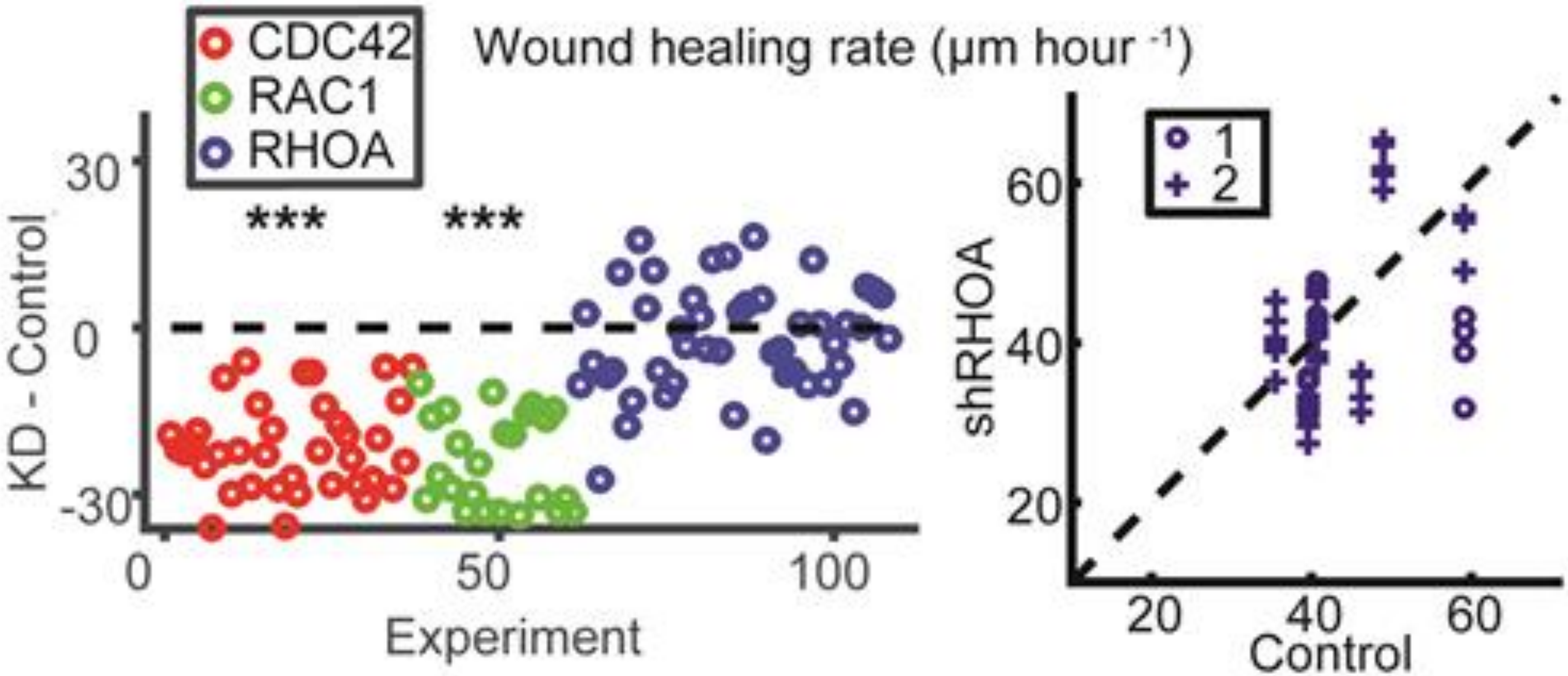
Break (15 minutes)

4. Unrelated promotion: DeBias (co-localization)

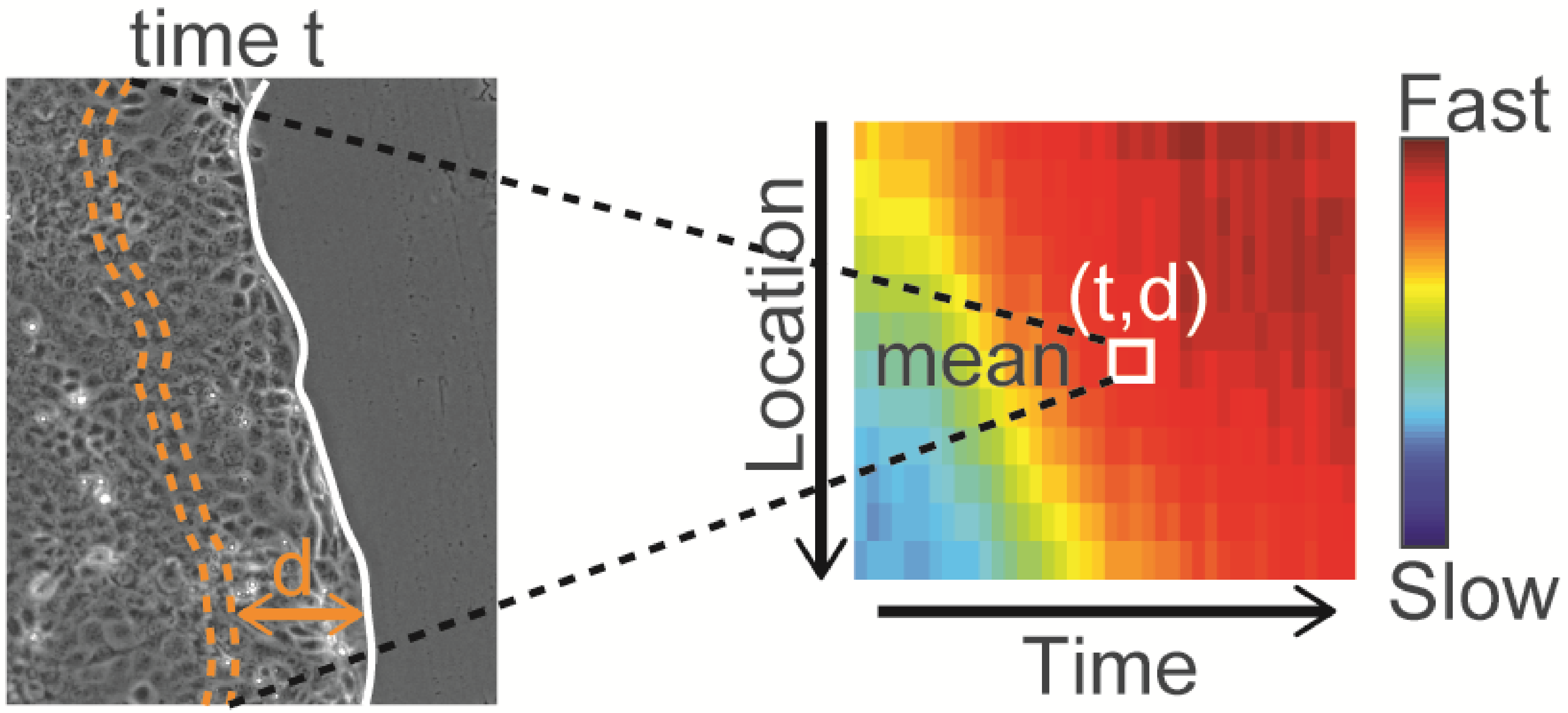
Rho-GTPases and their activators



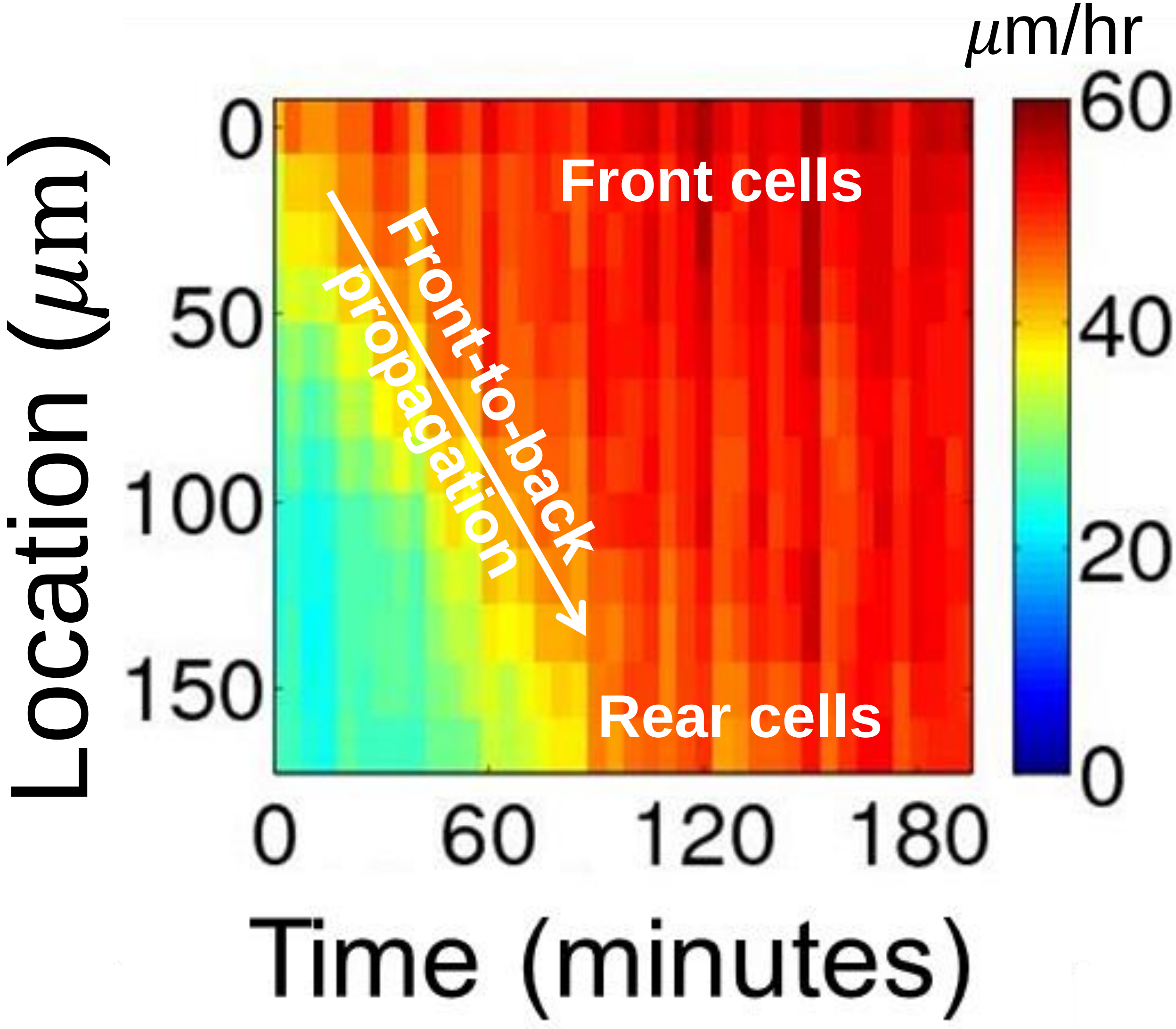
Down-regulation of Cdc42 and Rac1 but not RhoA disrupts monolayer migration



Spatiotemporal quantification



Visualizing front-to-back propagation

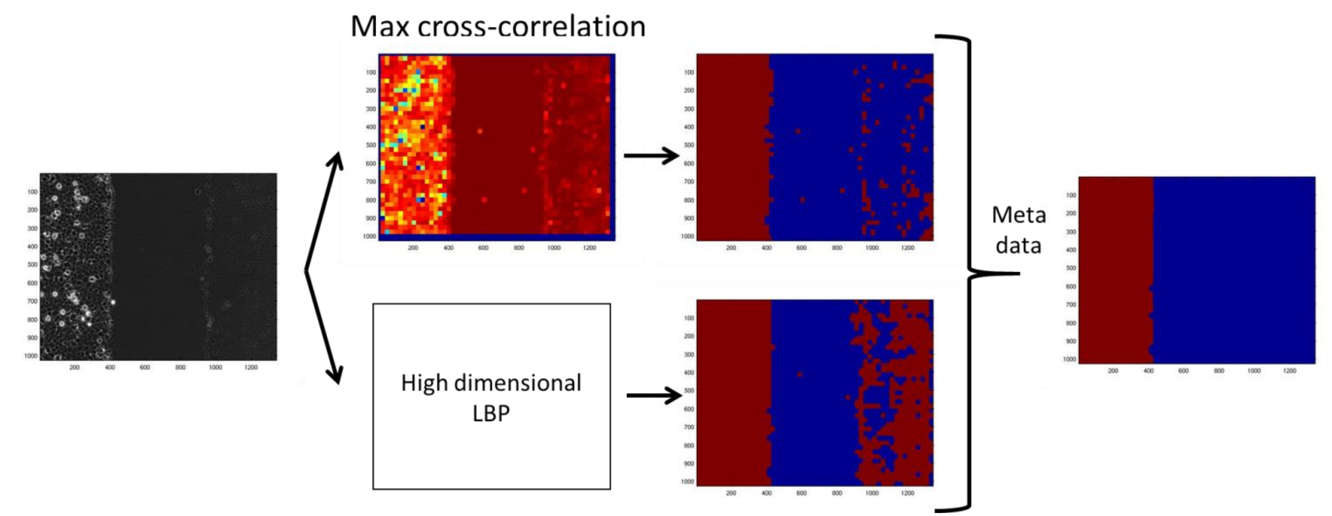


Two side notes

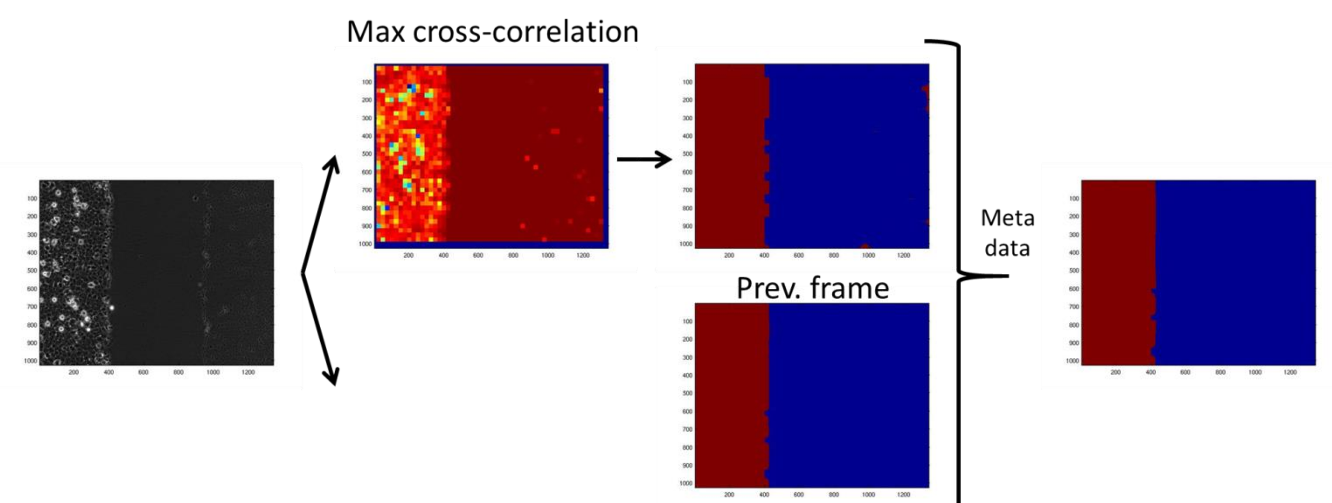
1. Segmentation
2. Flow fields vs. single cell tracking

Cellular/ background segmentation

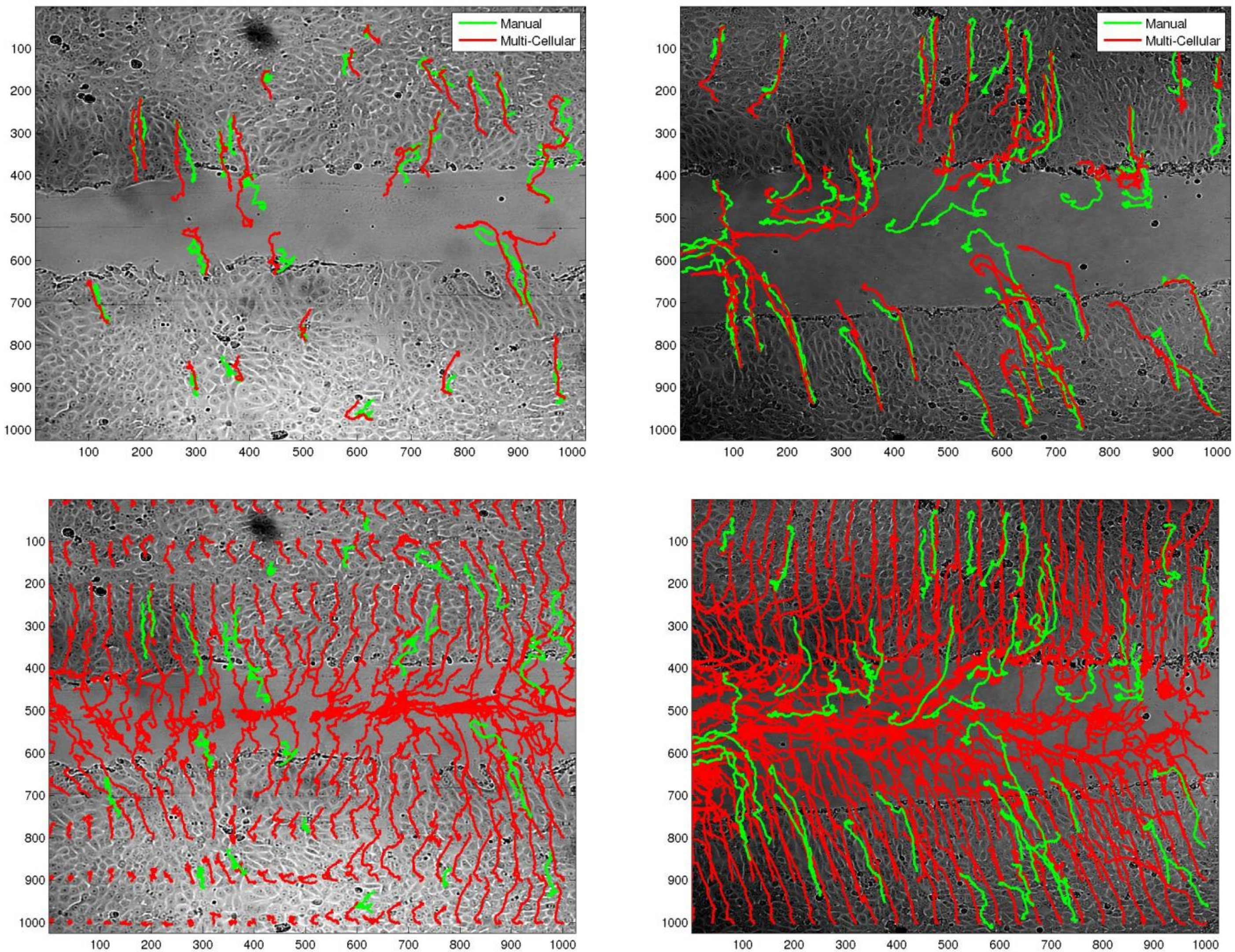
New Segmentation Algorithm: Frame 1



New Segmentation Algorithm: Frame > 1

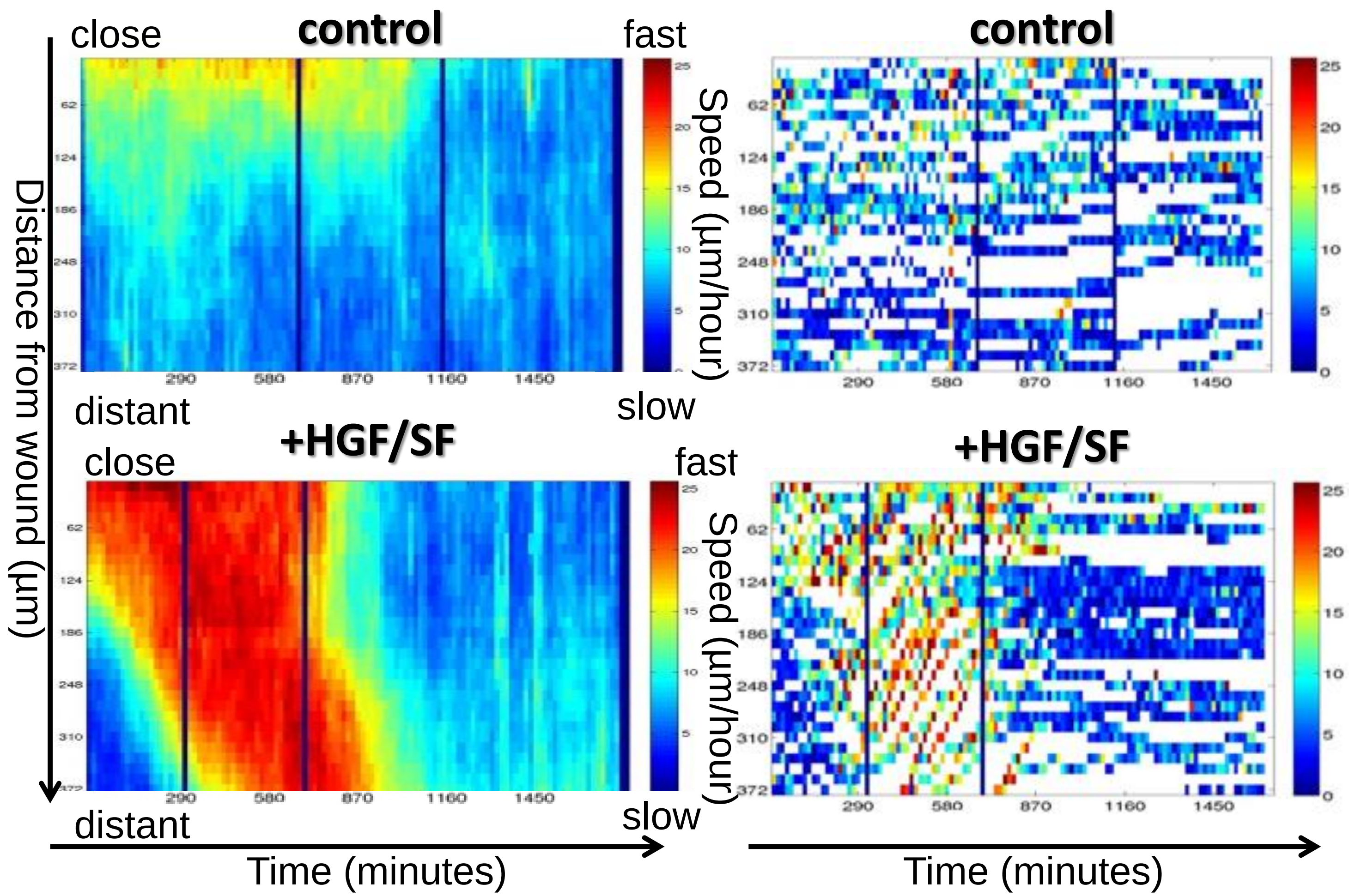


PIV versus (partial) cell tracking - exploiting Information from **all cells**



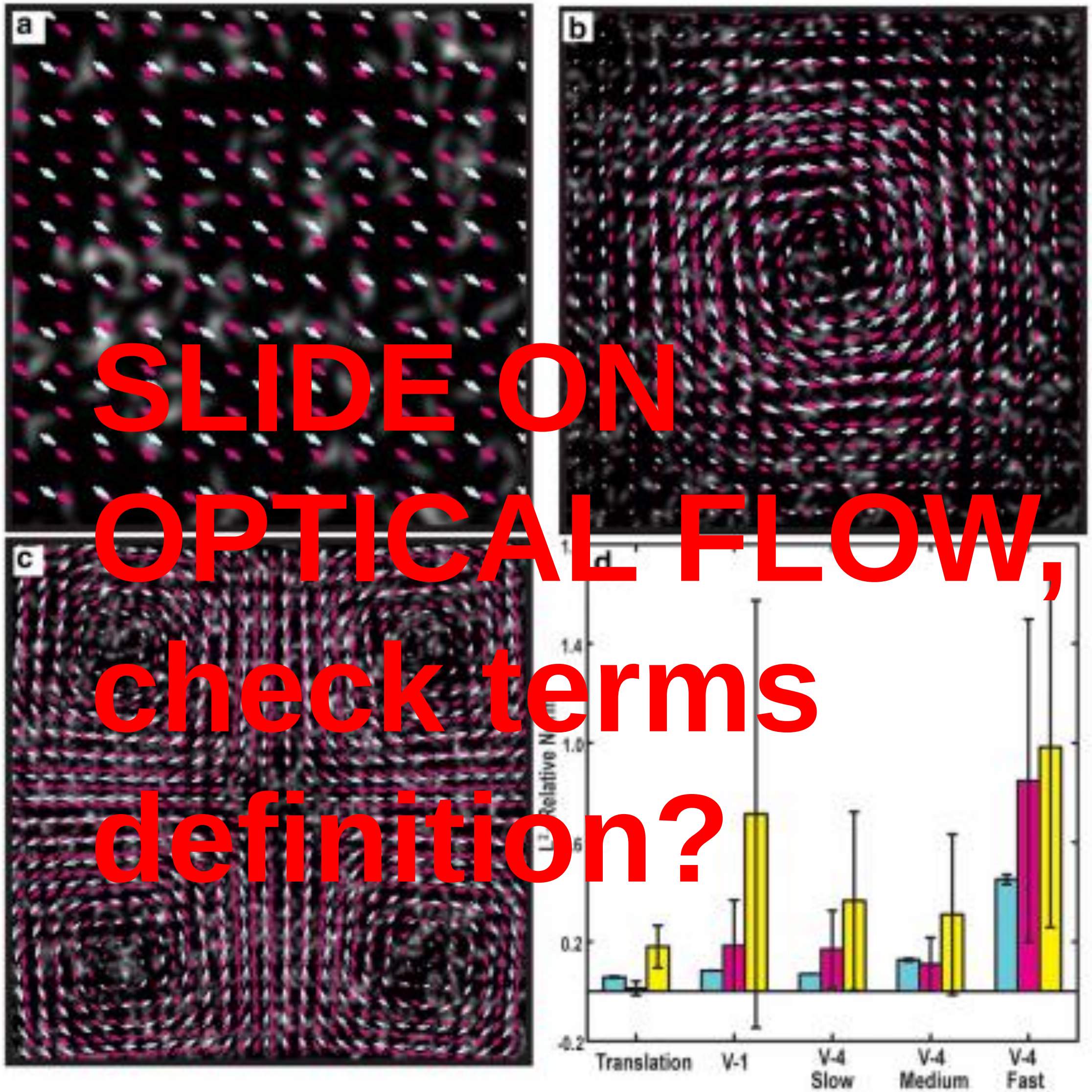
Zaritsky et al. (2012)

"Wisdom of Crowds"



Zaritsky et al. (2012)

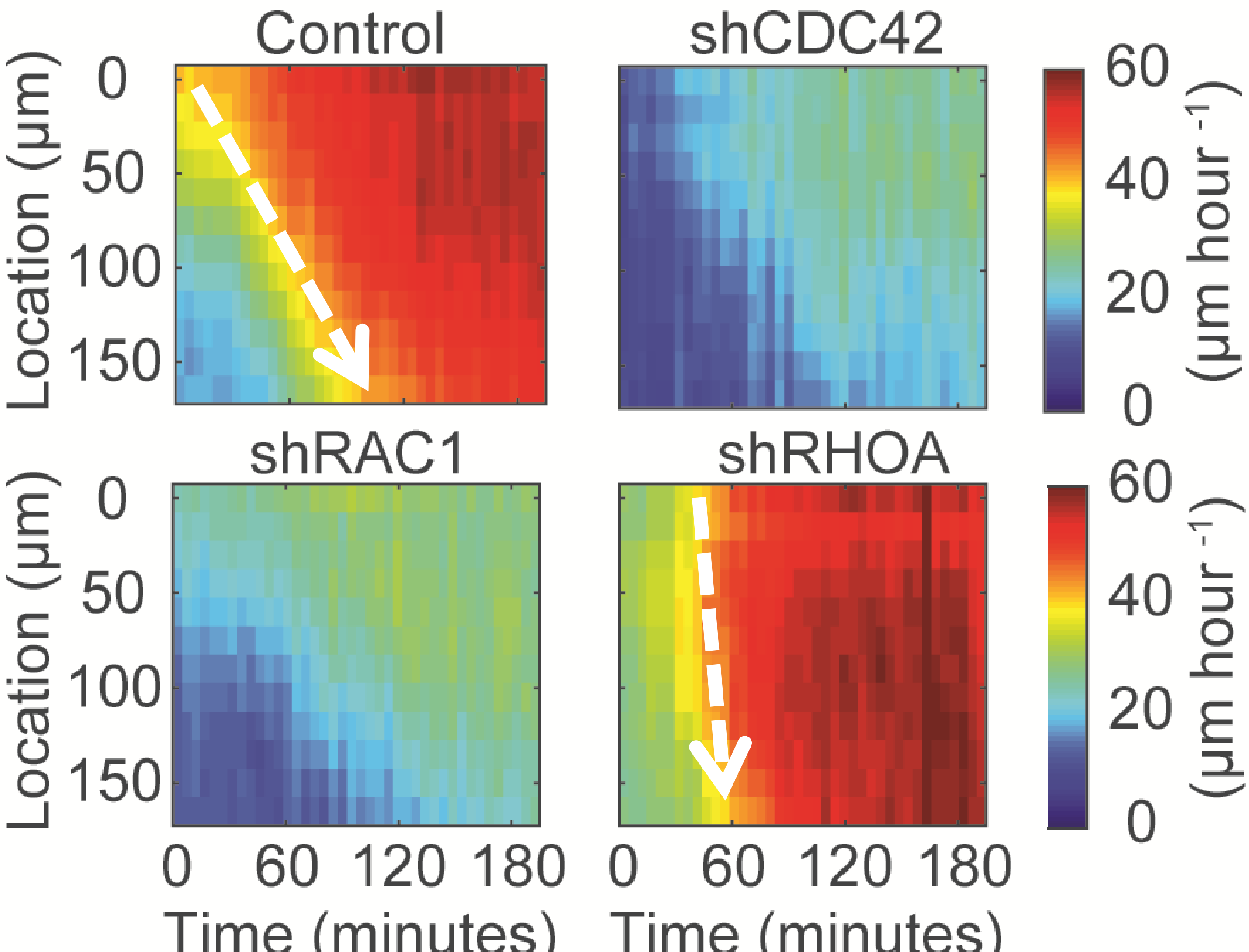
Optical flow versus PIV?



SLIDE ON OPTICAL FLOW, check terms definition?

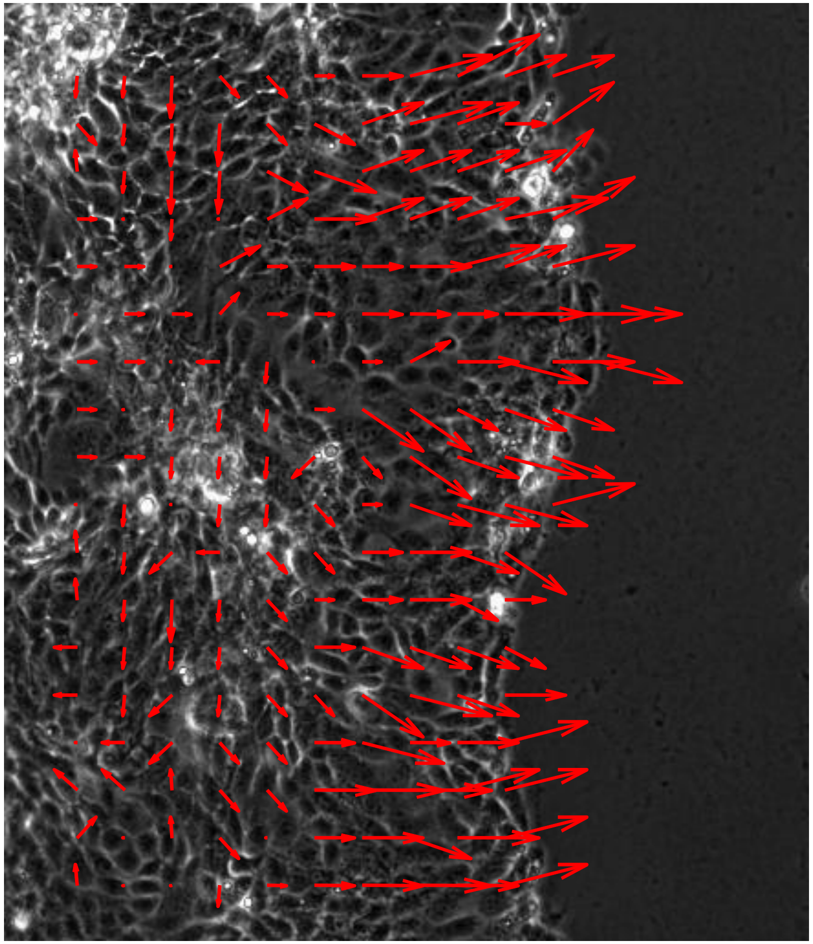
Vig et al. (2016)

Depleted RhoA enhances long-range communication

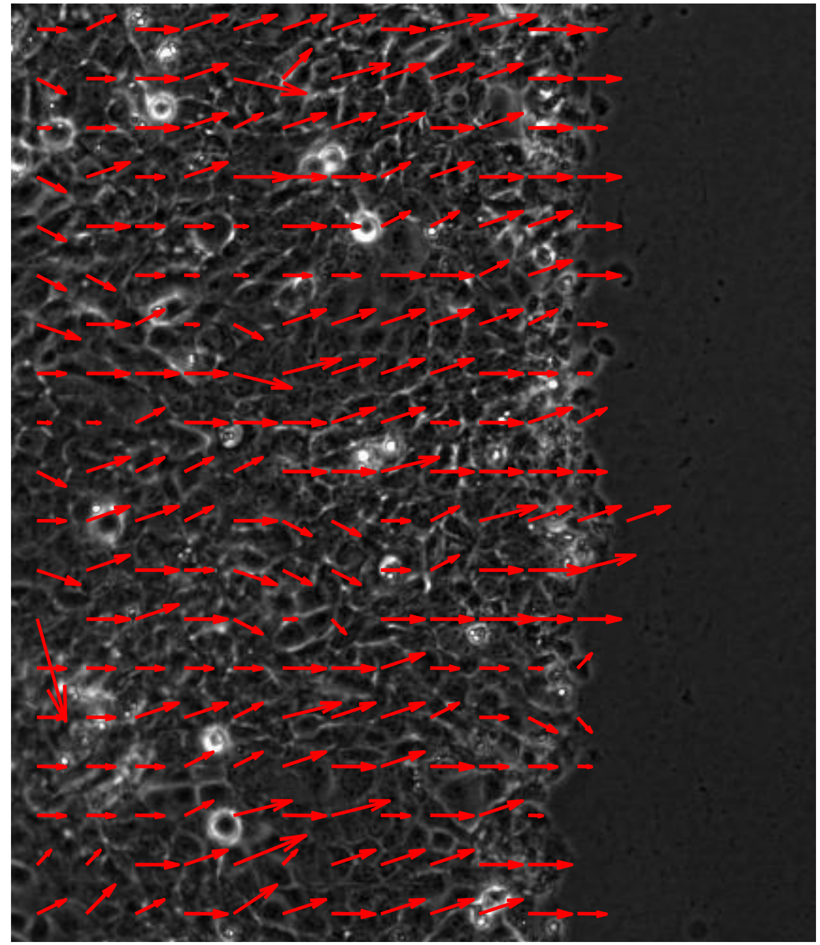


Depleted RhoA enhances long-range communication

Control



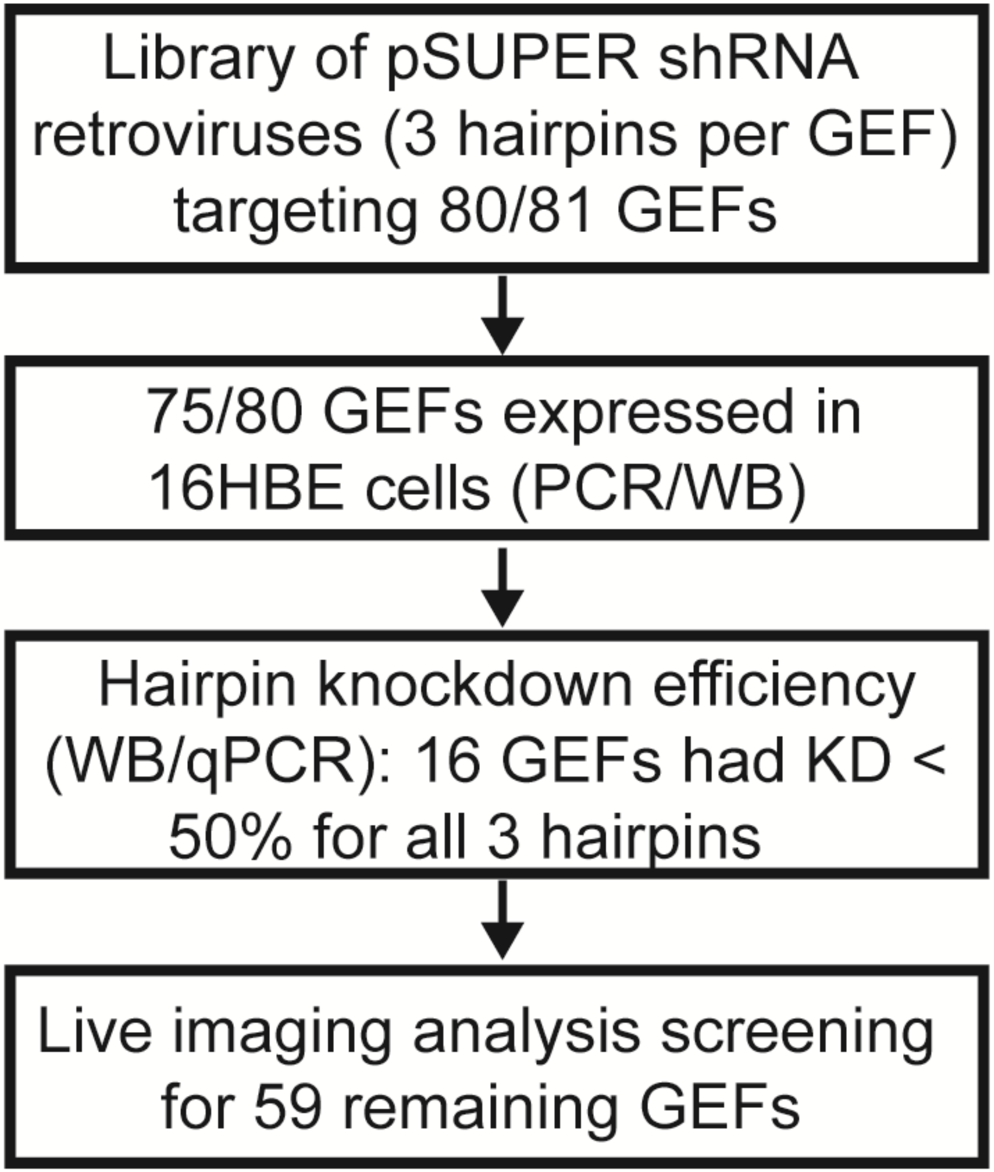
shRHOA



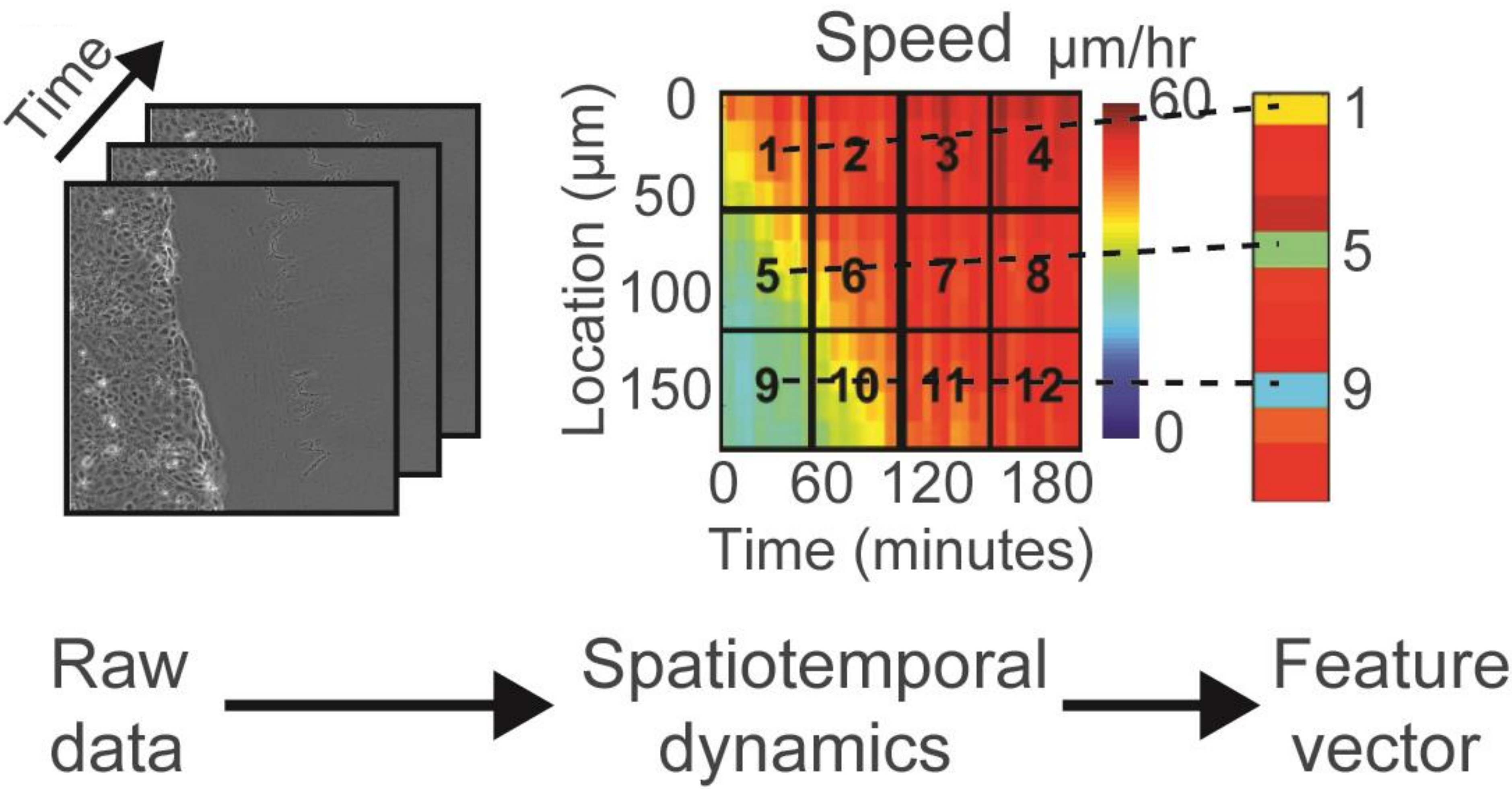
Comprehensive GEFs screen

- 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

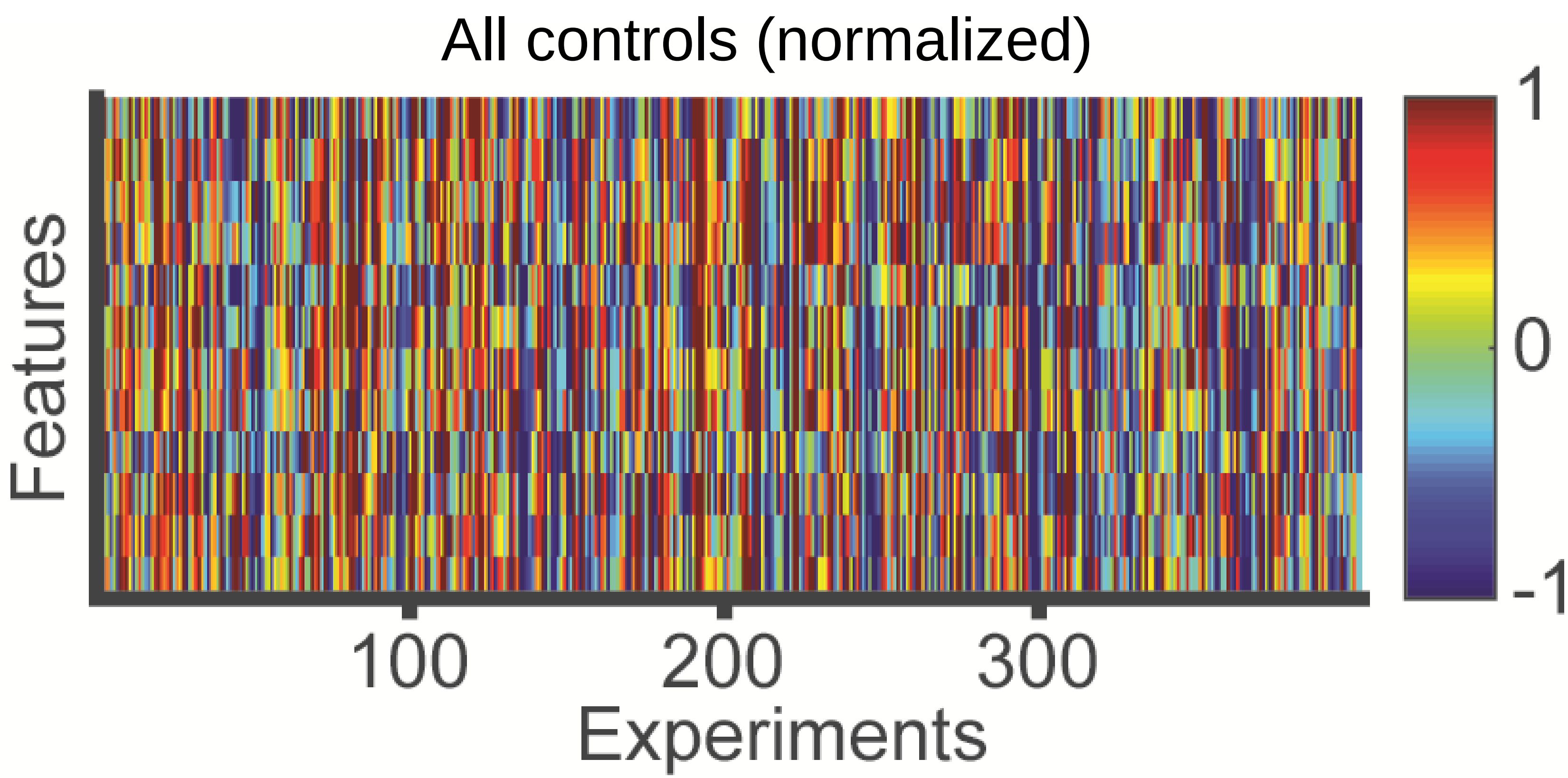
Comprehensive GEFs screen



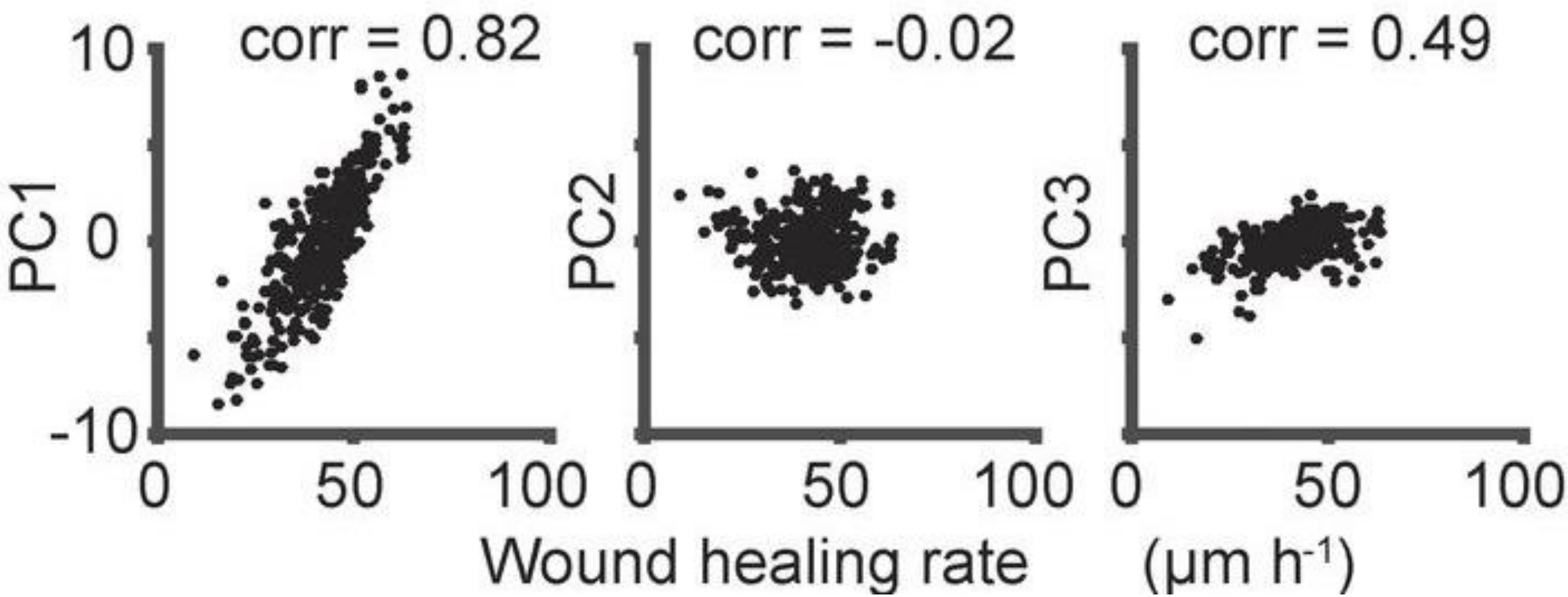
Encoding spatiotemporal dynamics



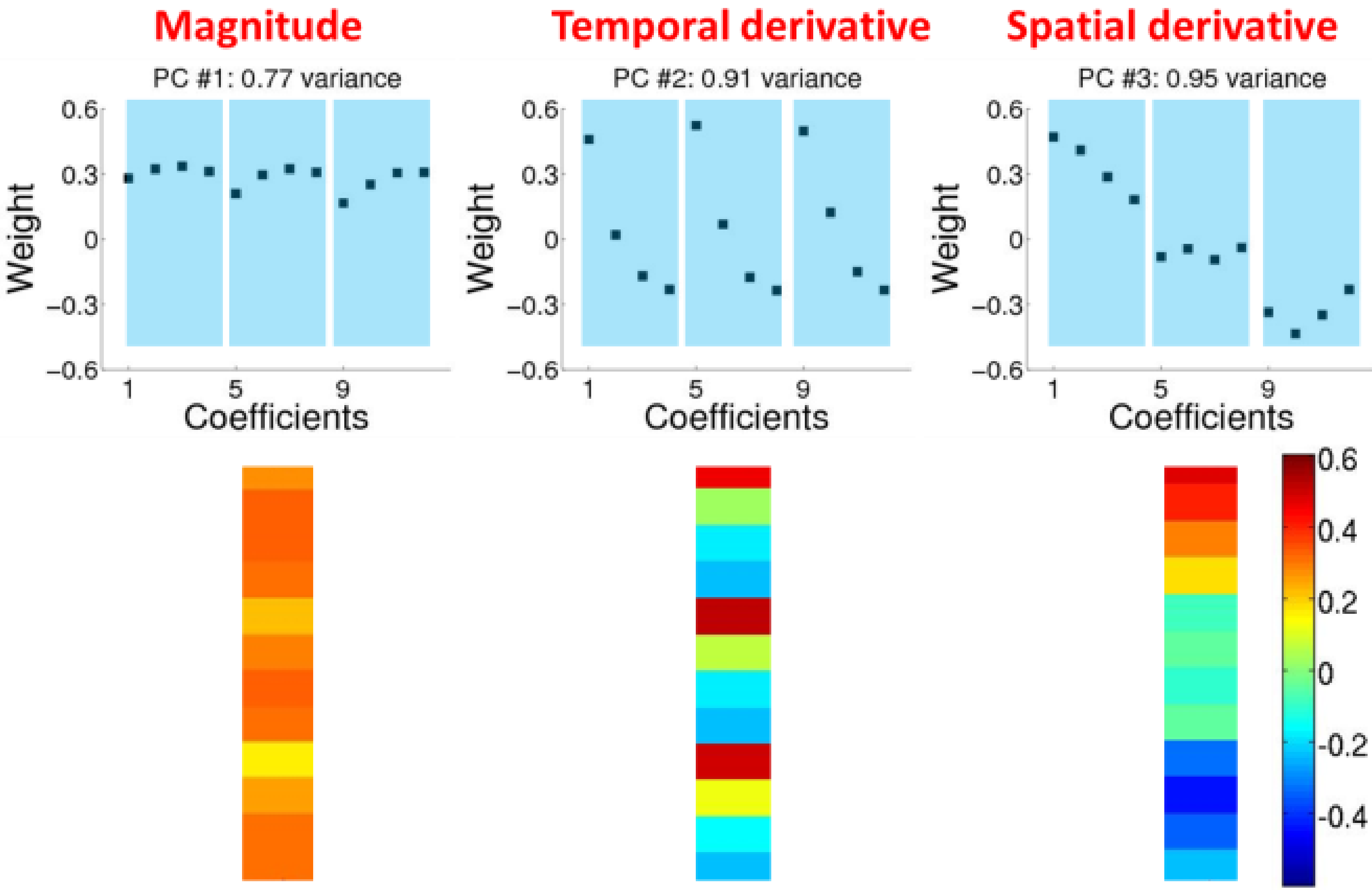
Information encoded in a wound healing experiment



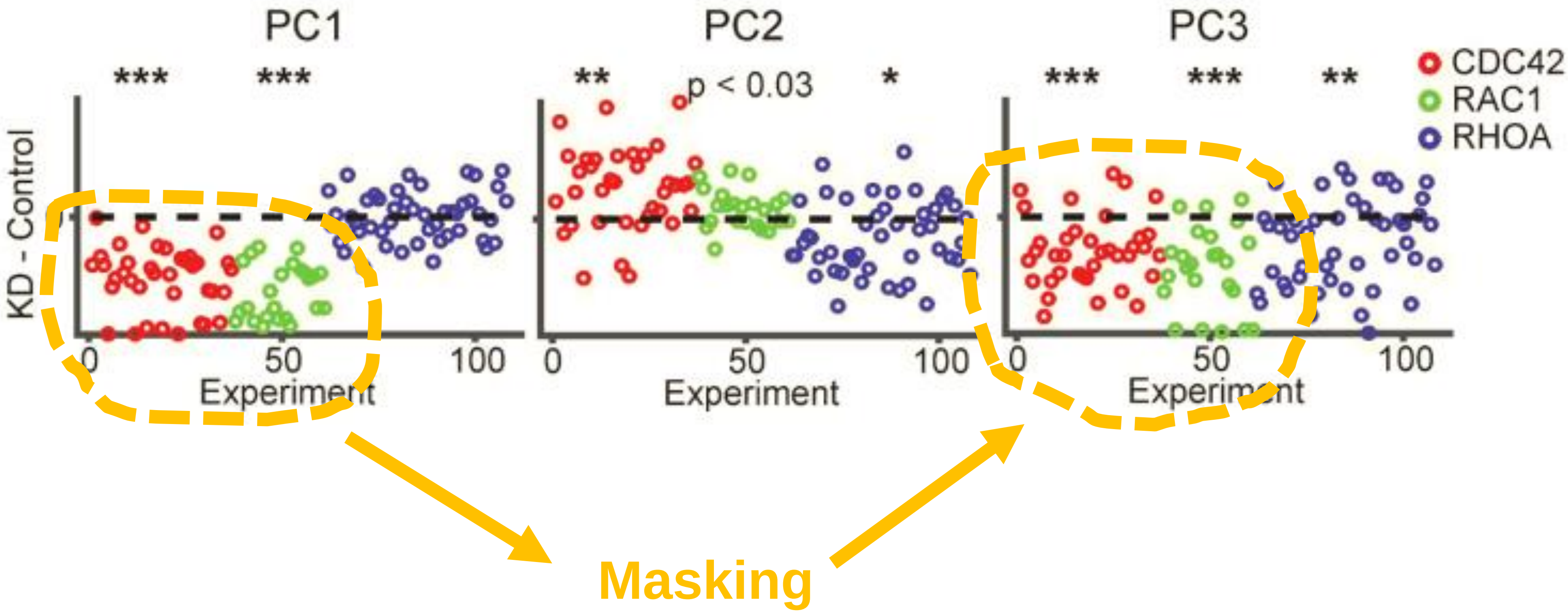
Association with monolayer migration rate



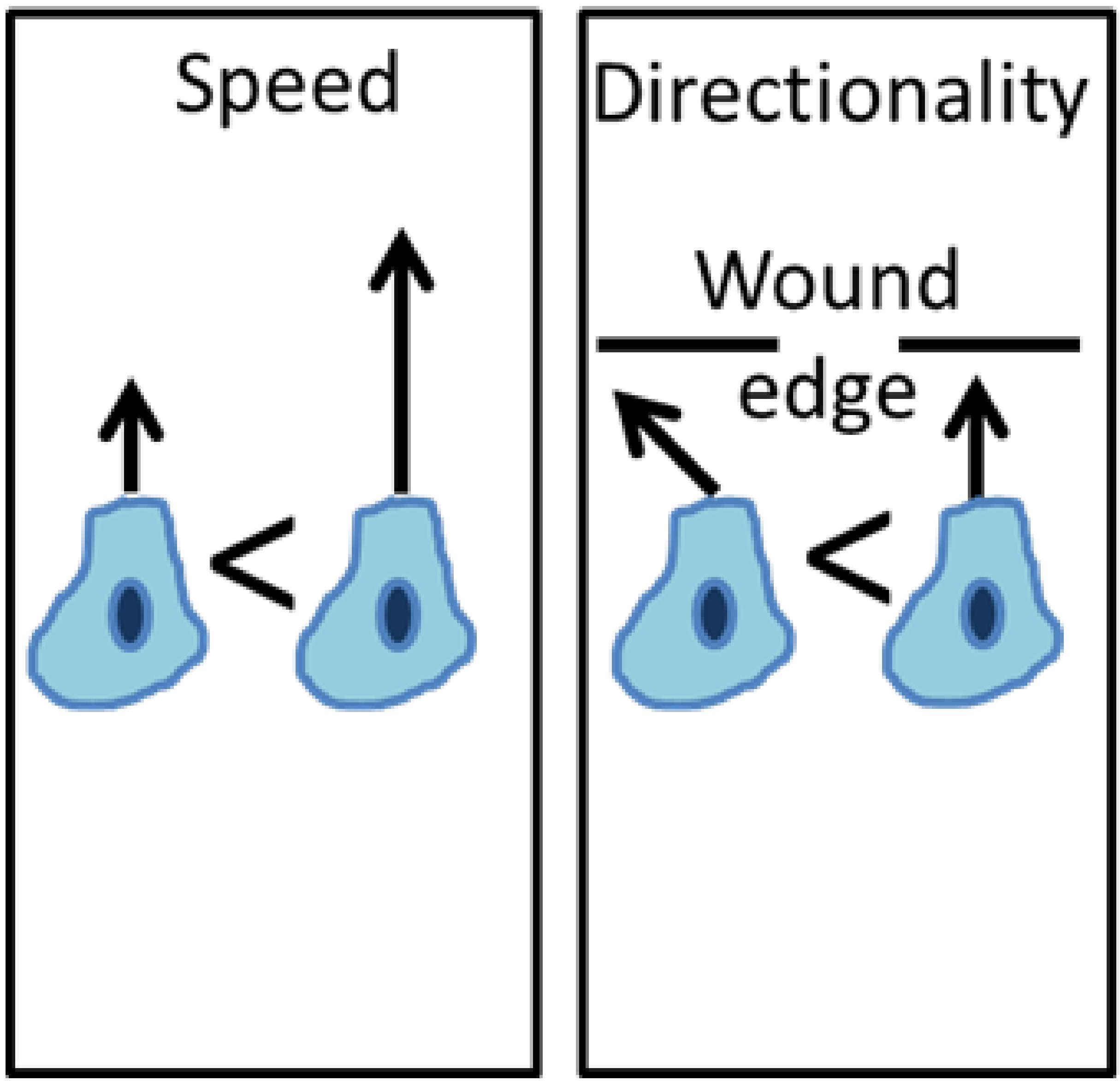
Reverse engineering: what information is encoded in an experiment?



Effect on PCs by depletion of canonical Rho GTPAses

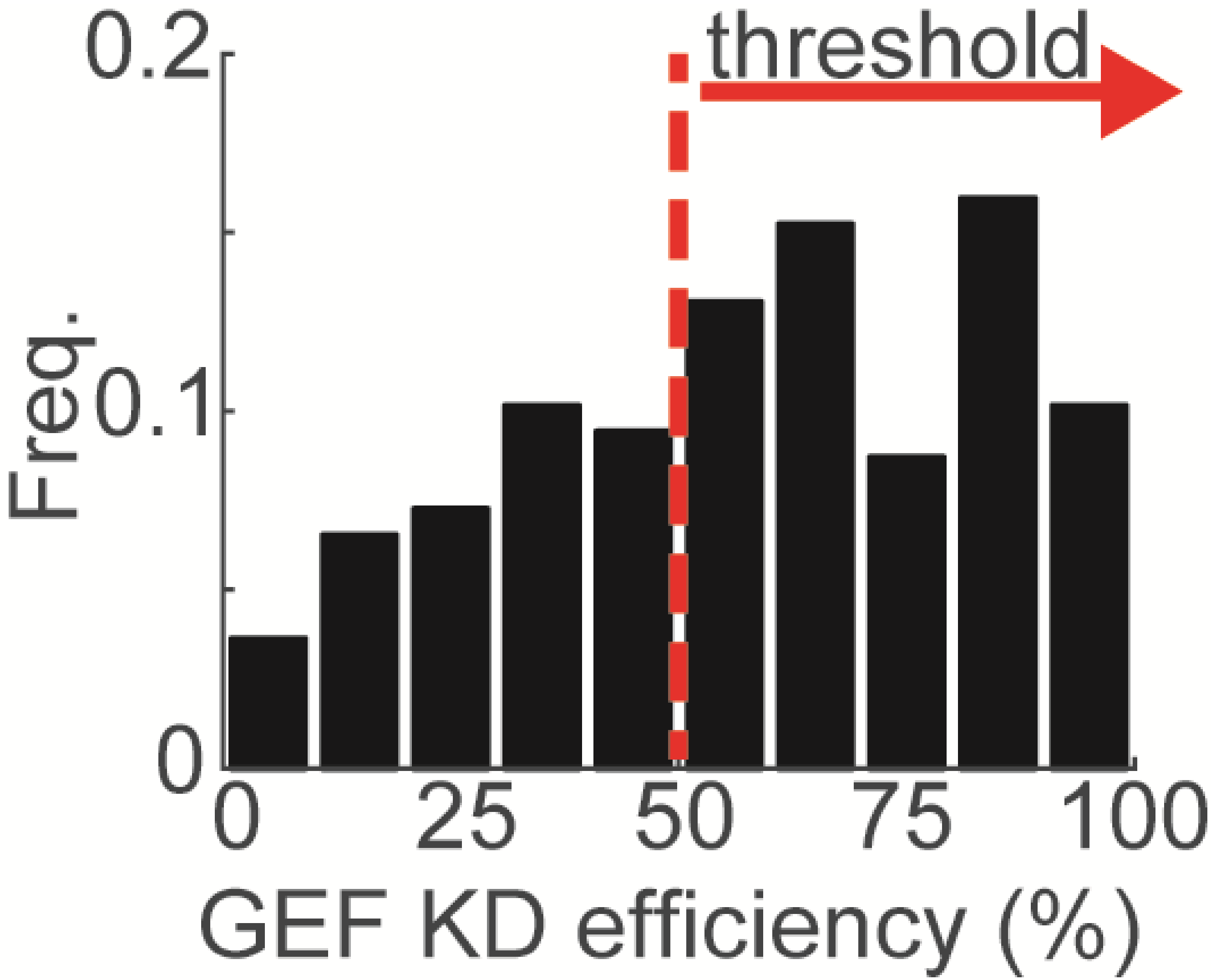


Measures

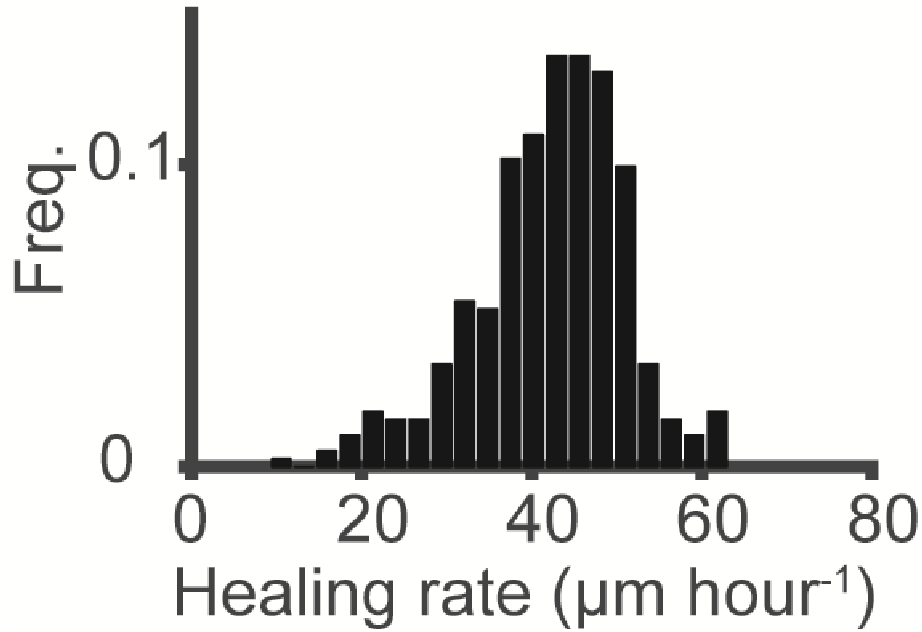


Screening methodology

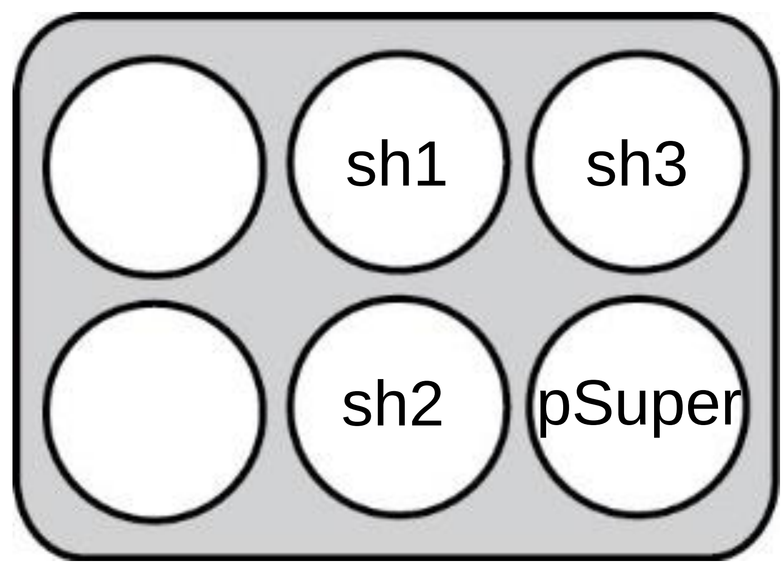
Knockdown efficiencies



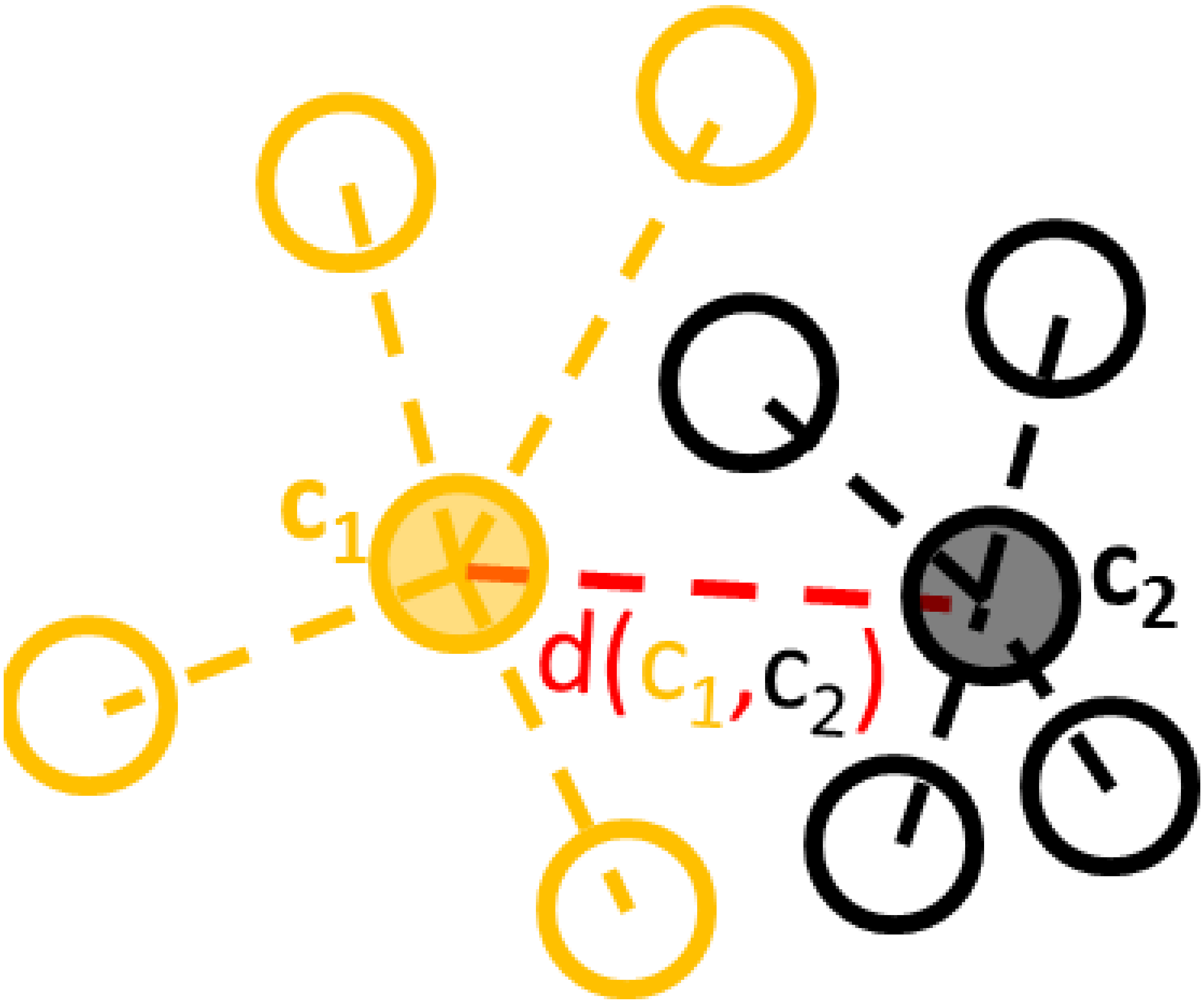
Inter-day variability in controls



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

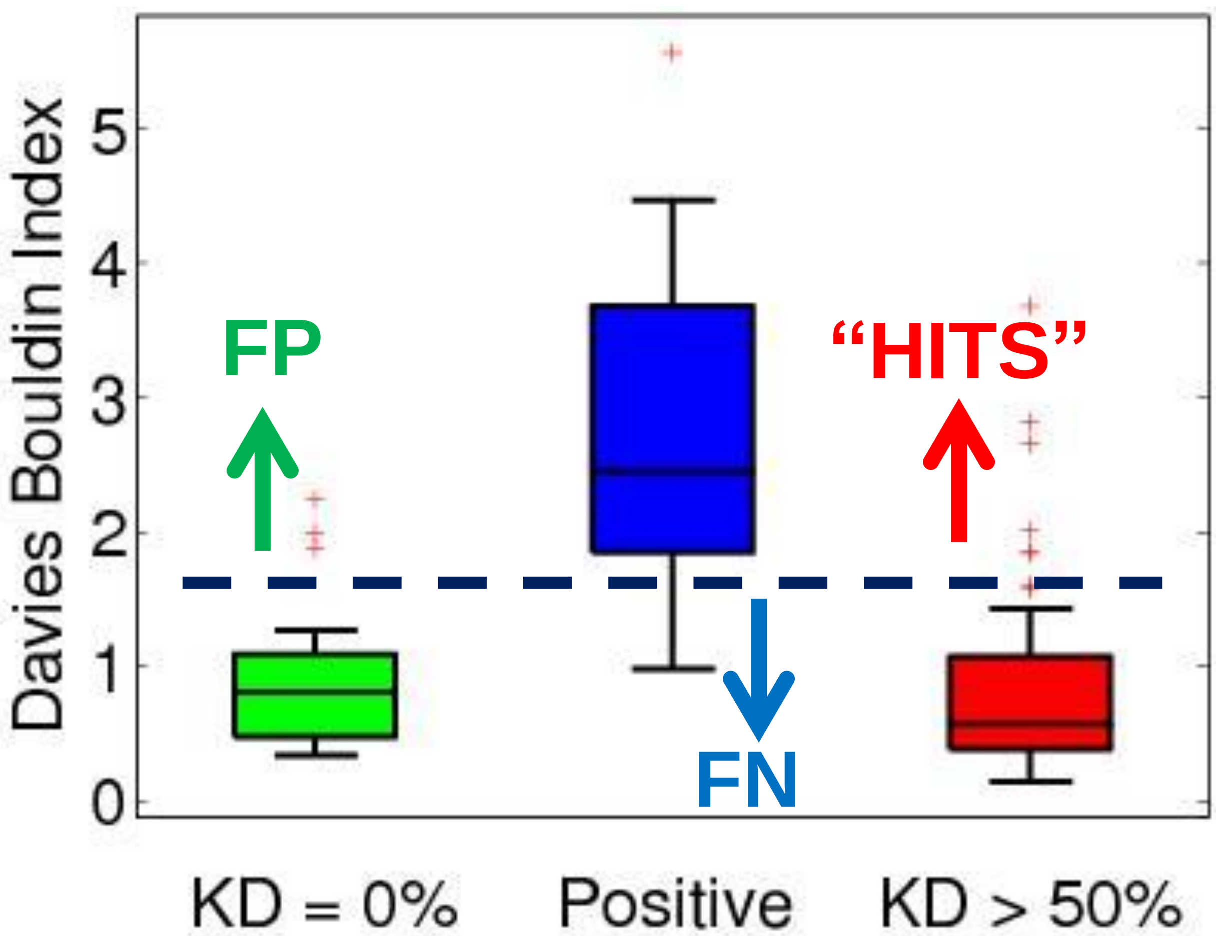


Scoring a knockdown phenotype

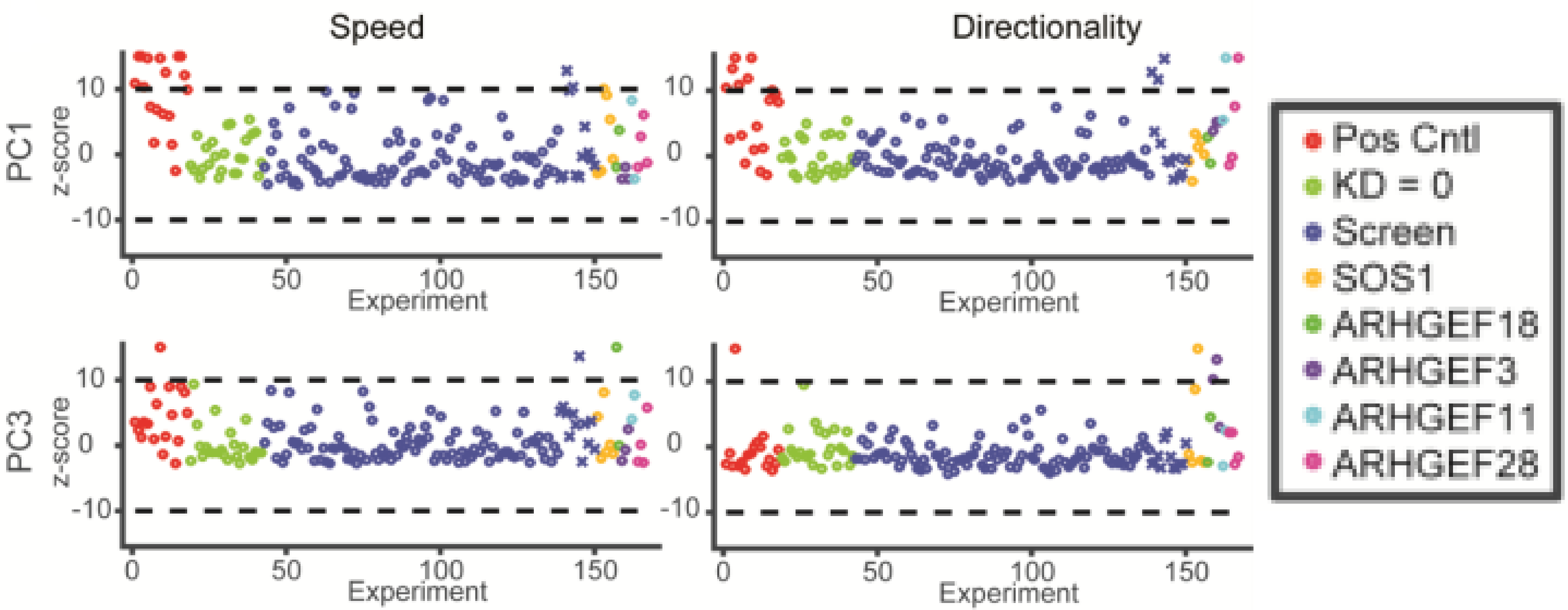


Quantifying off-target effects

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



Screen



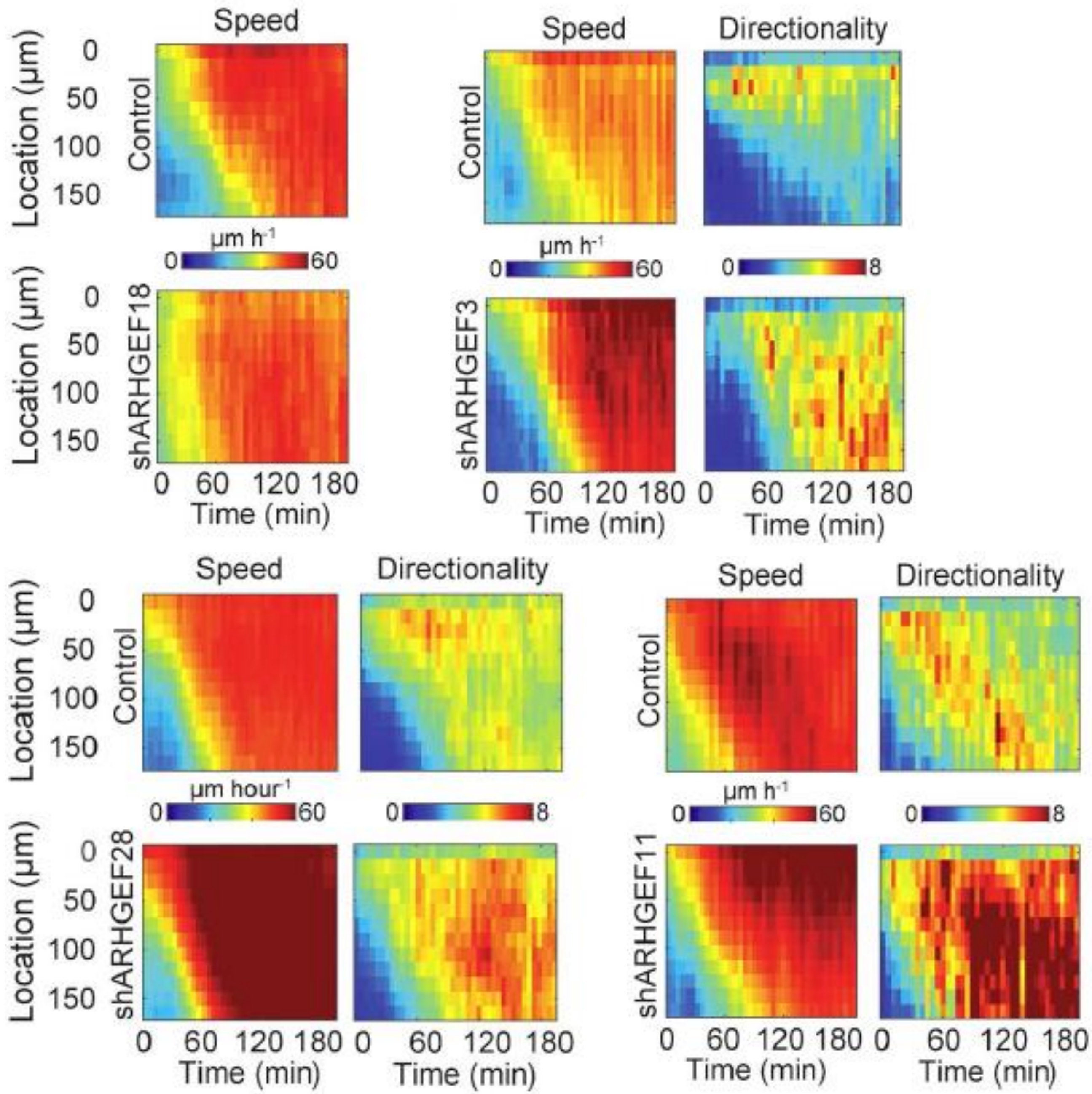
Speed
Directionality
PC1
PC3
z-score
10
0
-10
0
50
100
150
Experiment
Pos Cntl
KD = 0
Screen
SOS1
ARHGEF18
ARHGEF3
ARHGEF11
ARHGEF28

Screen hits

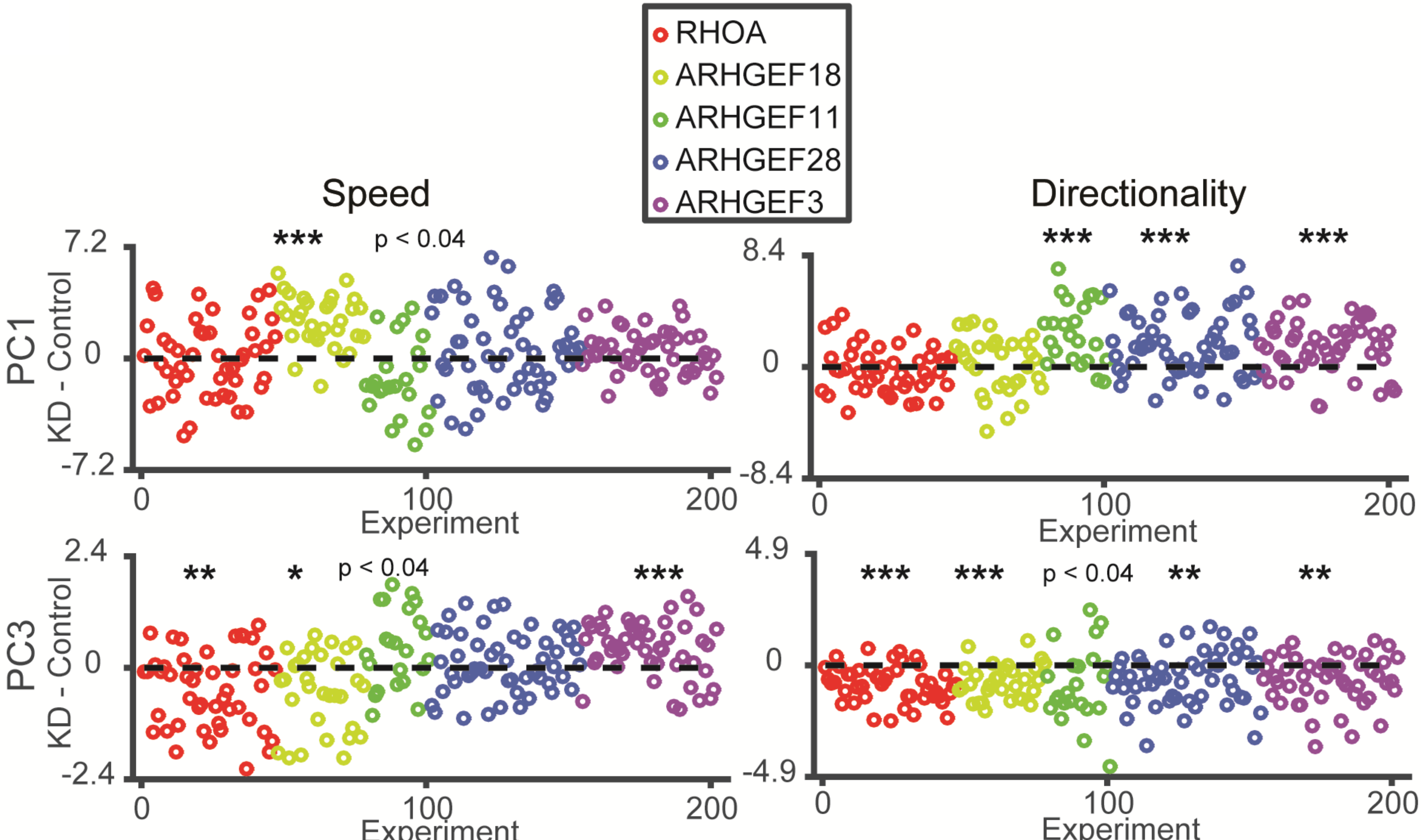
Condition	Long range communication (PC3)	Speed (PC1)	Directionality (PC1)
β-PIX		↓	↓
SOS1	✗	↓	↓
ARHGEF18	↑		
ARHGEF11			↑
ARHGEF28			↑
ARHGEF3	↑		
ARHGEF10			↑
TRIO		↓	↓
TUBA		↓	↓
ARHGEF9	✗		
DOCK10*			

* - hit in directionality PC2, details in legend

Screen hits - visualization



Validation
(and discovering new phenotypes)

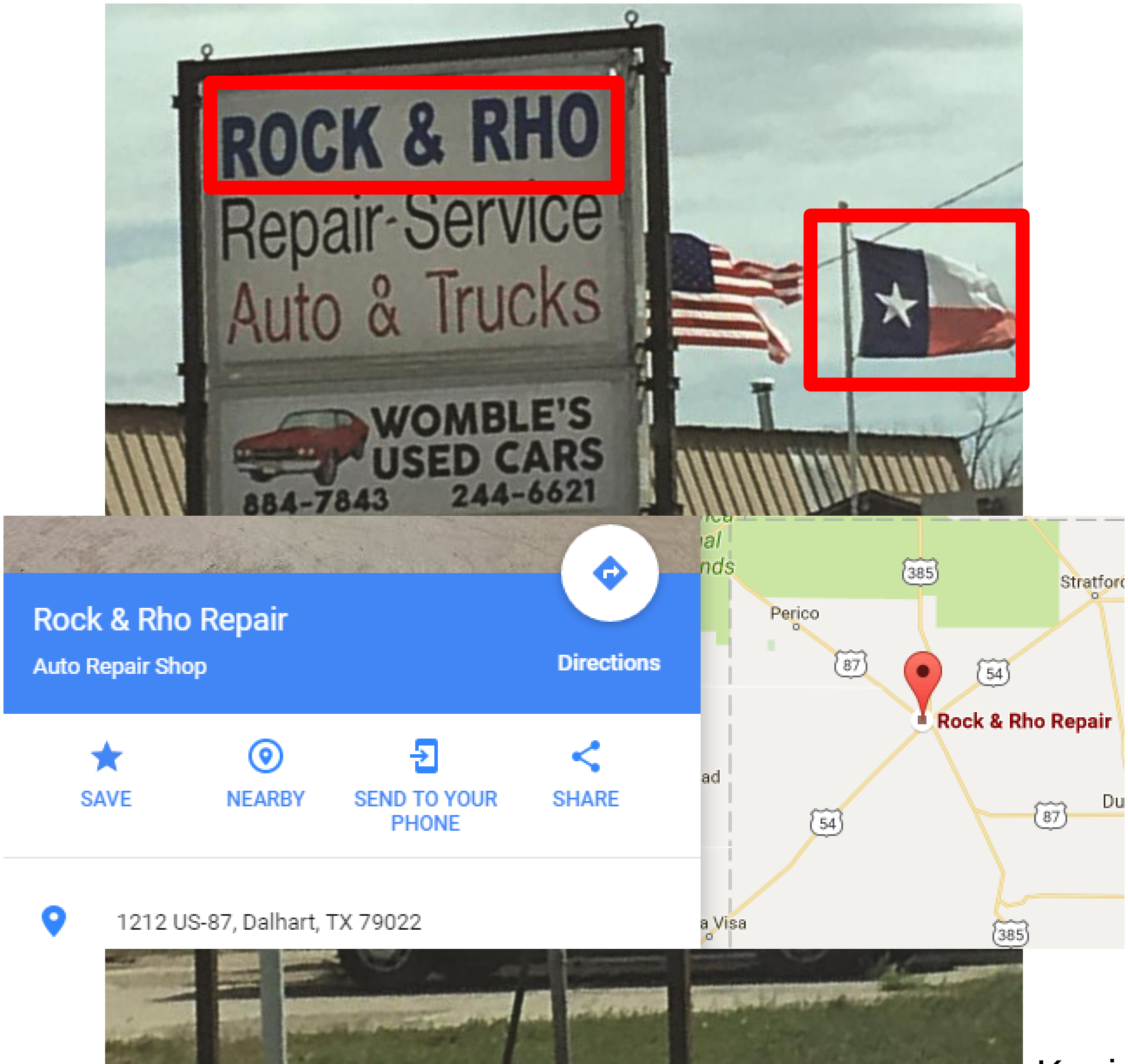


Greatest hits

RhoA GEFs inhibit long-range communication

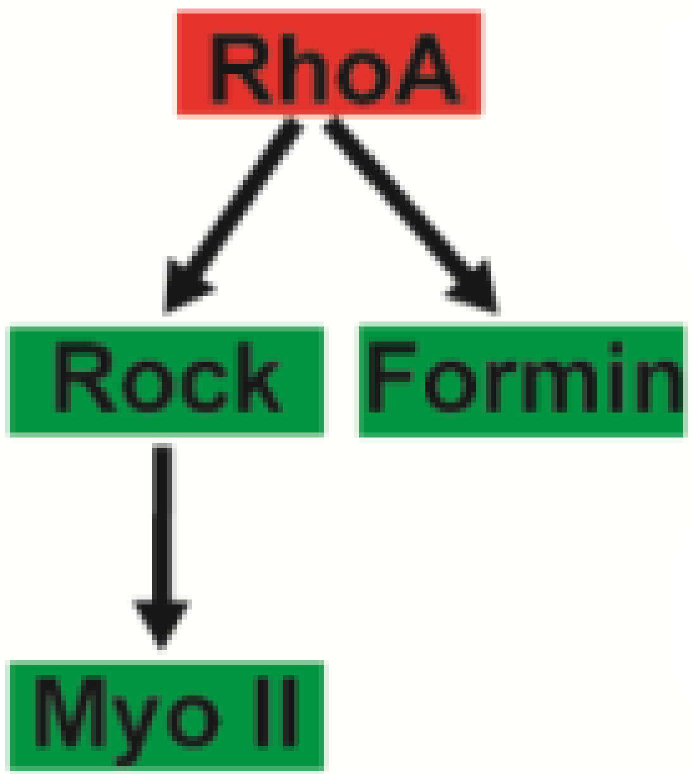
Condition	Short range communication (PC1 coordination)	Long range communication (PC3)	Speed (PC1)	Directionality (PC1)
SOS1-RAS	↓↓↓		↓↓↓	↓↓↓
ARHGEF3		↑↑		↑↑↑
ARHGEF11				↑↑↑
ARHGEF28	↑↑↑	↑↑		↑↑↑
ARHGEF18		↑↑↑	↑↑↑	
RHOA		↑↑↑		

What about Myosin?



Kevin Dean, @kD3AN

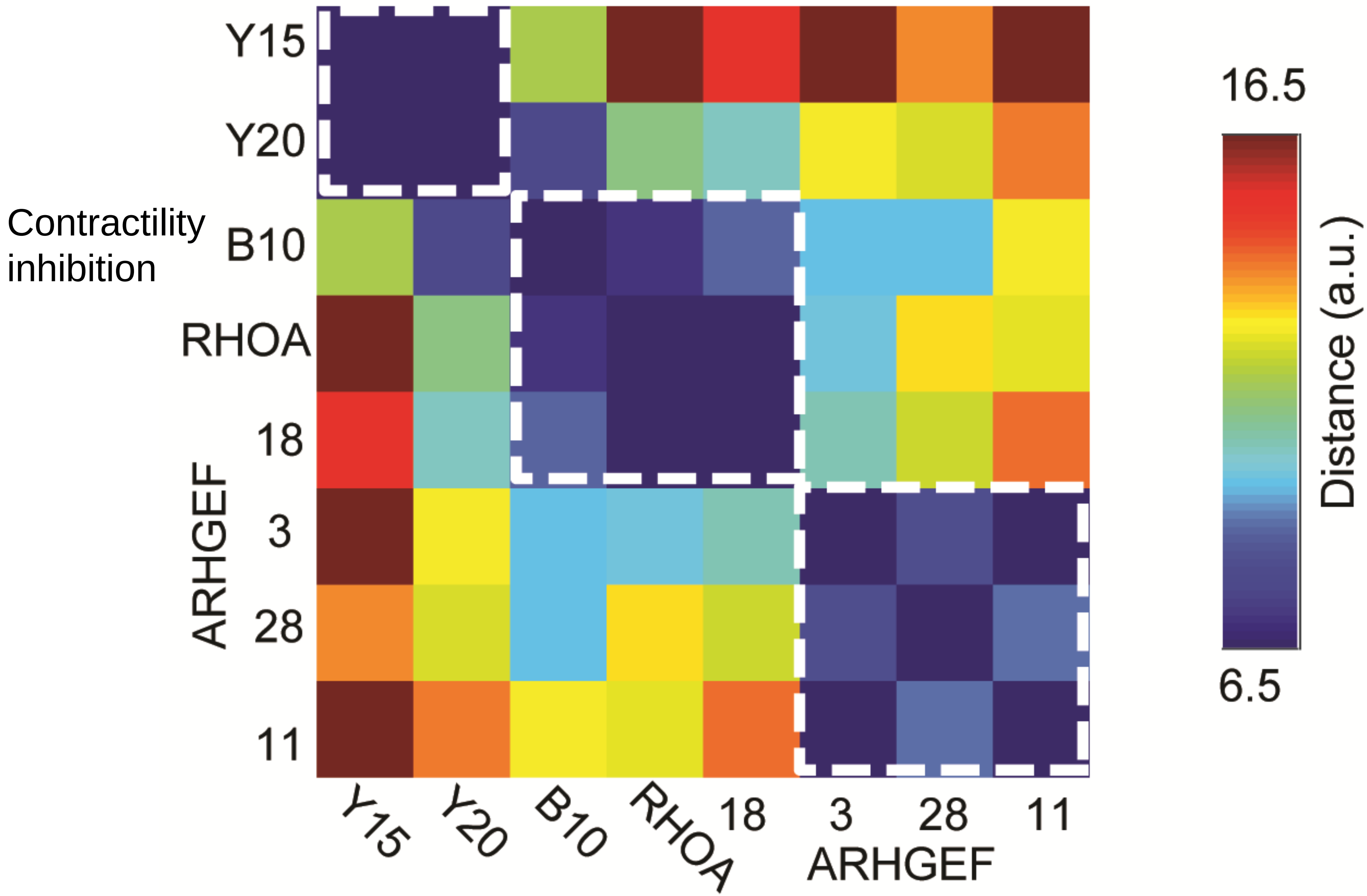
Downstream effectors of RhoA



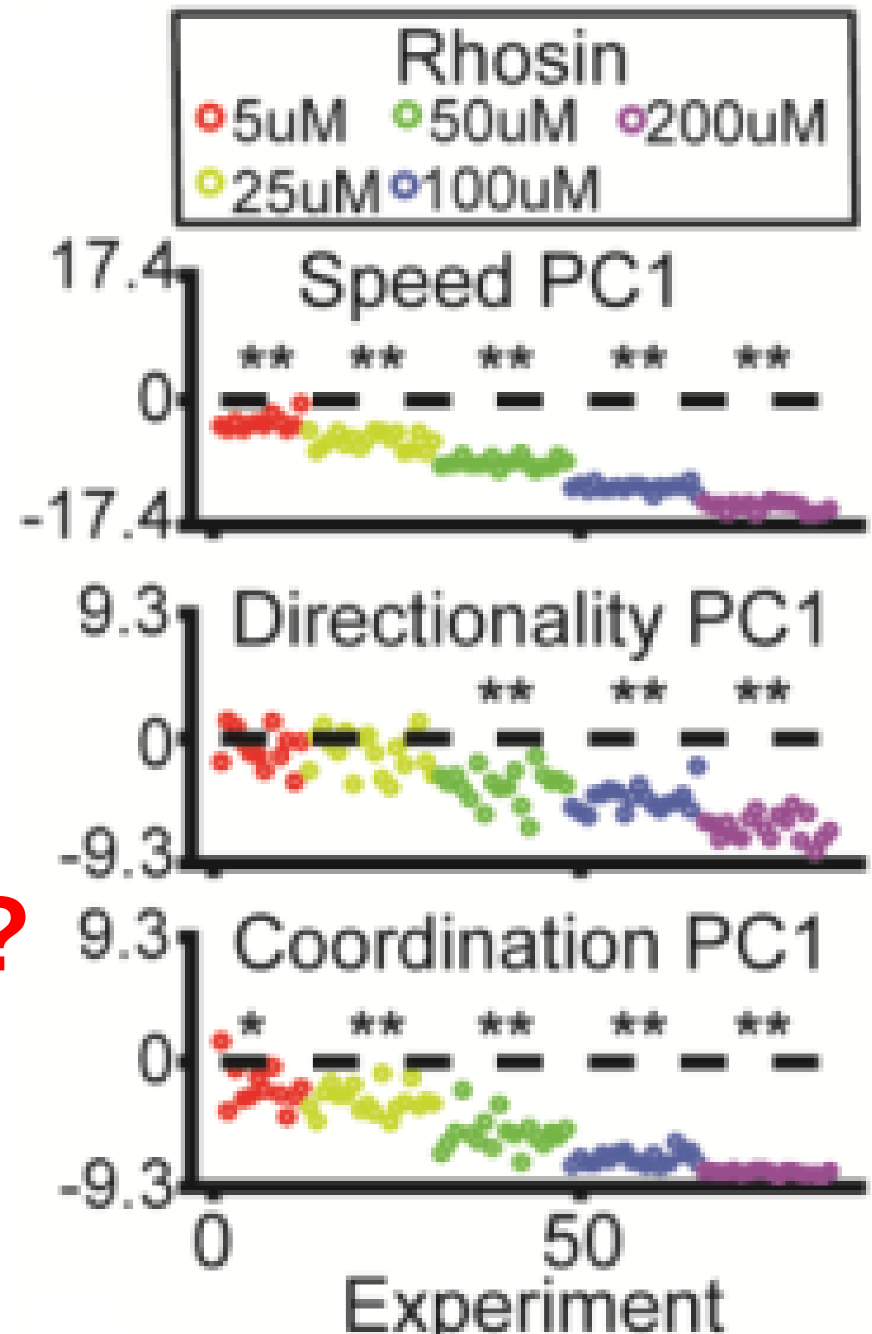
Actomyosin contractility disturbs intercellular communication downstream of the ARHGEF18 - RHOA pathway

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

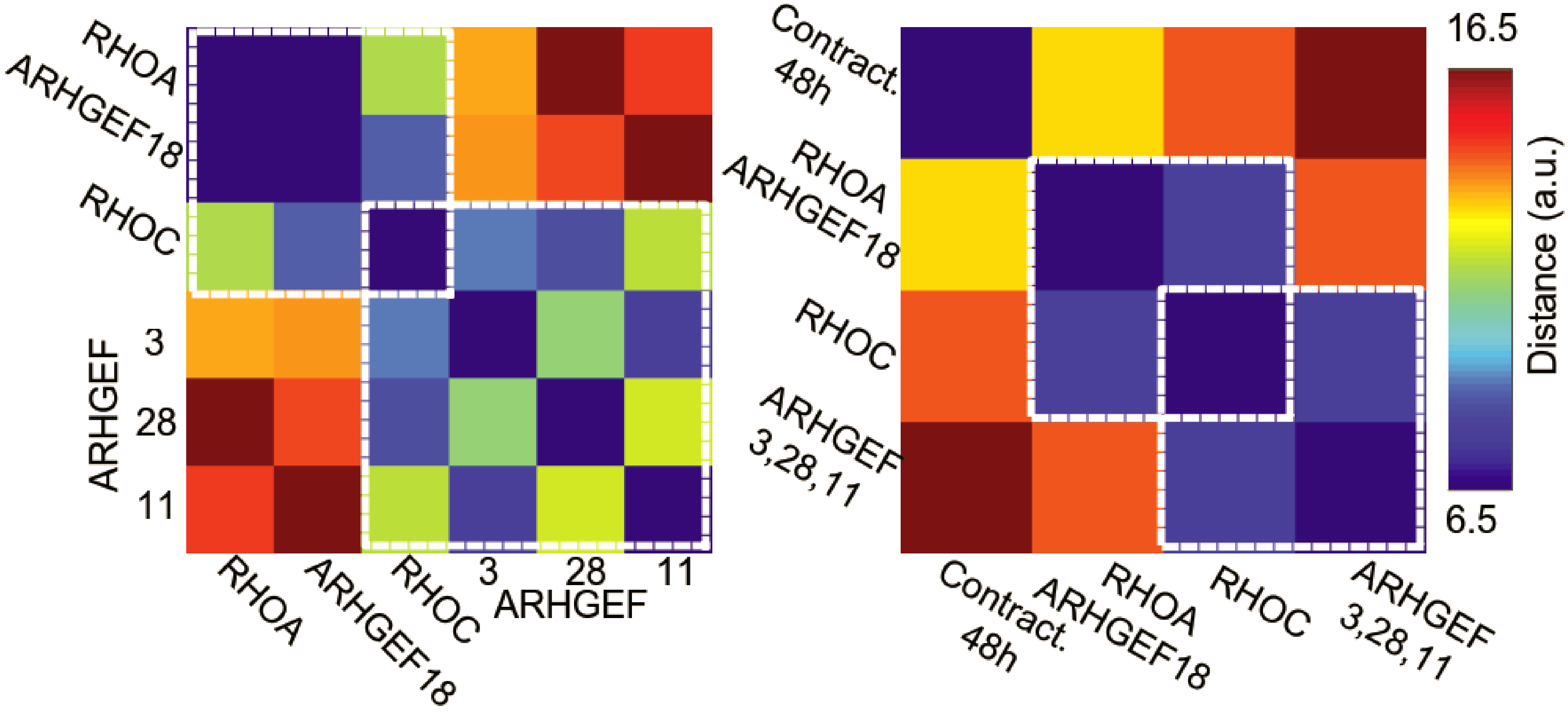


Rho isoforms are required for collective migration

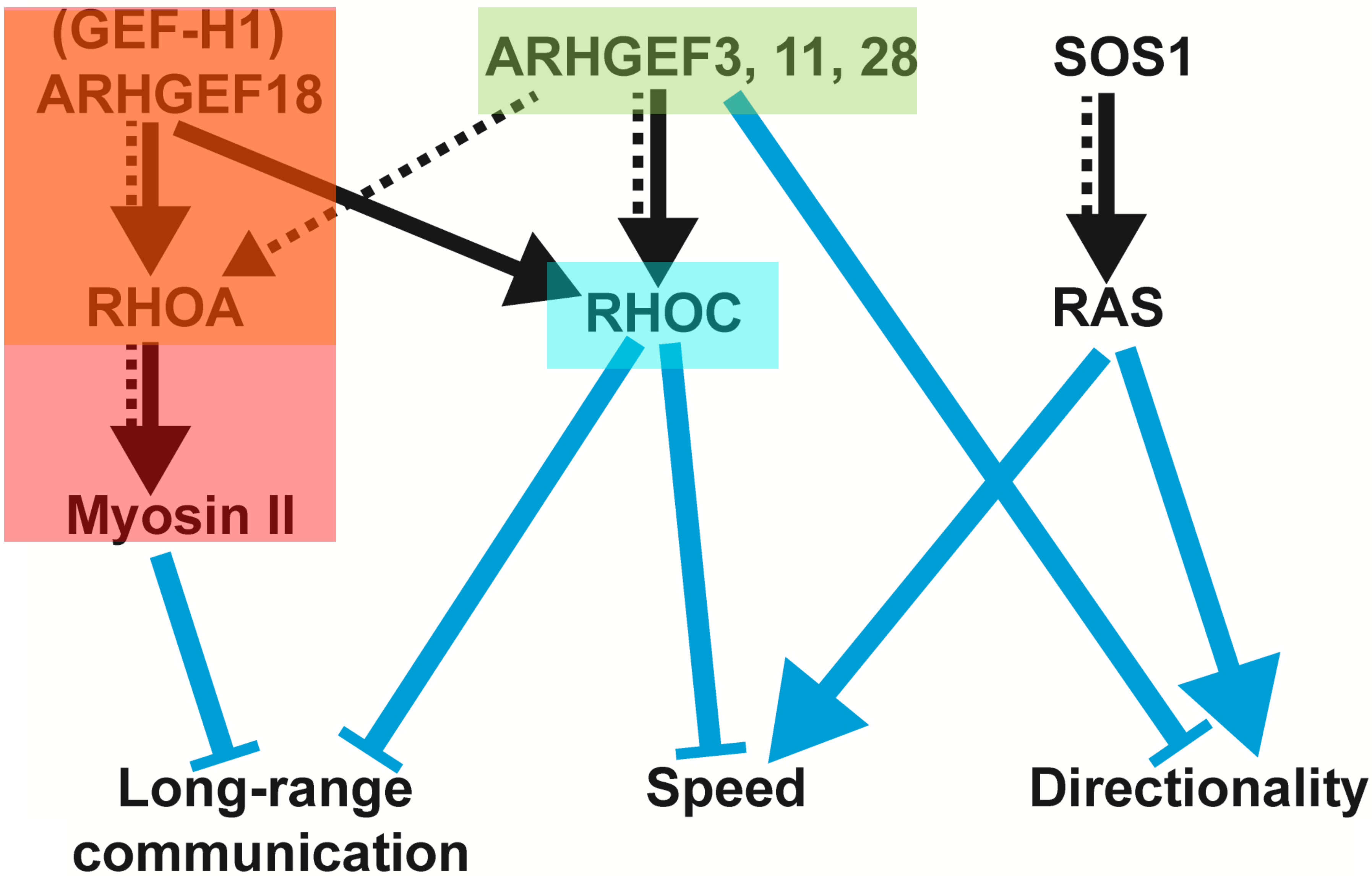


Reproduce this figure as exercise?

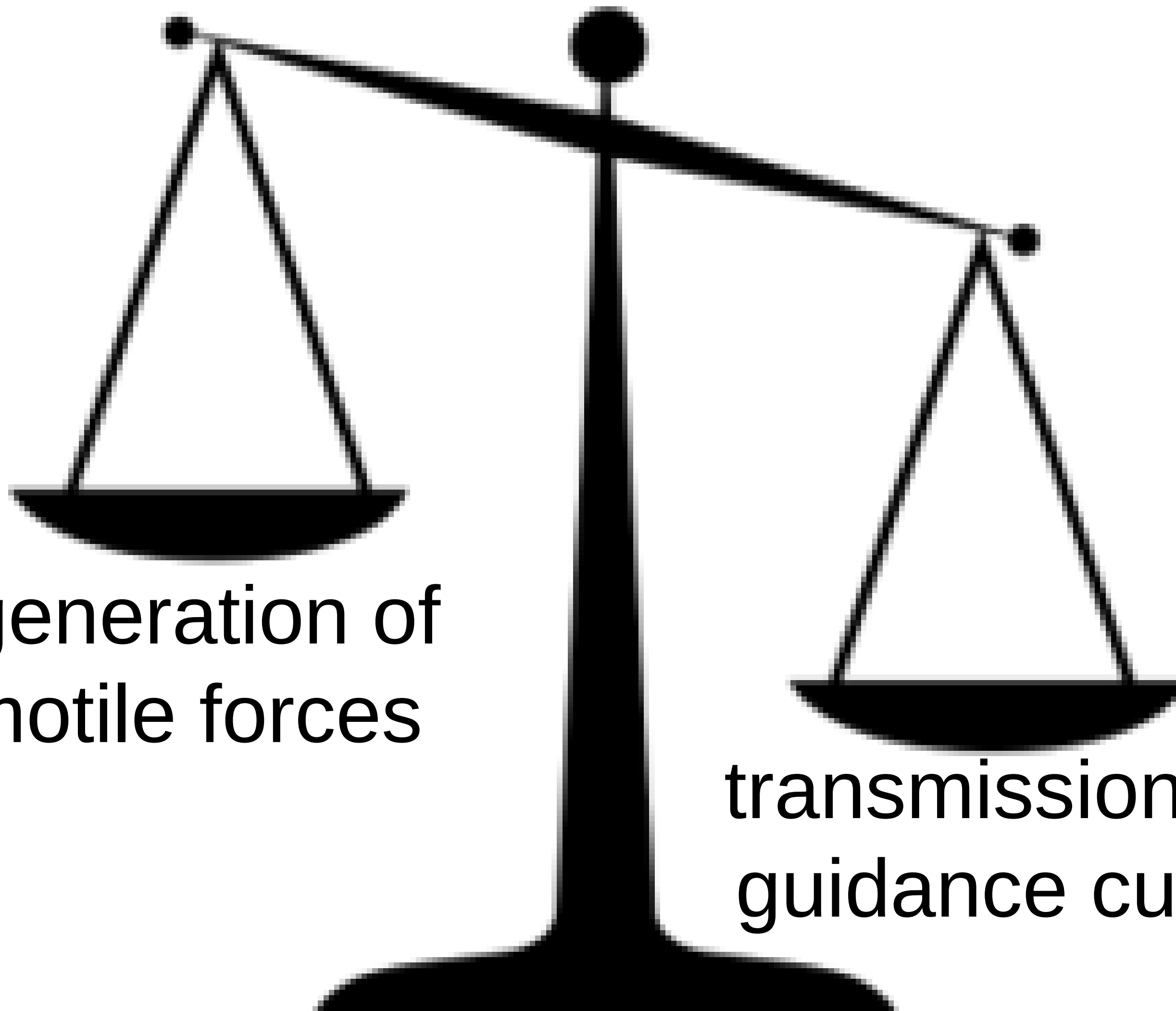
RhoC has an intermediate phenotype



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

Source code:

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

Acknowledgments

Yun-Yu Tseng

Ángeles Rabadán

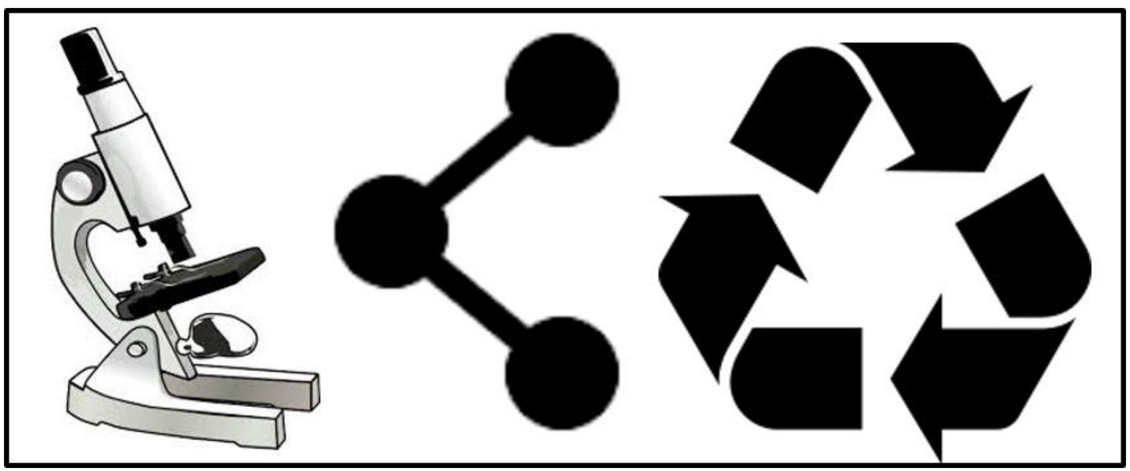
Shefali Krishna

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!

ibec Institute for bioengineering of Catalonia

Memorial Sloan Kettering Cancer Center

Xavier Serra-Picamal

Xavier Trepat

Yun-Yu Tseng

Angeles Rabadan

Joachim Spatz

Tamal Das

Exercise

1. Execute the workflow on a single video, visualize kymographs
2. Transform using pre-determined PCA and compare KD to control (Rhosin?)
3. Tweak one component:
   – Cell/background segmentation
   – PIV
   – Direct calculation of spatial / temporal derivatives

https://github.com/DanuserLab/MonolayerKymographs

Agenda

1. Collective cell migration
2. Explicit detection of coordinated clusters (+ exercise)

Break (20 minutes)

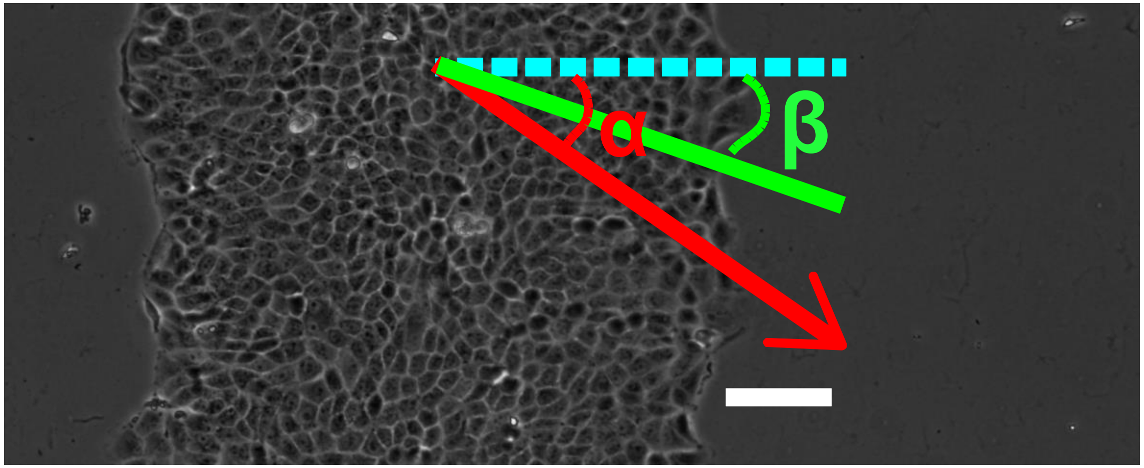
3. GEF screen experimental & analytic design (+ exercise)

Break (20 minutes)

4. **Unrelated promotion: DeBias (co-localization)**

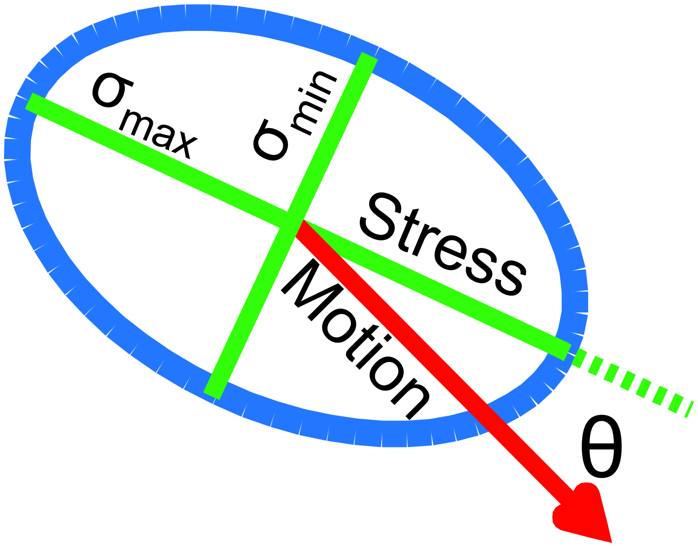
Motion-stress alignment

Velocity angle,
stress orientation



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

Motion-stress
alignment

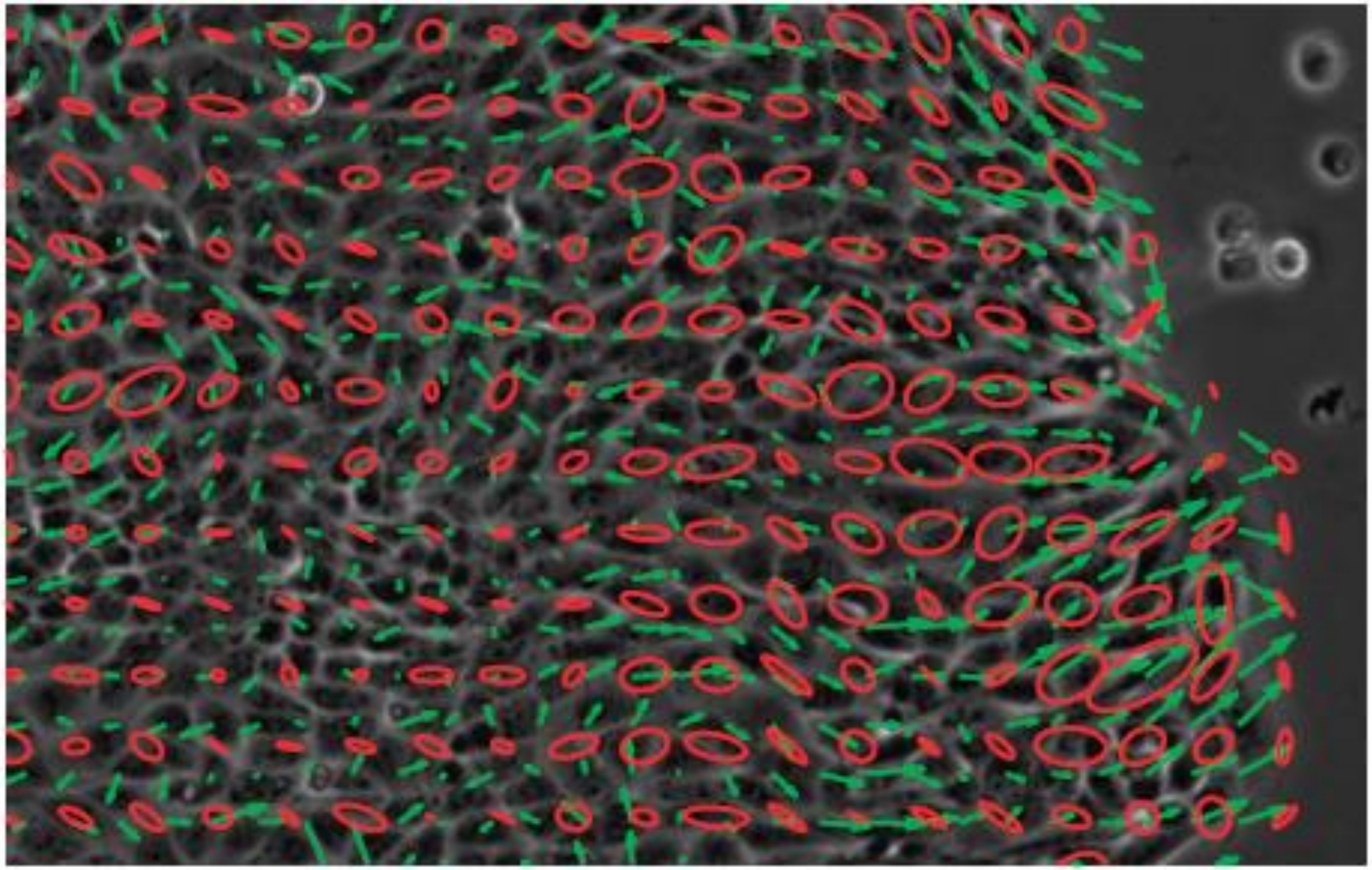


$0 \leq \theta \leq 90$

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

Plithotaxis

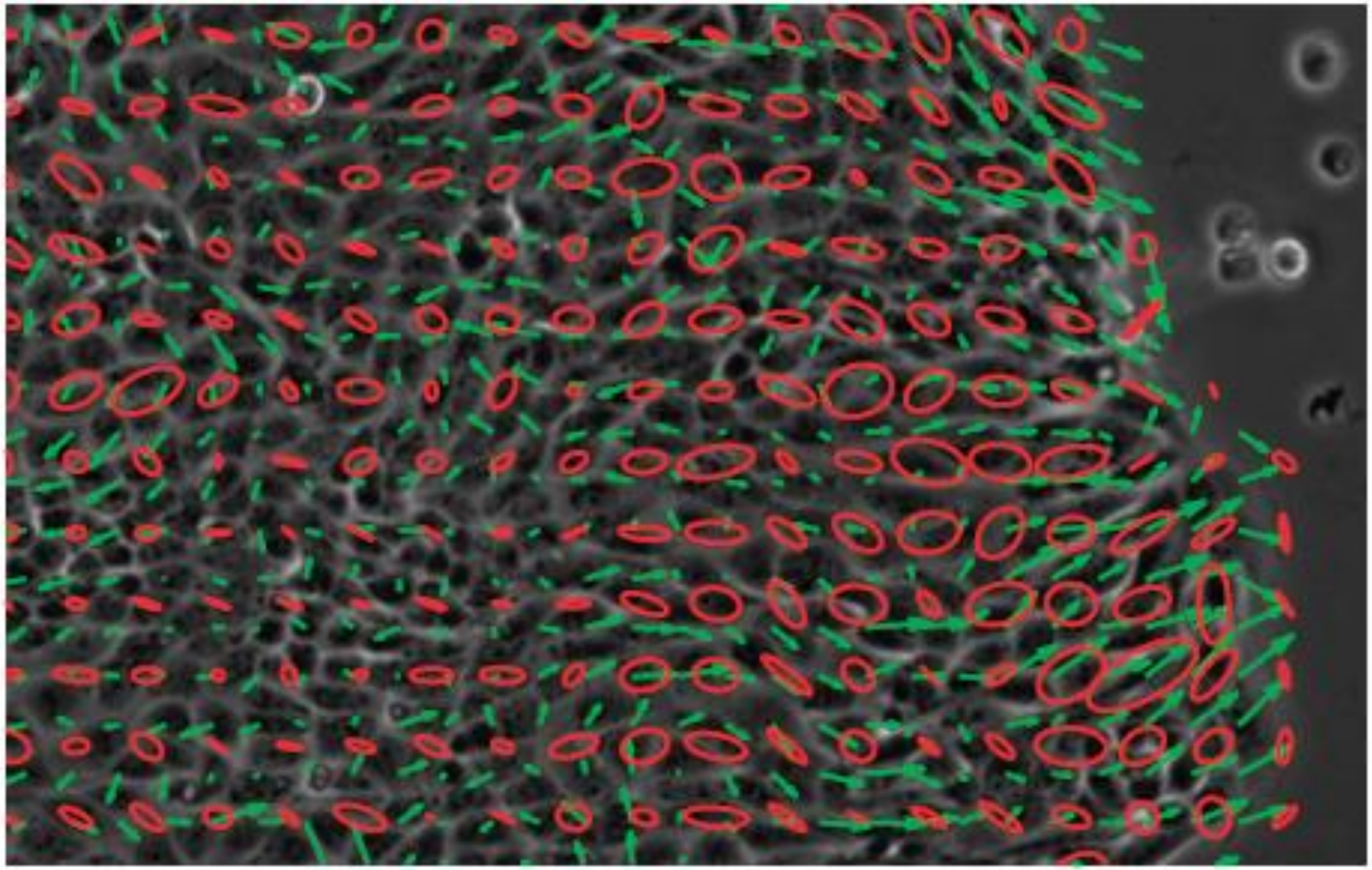
"tendency for each individual cell within a monolayer to migrate along the local orientation of the maximal principal stress."



Tambe et al. (2011)
Trepat and Fredberg (2011)
Serra-Picamal and Conte et al. (2012)

Plithotaxis

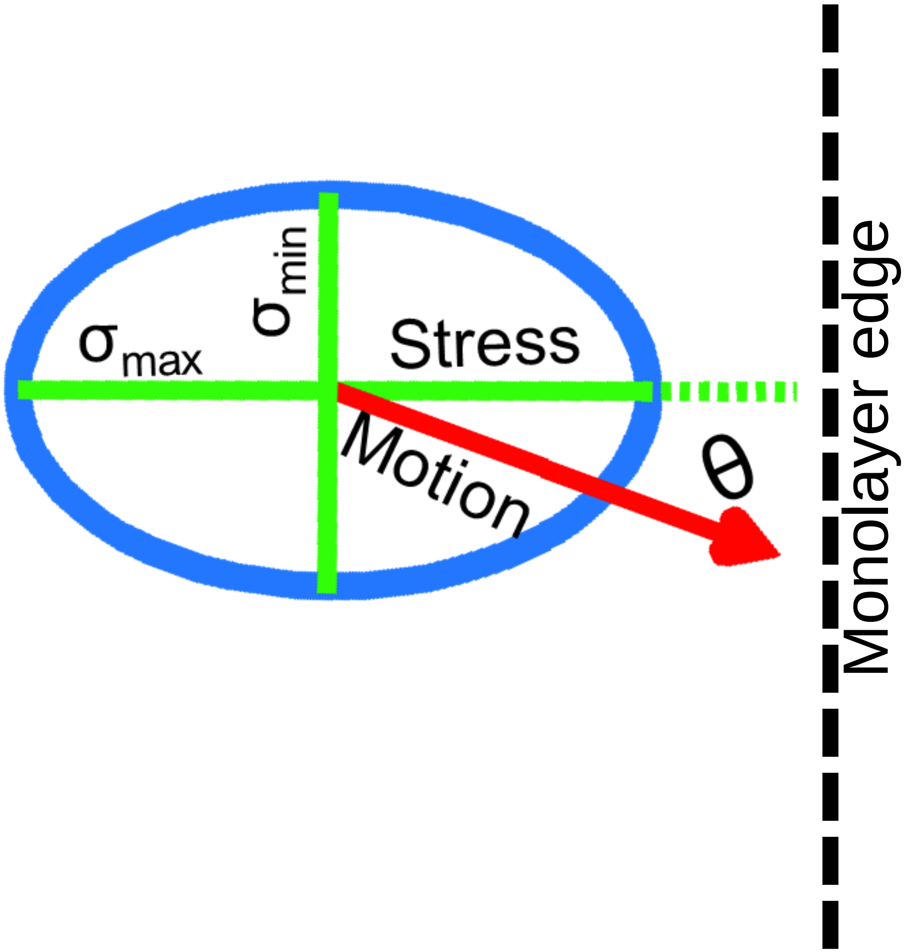
“tendency for **each individual** cell within a monolayer to migrate along the **local orientation** of the maximal principal stress.”



Tambe et al. (2011)
Trepat and Fredberg (2011)
Serra-Picamal and Conte et al. (2012)

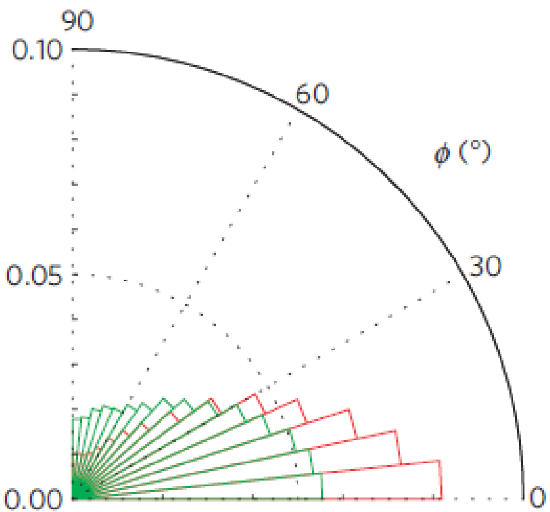
Plithotaxis?

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

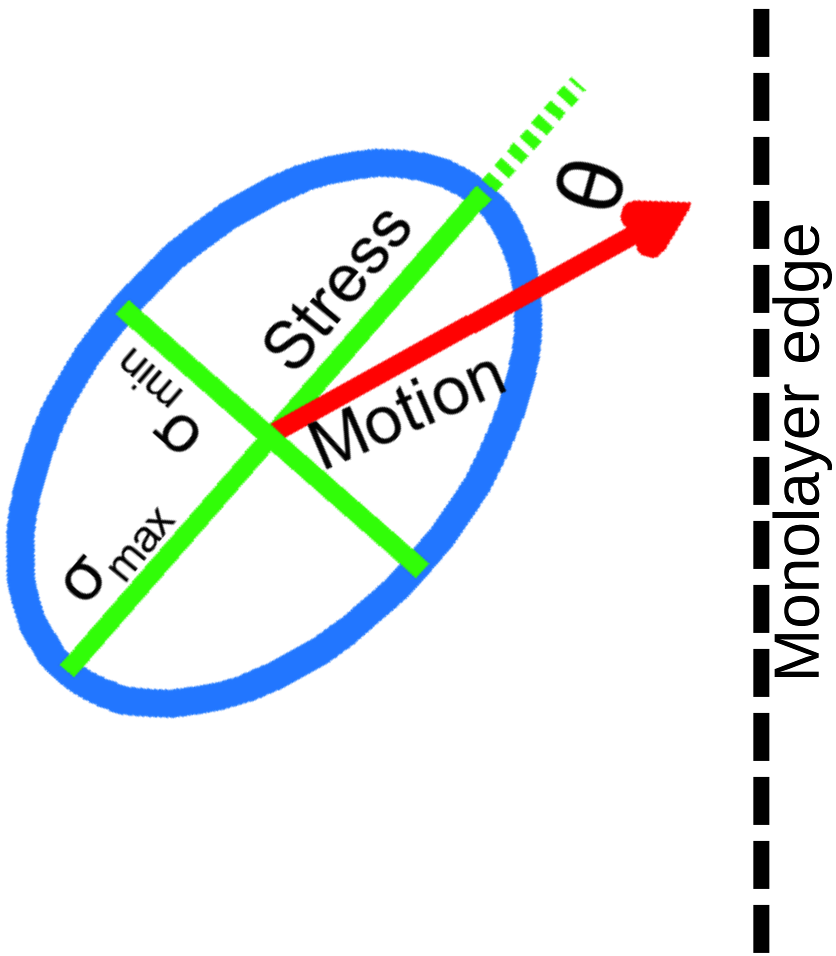


Plithotaxis?

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

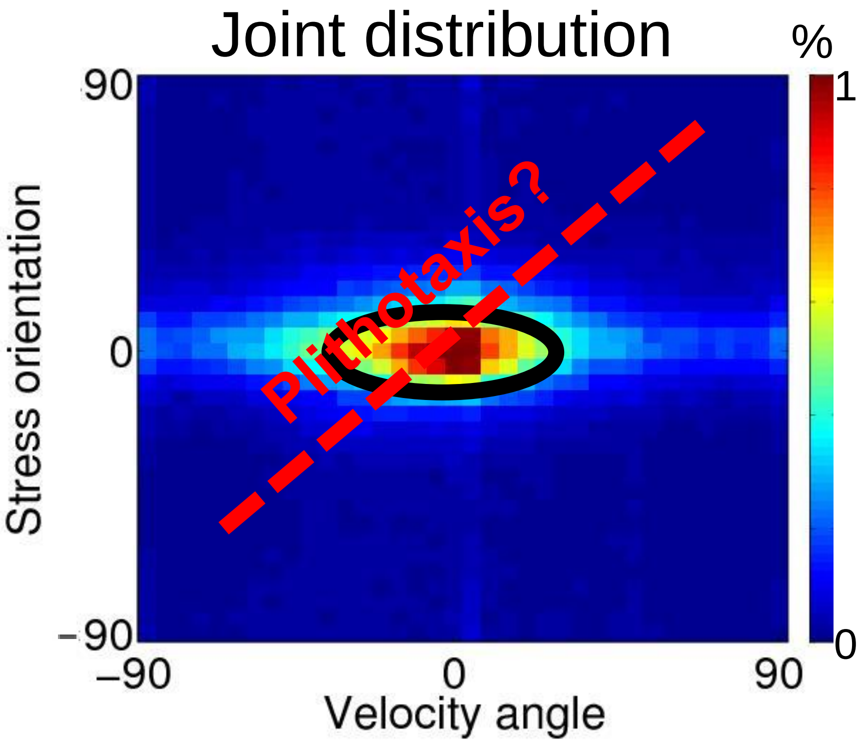


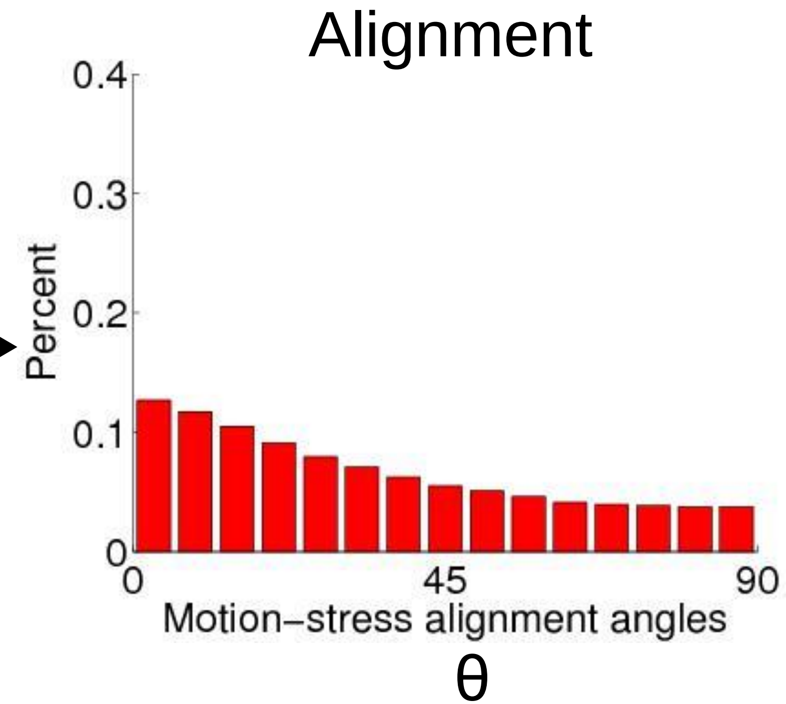
Serra-Picamal and Conte et al. (2012)



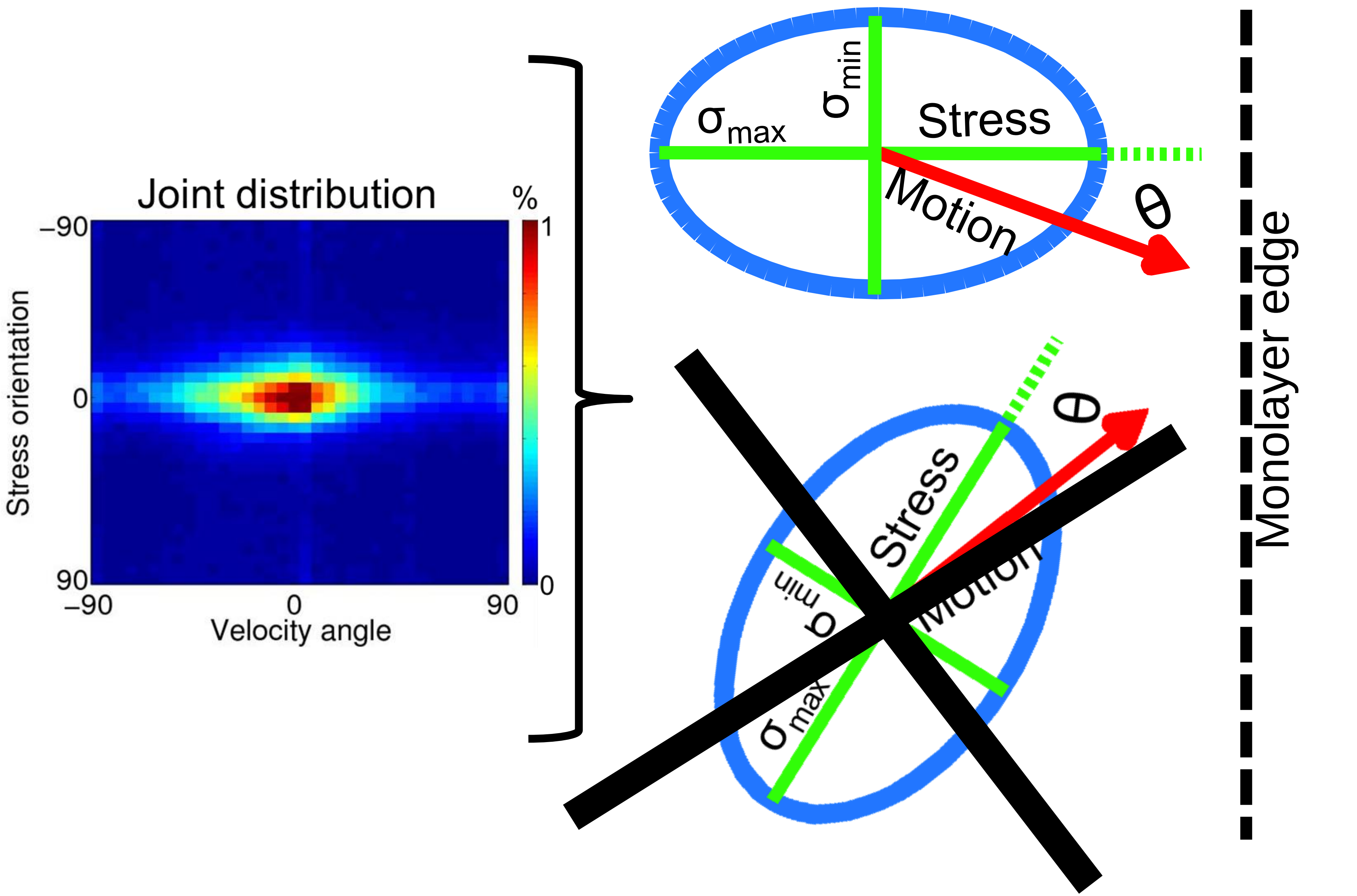
The roles of (global) monolayer geometry versus (local) plithotaxis in inducing motion-stress alignment

Motion-Stress Alignment ≠ Plithotaxis





No Evidence for Plithotaxis!

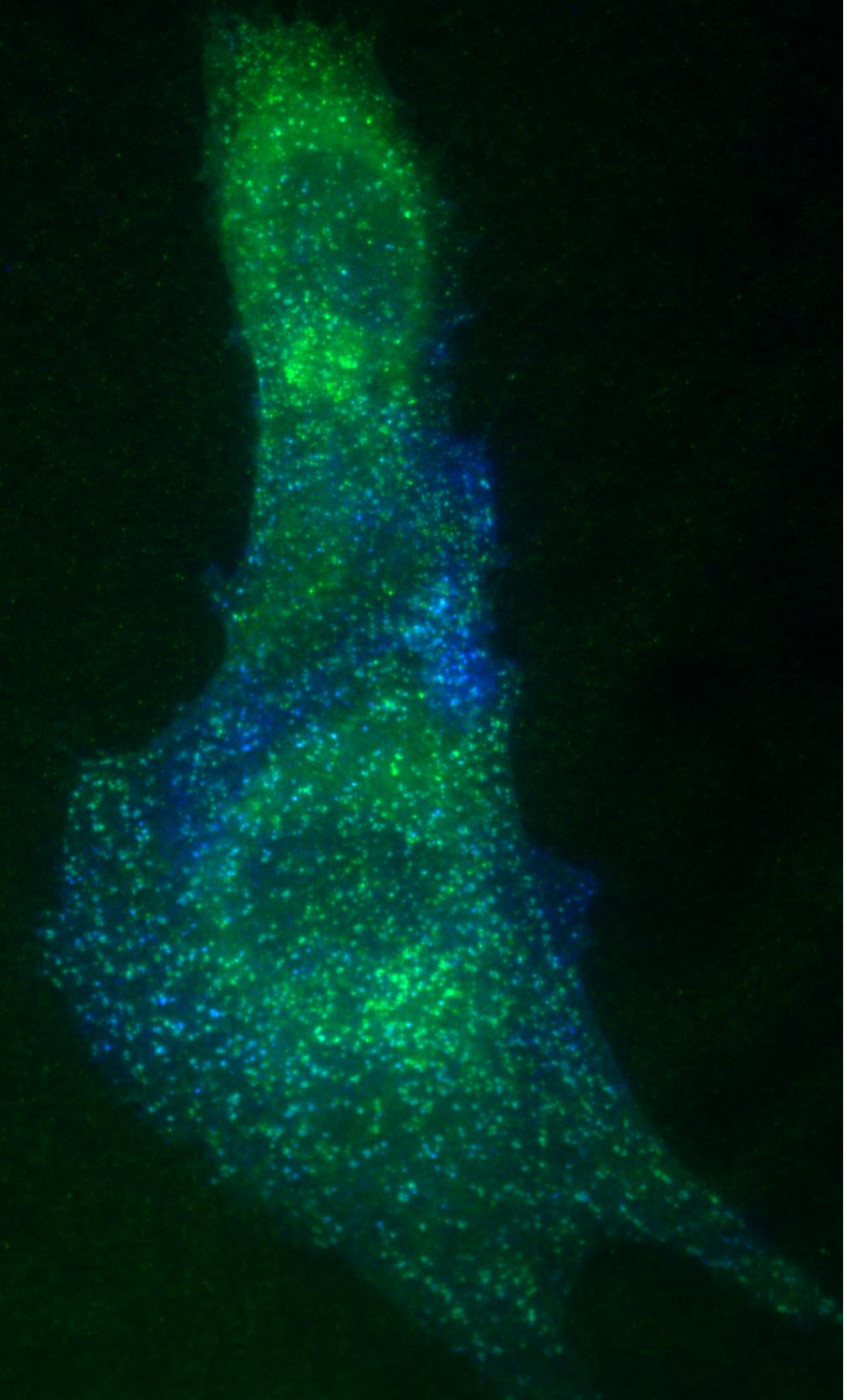


Components of motion-stress alignment

?

Observed motion-stress alignment = Global contribution (geometry) + Local contribution (plithotaxis)

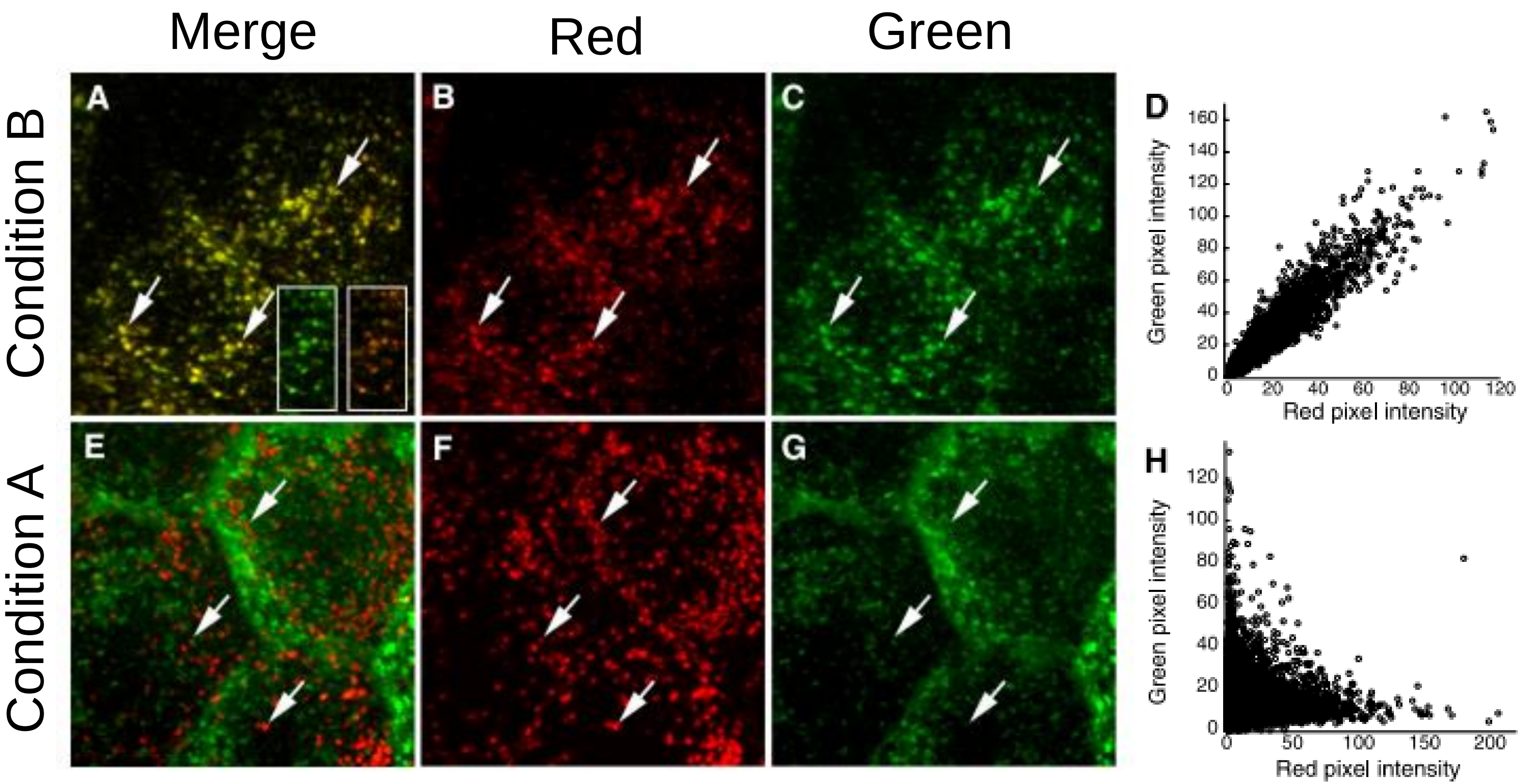
The interplay between development of quantitative tools ("hammers“) and identifying open important questions in cell biology ("nails“)



Decoupling global biases and local interactions between cell biological variables

DeBias

Quantifying protein-protein co-localization



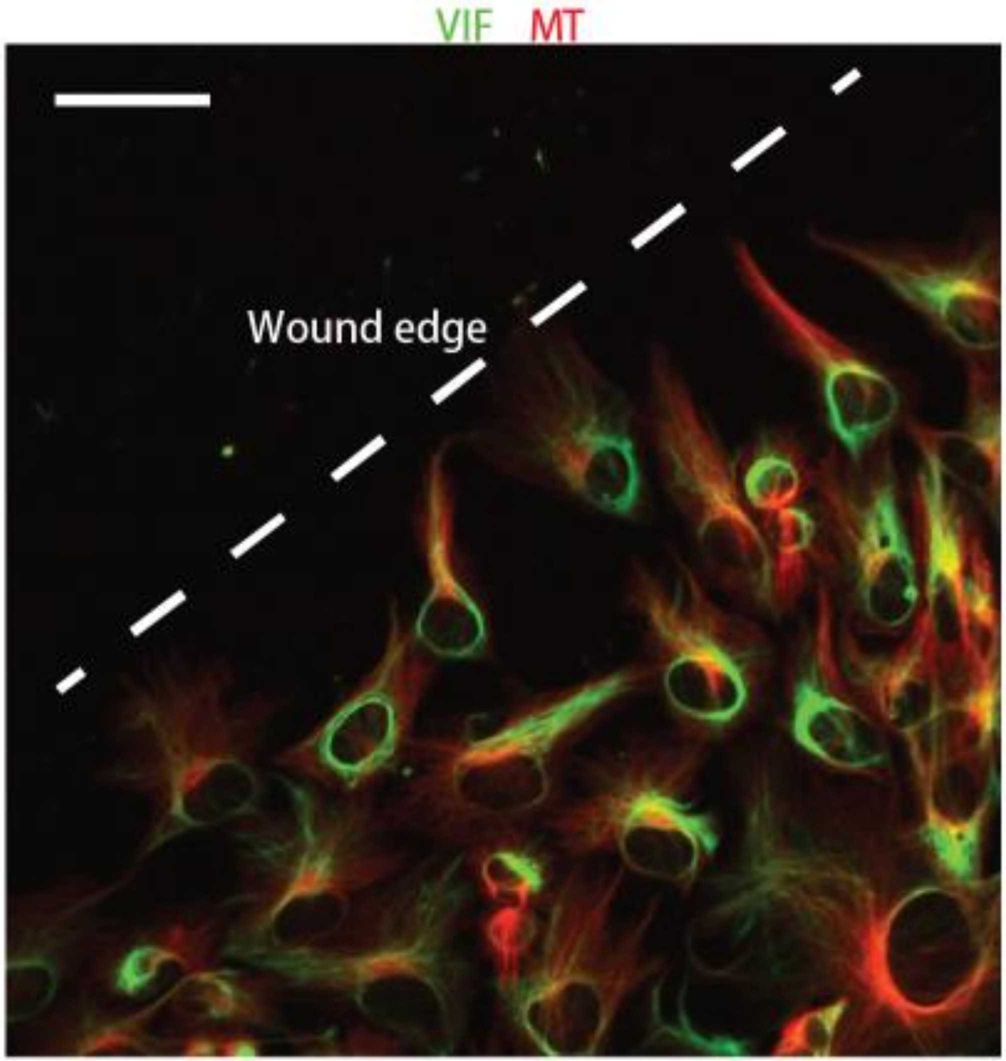
Dunn et al. (2011)

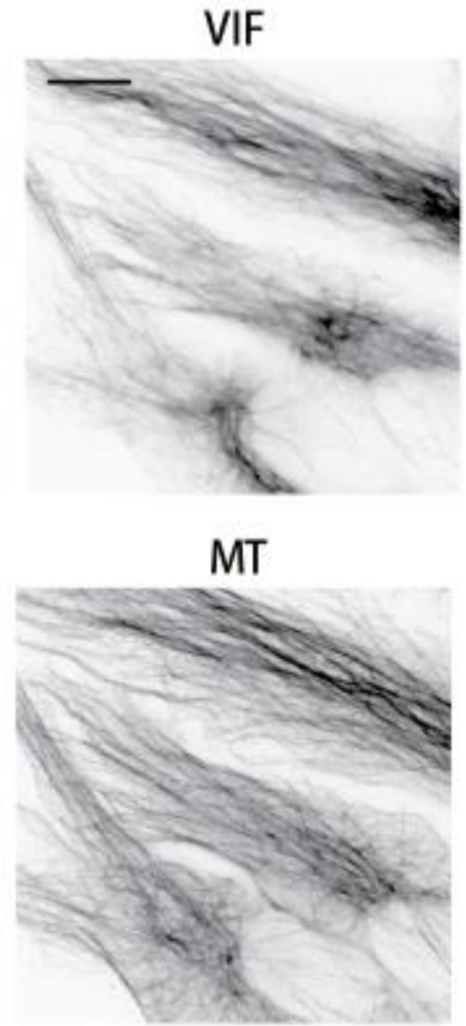
What additional information is hidden in co-localization data?

DeBias

Co-orientation of intracellular cytoskeletal networks in migrating cells

Vimentin provides a structural template for microtubule growth

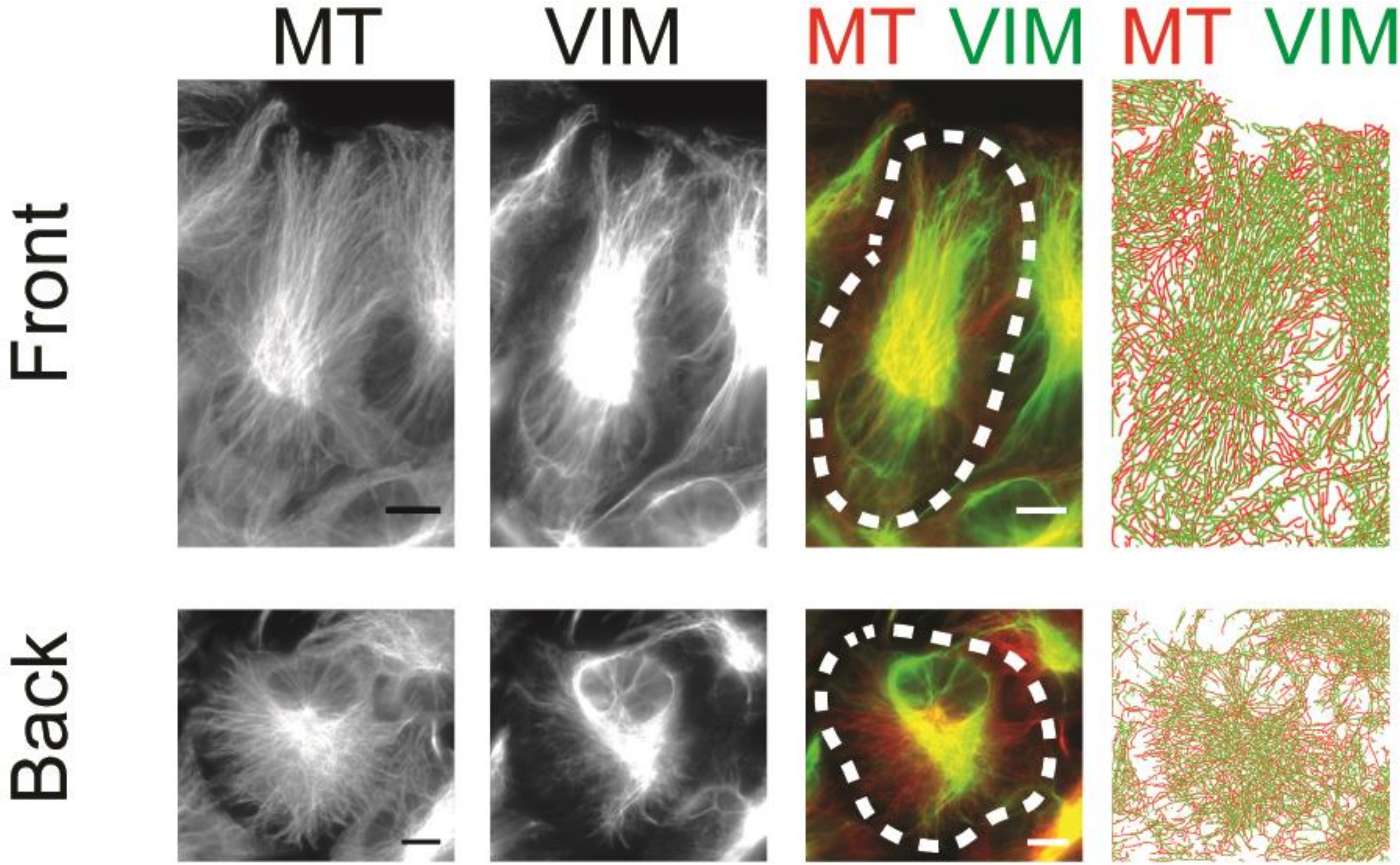




Genome-edited Retinal Pigment Epithelial (RPE) cells

Gan, Ding and Burckhardt et al. (2016)

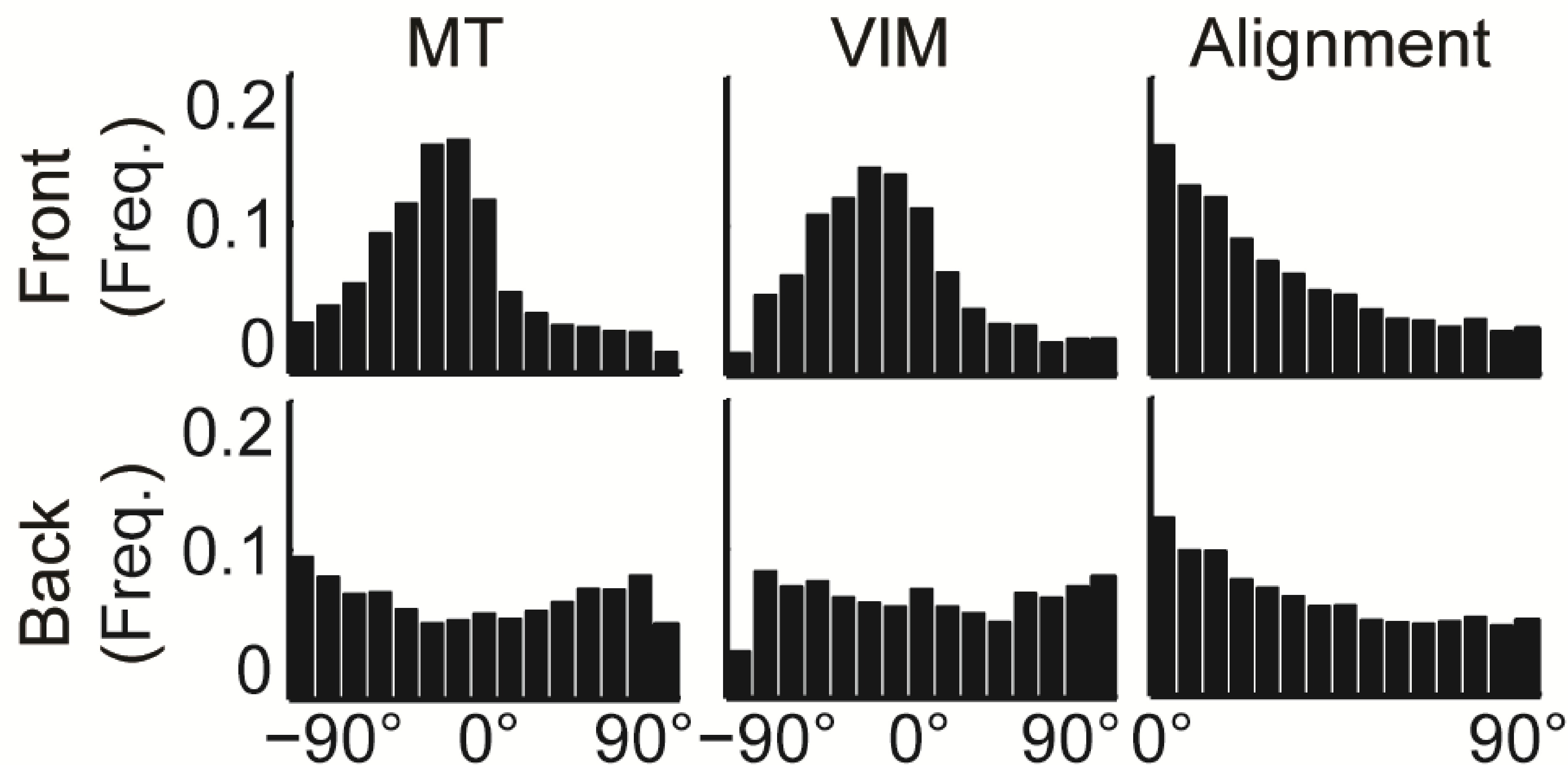
A relation between cell polarity and vimentin-microtubule interaction?



Zhuo Gan

Polarity-independent interaction of vimentin and microtubules

Polarity-independent interaction of vimentin and microtubules



What do we want to achieve?

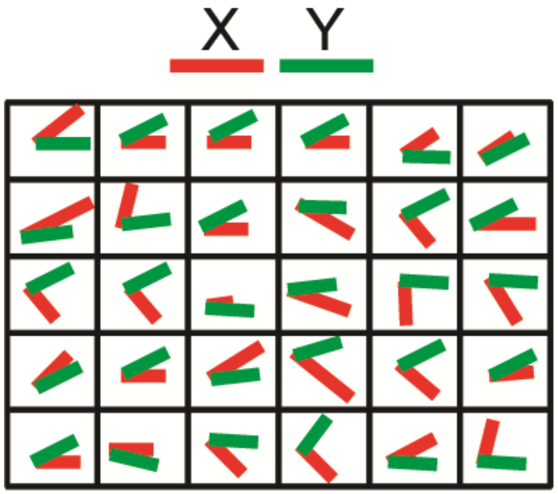
- Simultaneous investigation of mechanisms that drive global bias and local interactions

How?

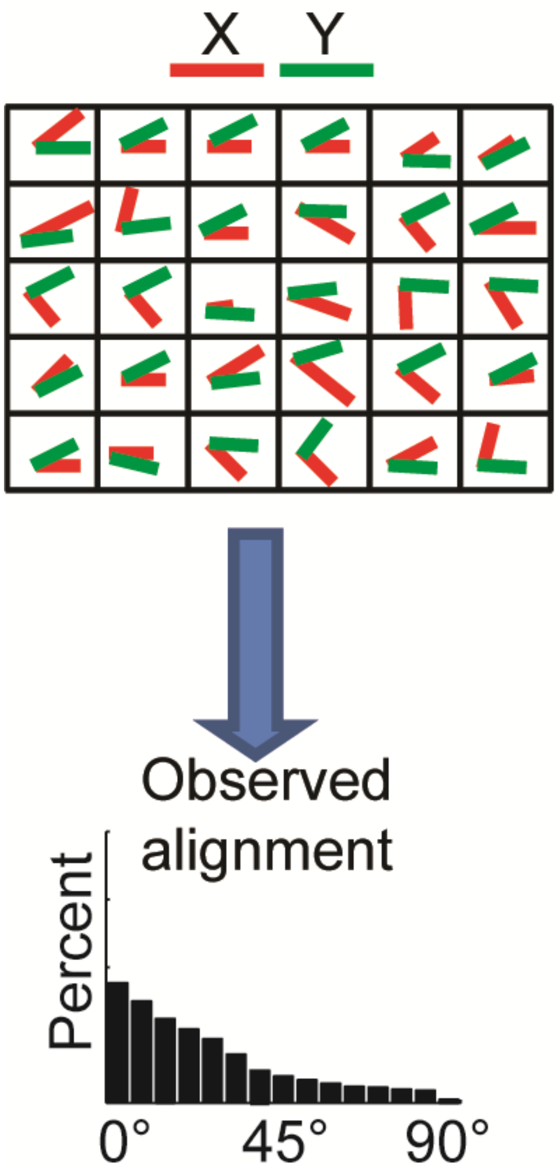
- By modeling the observed agreement between matched variables as the cumulative global and local components

$$\boxed{\text{Observed colocalization}} = \boxed{\text{Global bias}} + \boxed{\text{Local interaction}}$$

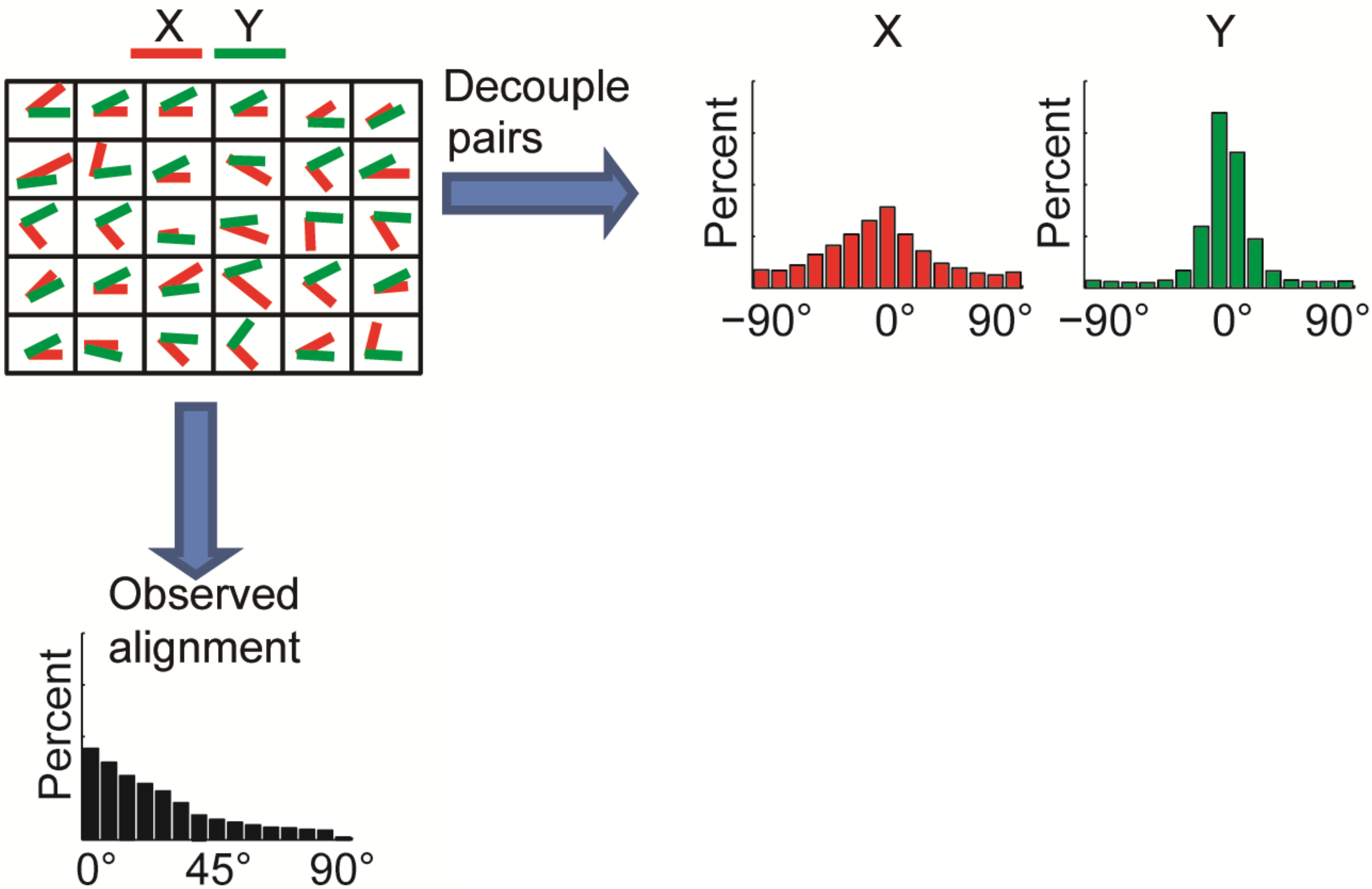
DeBias



DeBias

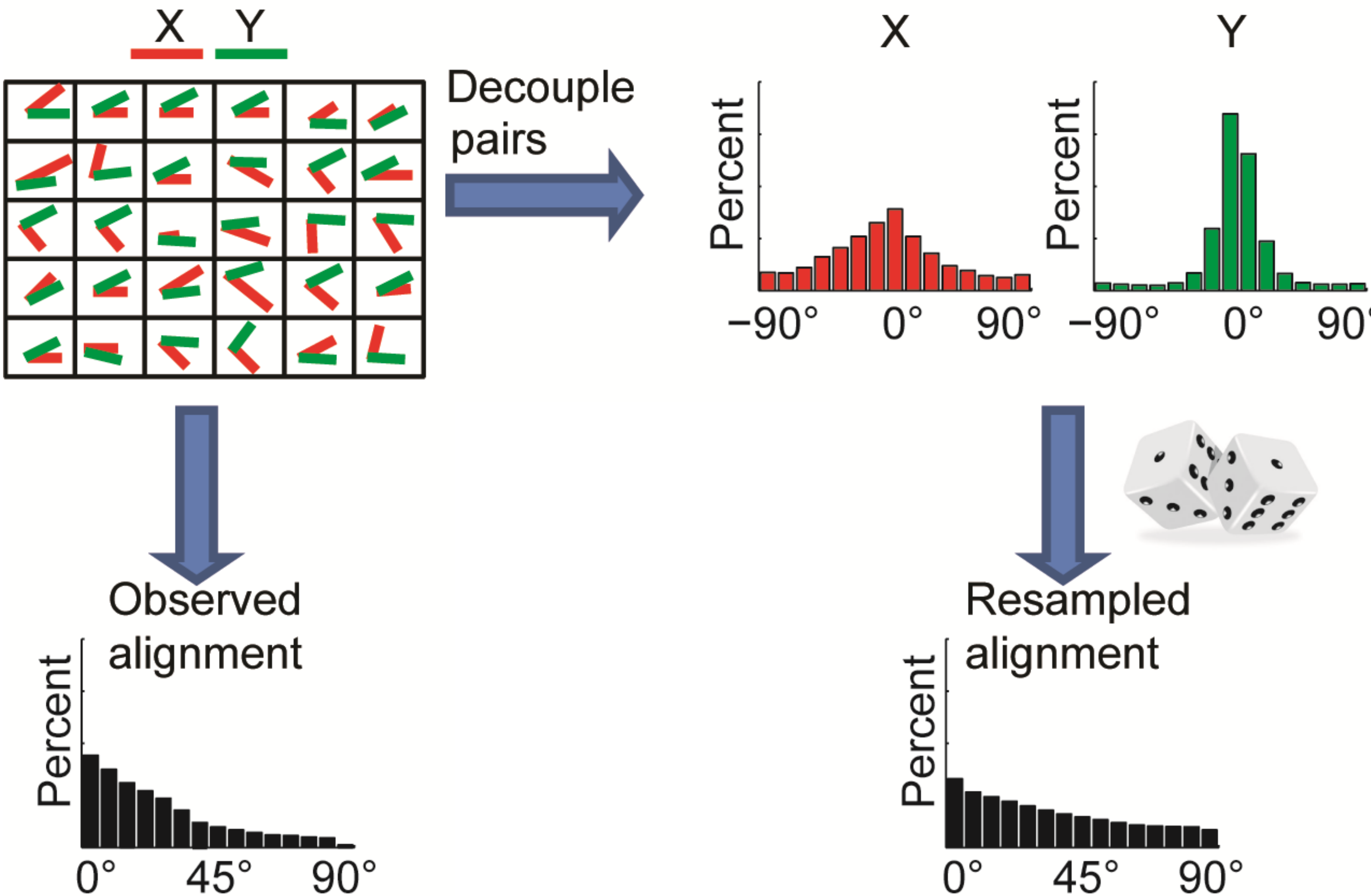


DeBias



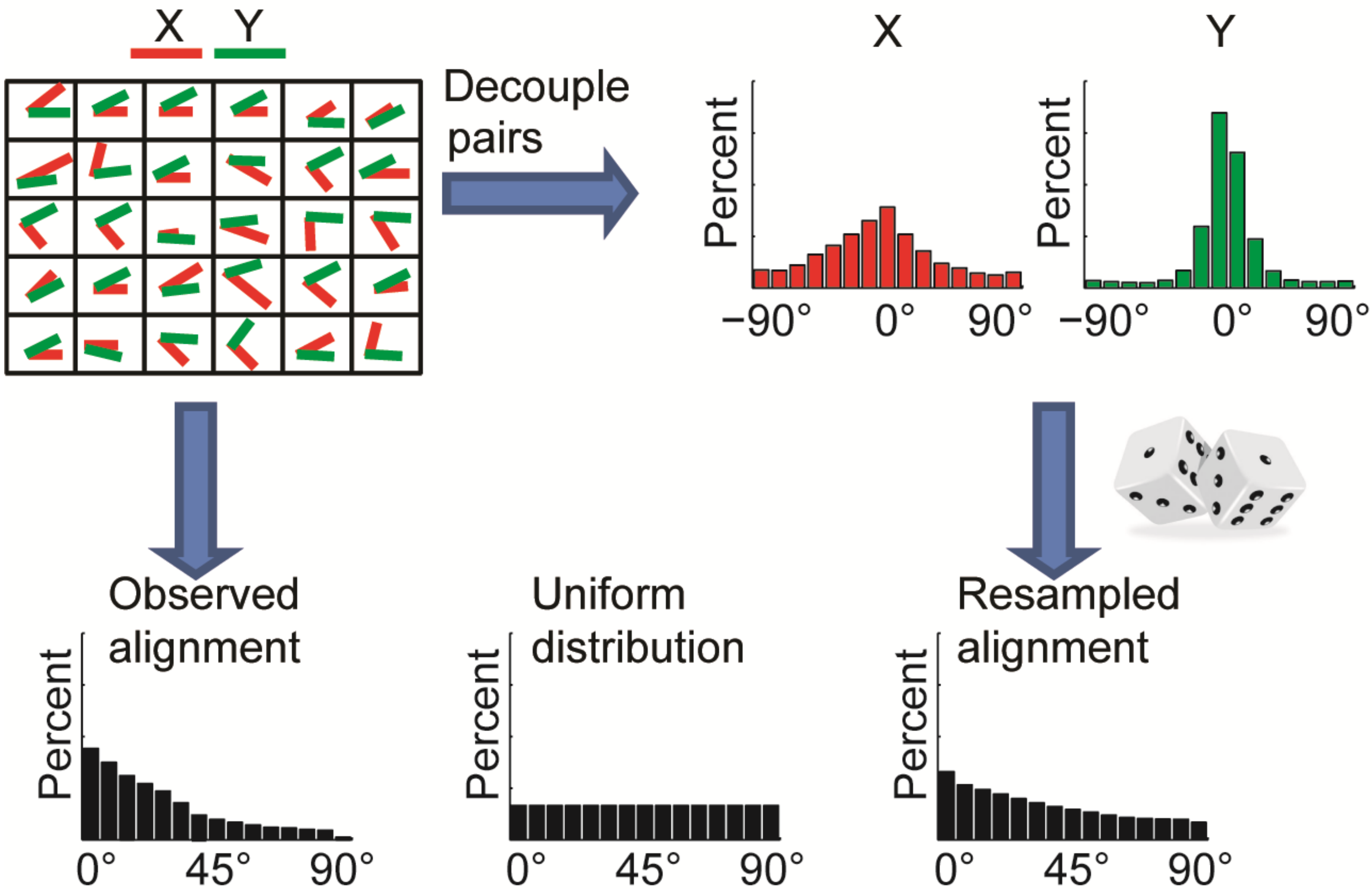
X
Y
Decouple pairs
X
Y
Percent
−90°
0°
90°
Percent
−90°
0°
90°
Observed alignment
Percent
0°
45°
90°

DeBias

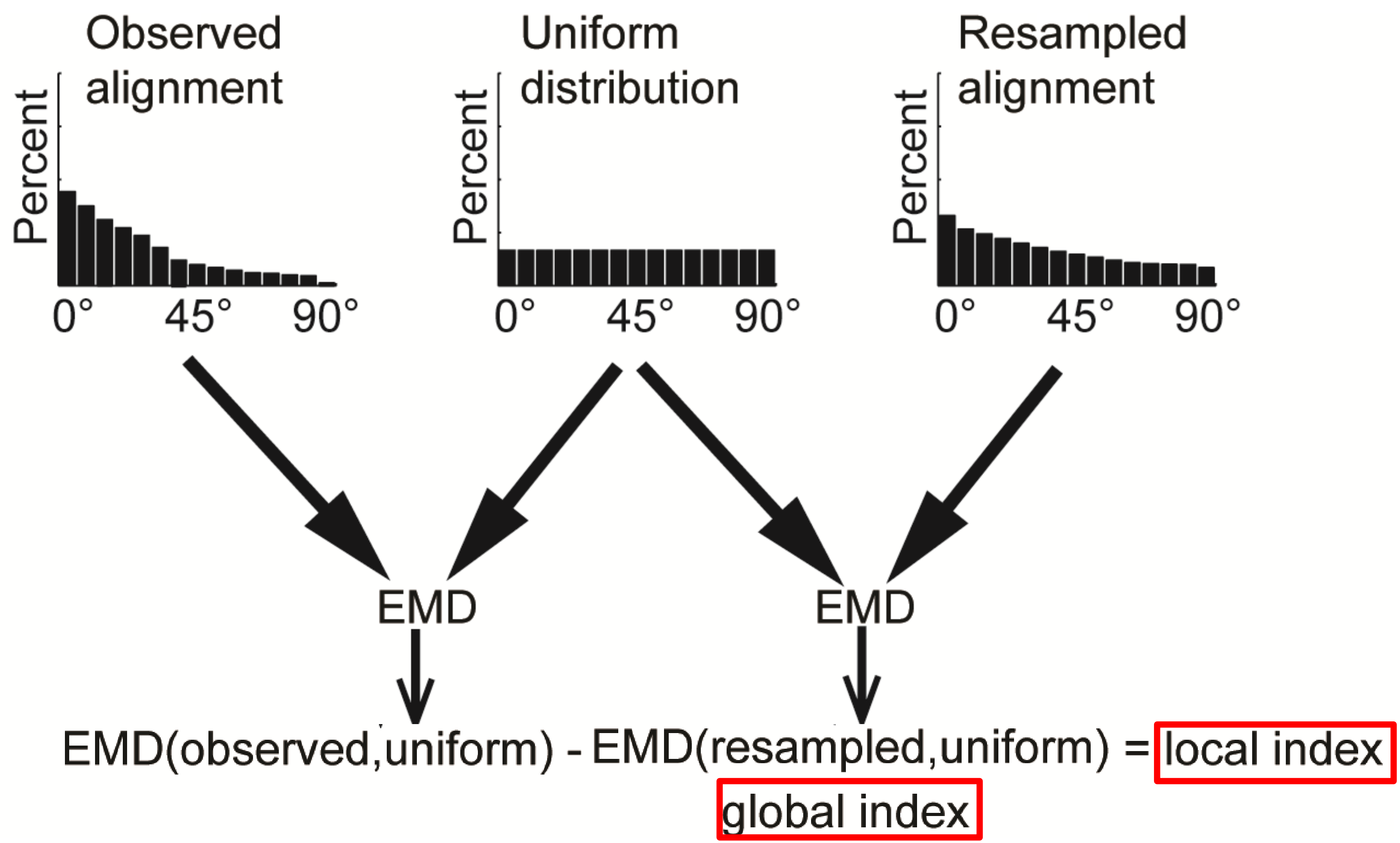


X
Y
Decouple pairs
X
Y
Percent
−90°
0°
90°
Percent
−90°
0°
90°
Observed alignment
Percent
0°
45°
90°
Resampled alignment
Percent
0°
45°
90°

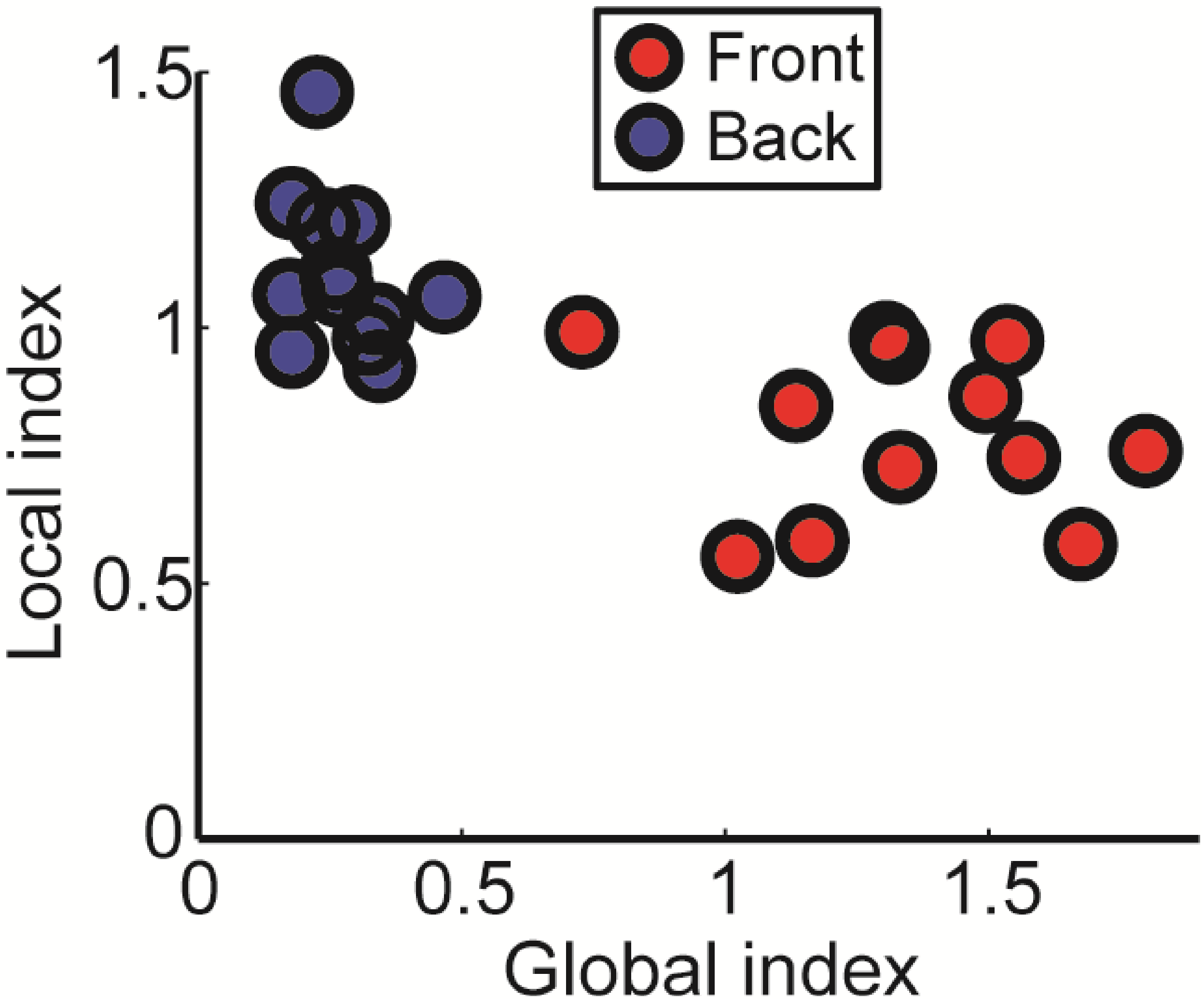
DeBias


X
Y
Decouple pairs
X
Y
Percent
−90°
0°
90°
Percent
−90°
0°
90°
Observed alignment
Percent
0°
45°
90°
Uniform distribution
Percent
0°
45°
90°
Resampled alignment
Percent
0°
45°
90°

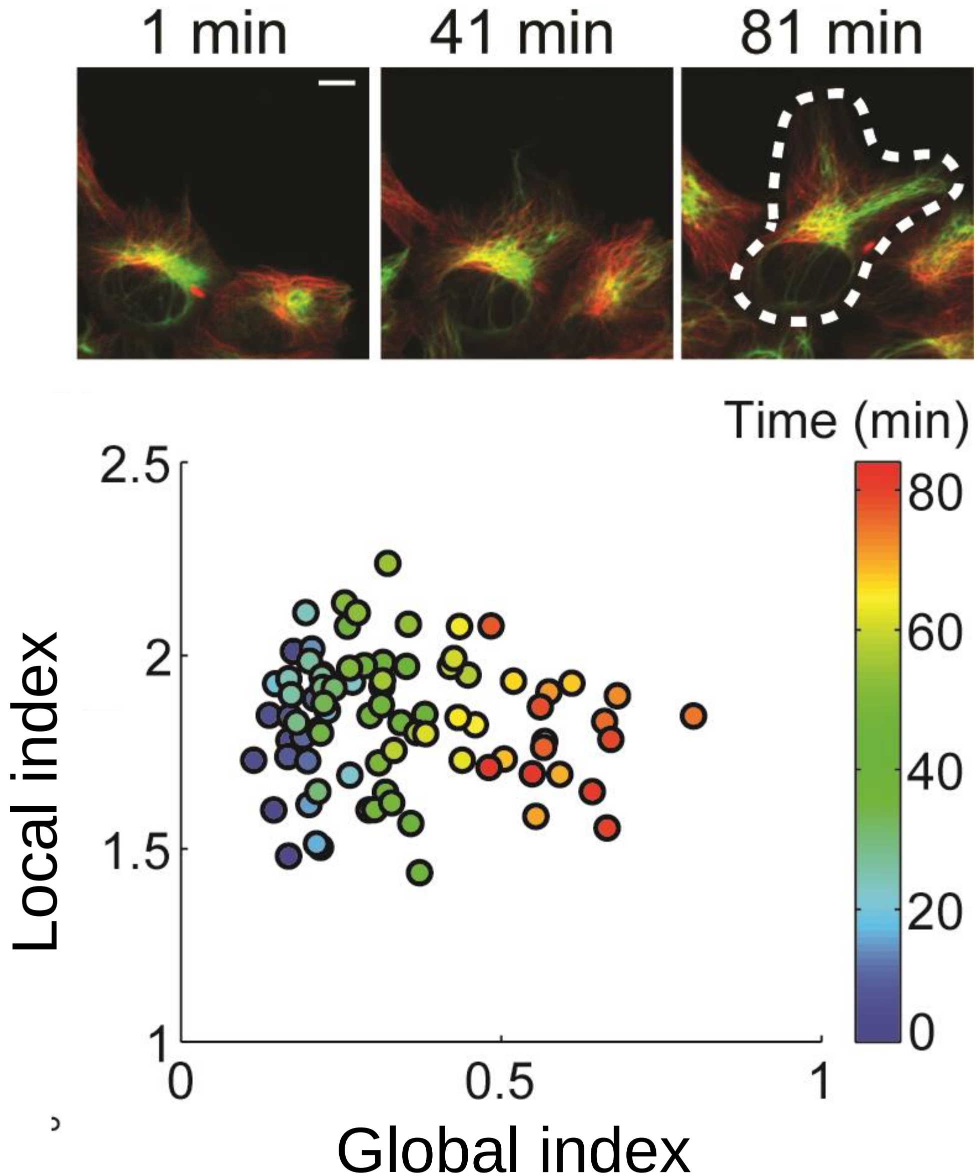
DeBias


Observed alignment
Uniform distribution
Resampled alignment
Percent
Percent
Percent
0° 45° 90°
0° 45° 90°
0° 45° 90°
EMD
EMD
EMD(observed,uniform) - EMD(resampled,uniform) = local index
global index

Polarity-independent interaction of vimentin and microtubules



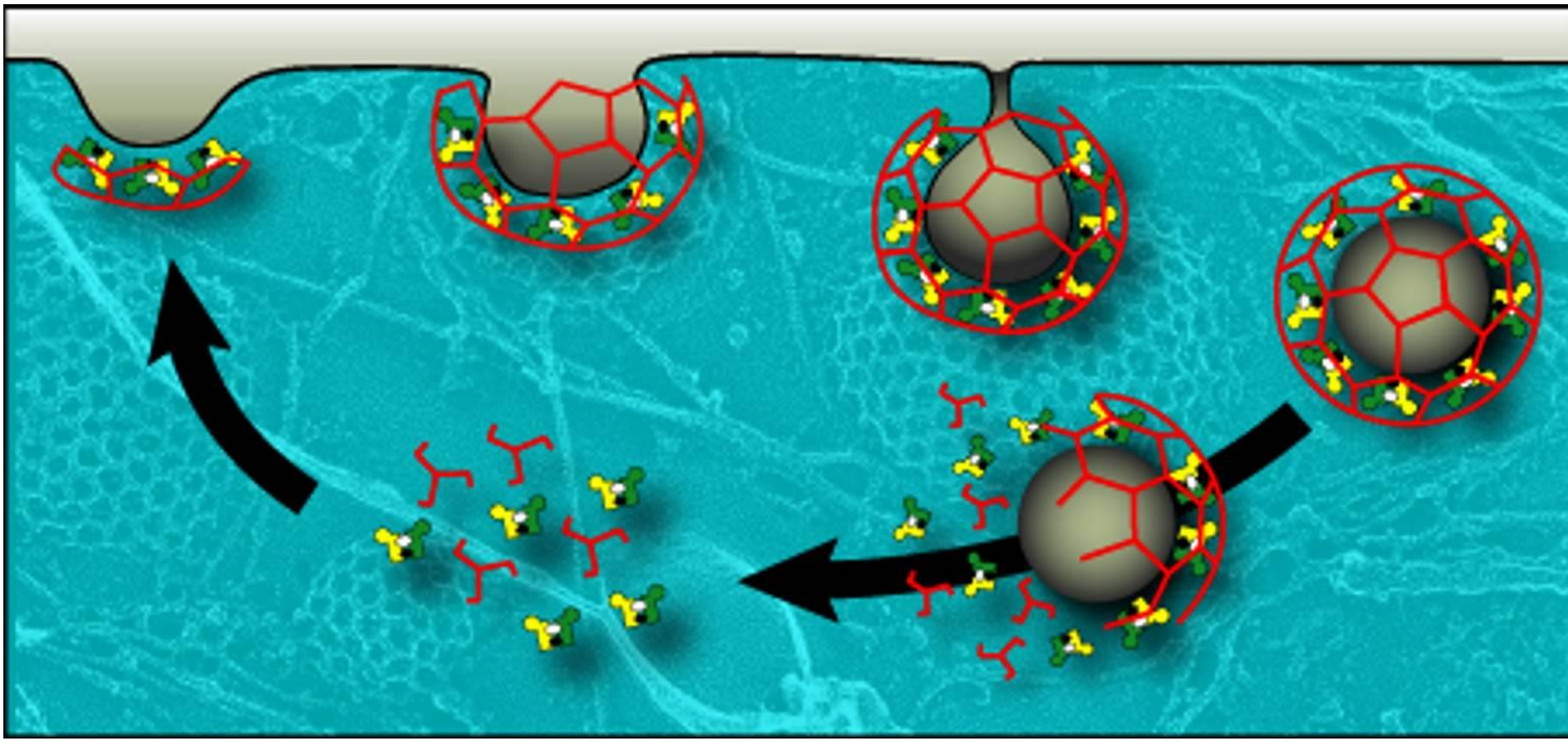
Polarity-independent interaction of vimentin and microtubules



Global bias: cell polarity

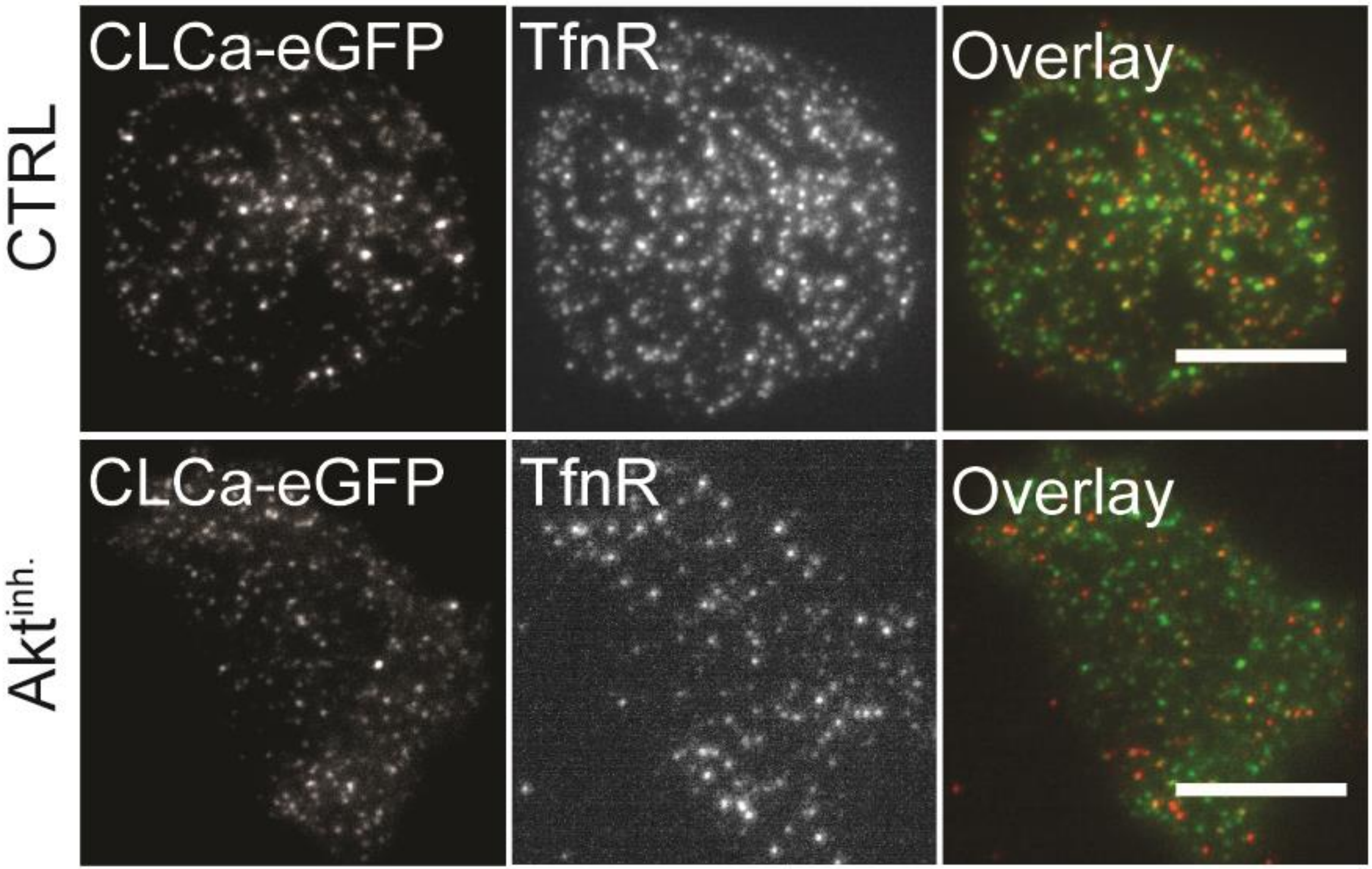
Inferring co-localization and predicting dynamics from fixed cells during clathrin-mediated endocytosis (CME)

Maturation of a clathrin coated pit



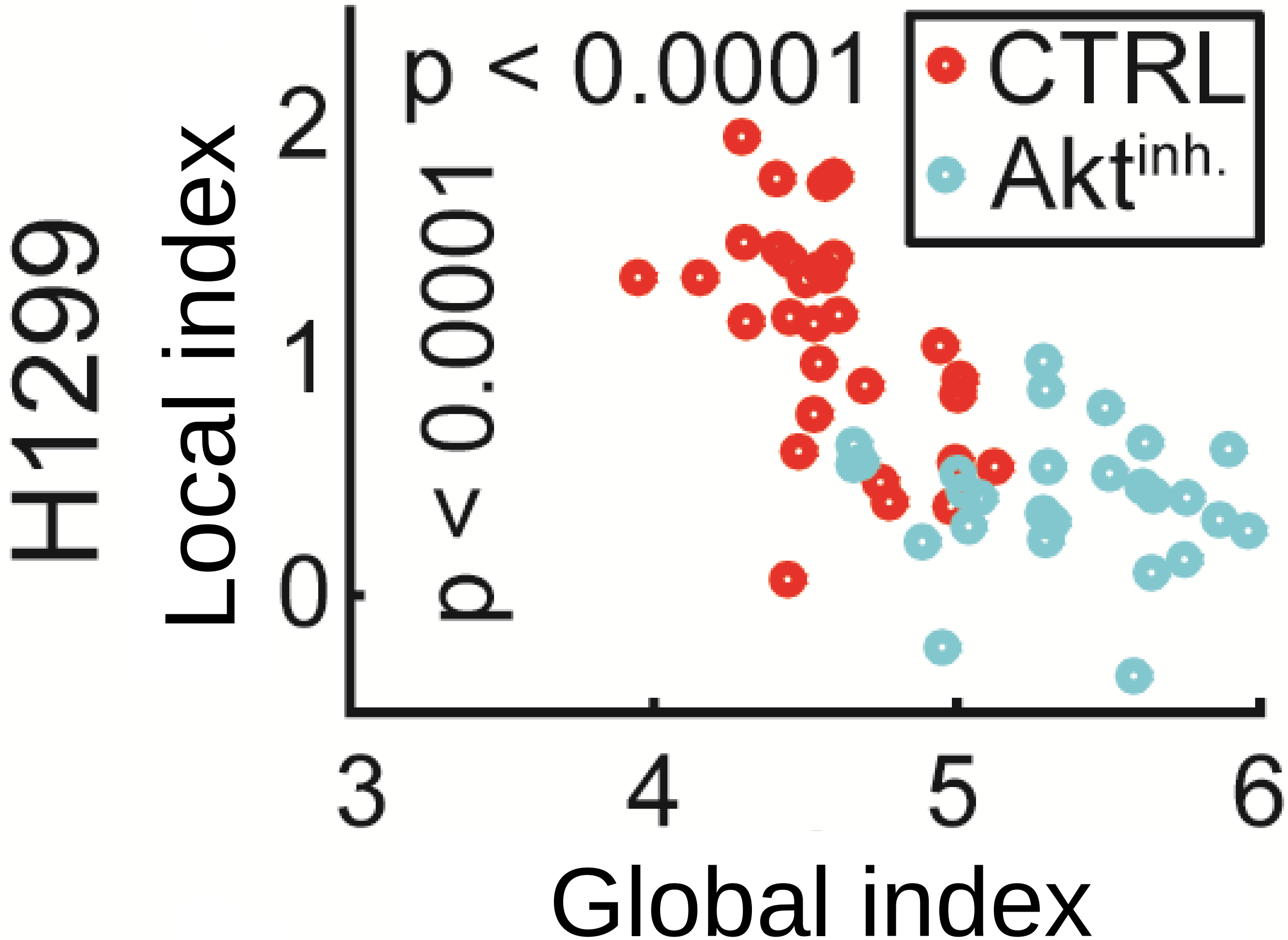
Marcel Mettlen

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

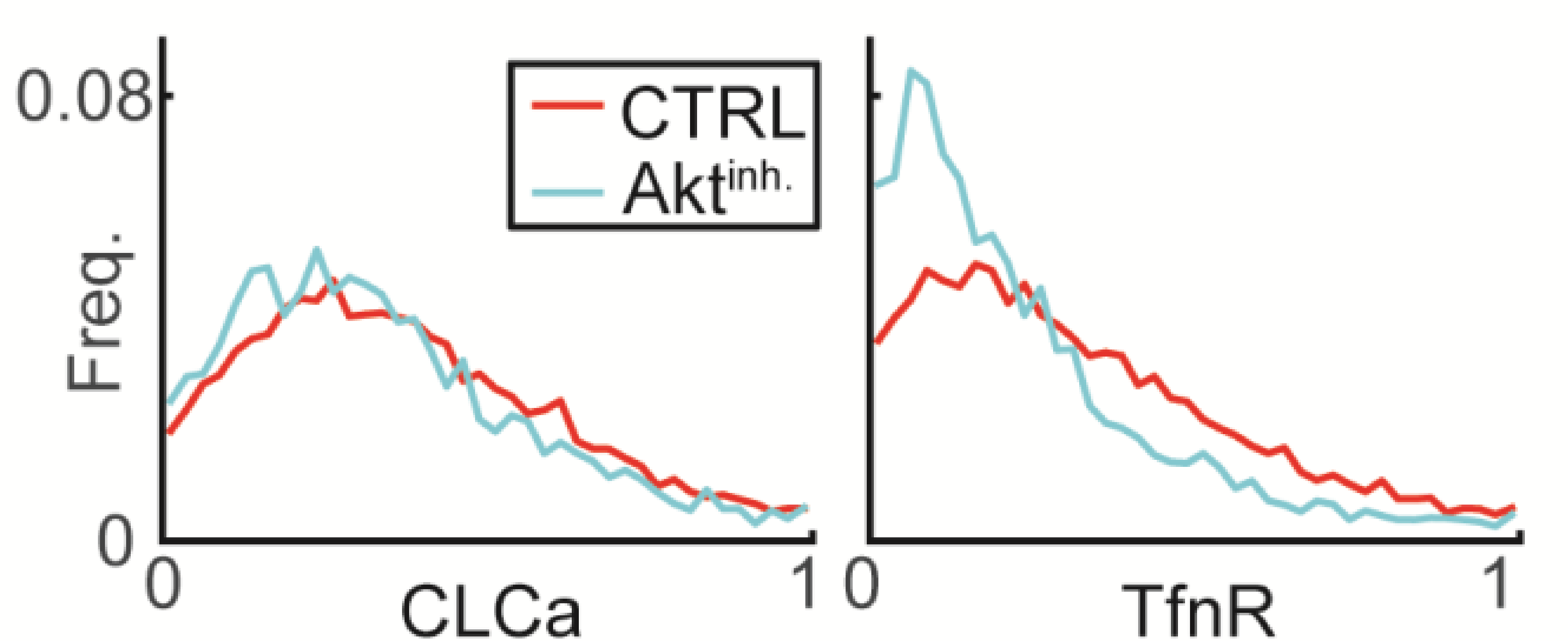


Carlos Reis, H1299 cells

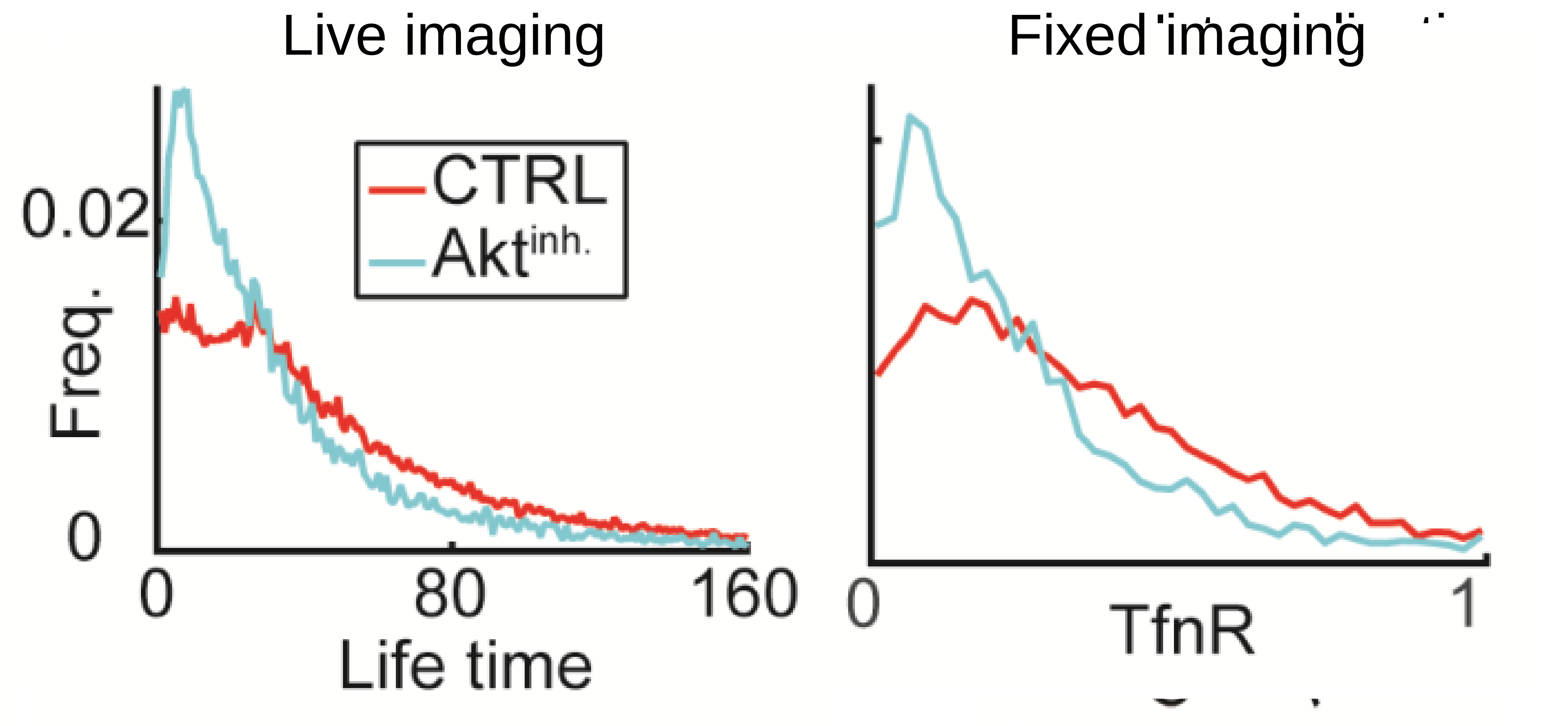
Inferring co-localization and predicting dynamics from fixed cells during clathrin-mediated endocytosis (CME)



Global bias: reduced TfnR in CCPs upon Akt inhibition

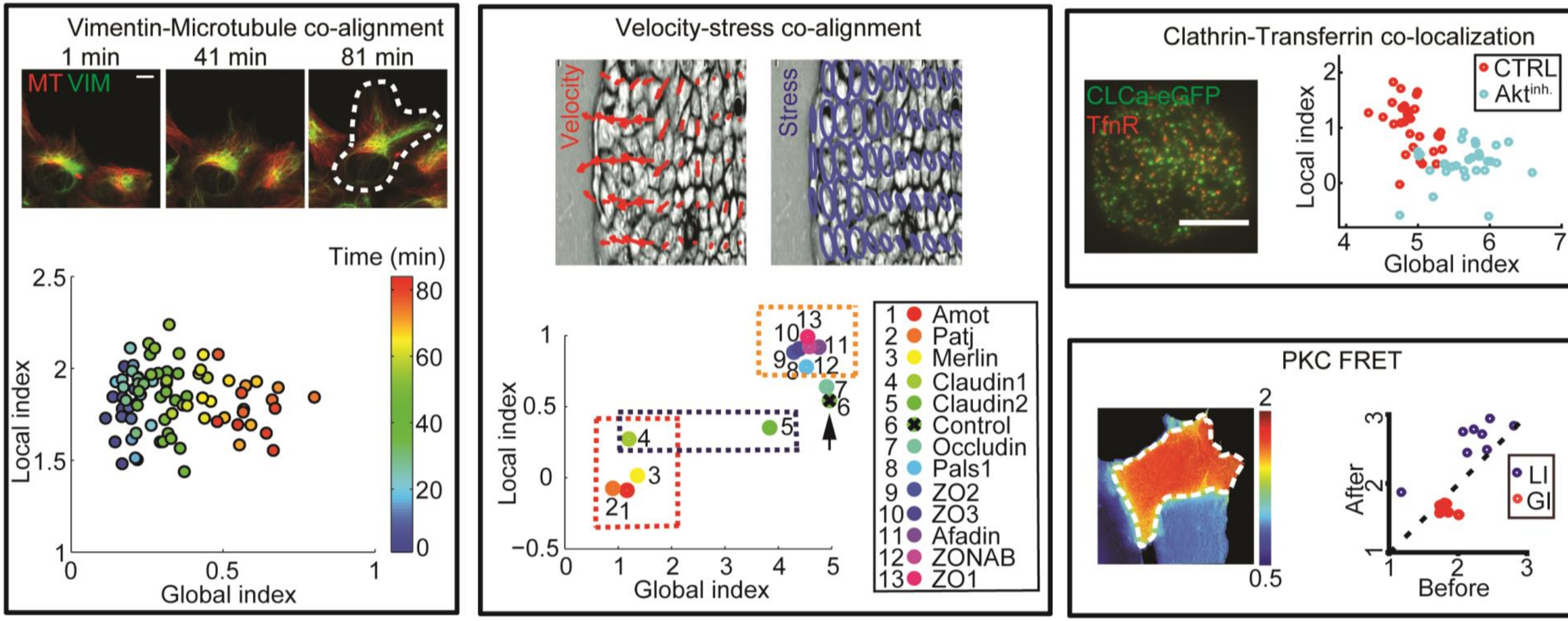


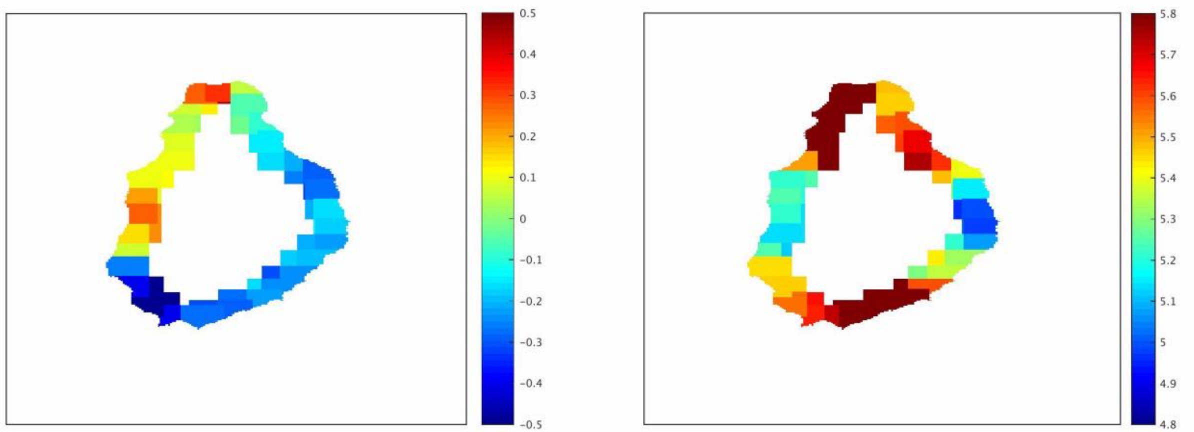
More CCPs containing less TfnR alter CCPs dynamics upon AKT inhibition



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

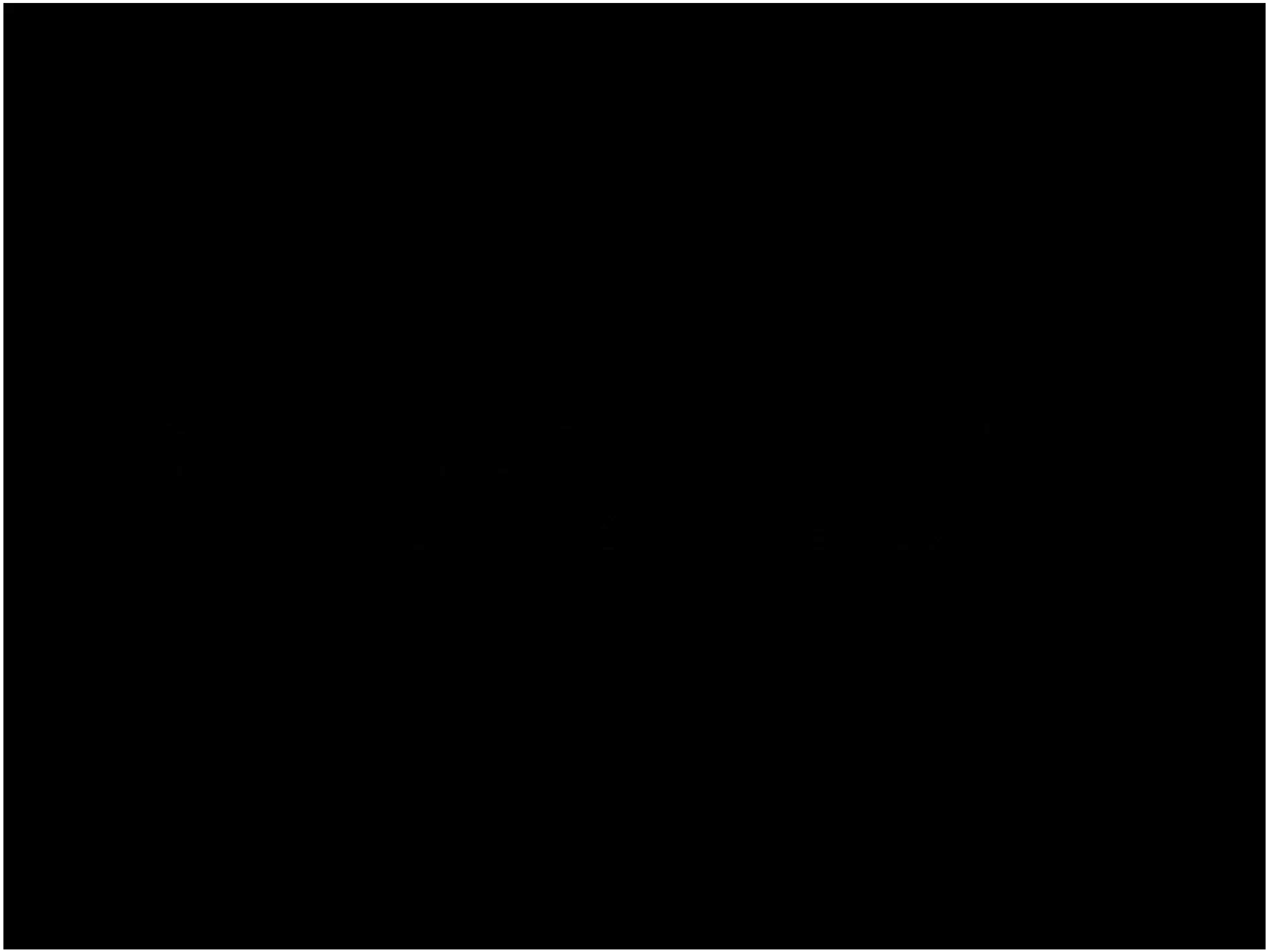
Webserver:

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

Source code:

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

Take home message
DeBias enables identifying the gorilla



Simons and Chabris (1999)

Acknowledgments

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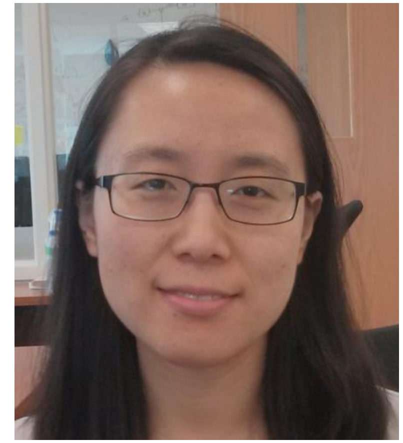

Uri Obolski, (Theory)

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Zhuo Gan, (Vimentin, PKC)

Yi Du (Webserver)

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Thank you!

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