De novo and sprouting blood vessel growth

role of stochastic cell motility

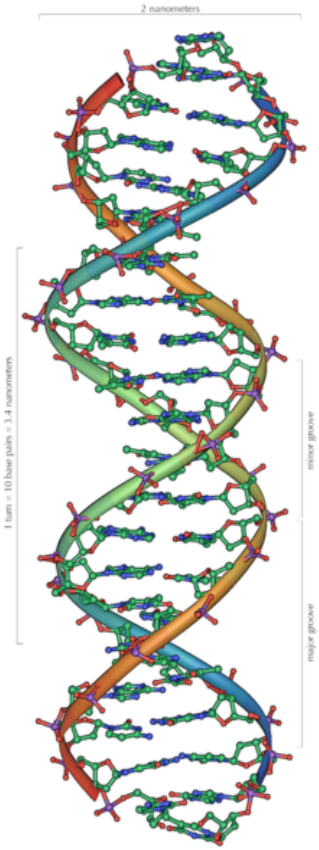
Roeland Merks
Biomodeling & Biosystems Analysis

CWI - Life Sciences
and Netherlands Institute for Systems Biology

Workshop *Nonlinear Dynamics of Natural Systems+*
Eindhoven, April 13, 2010

Biological development

DNA + fertilized egg cell -> 3D shape + function



Wikipedia

Wikipedia

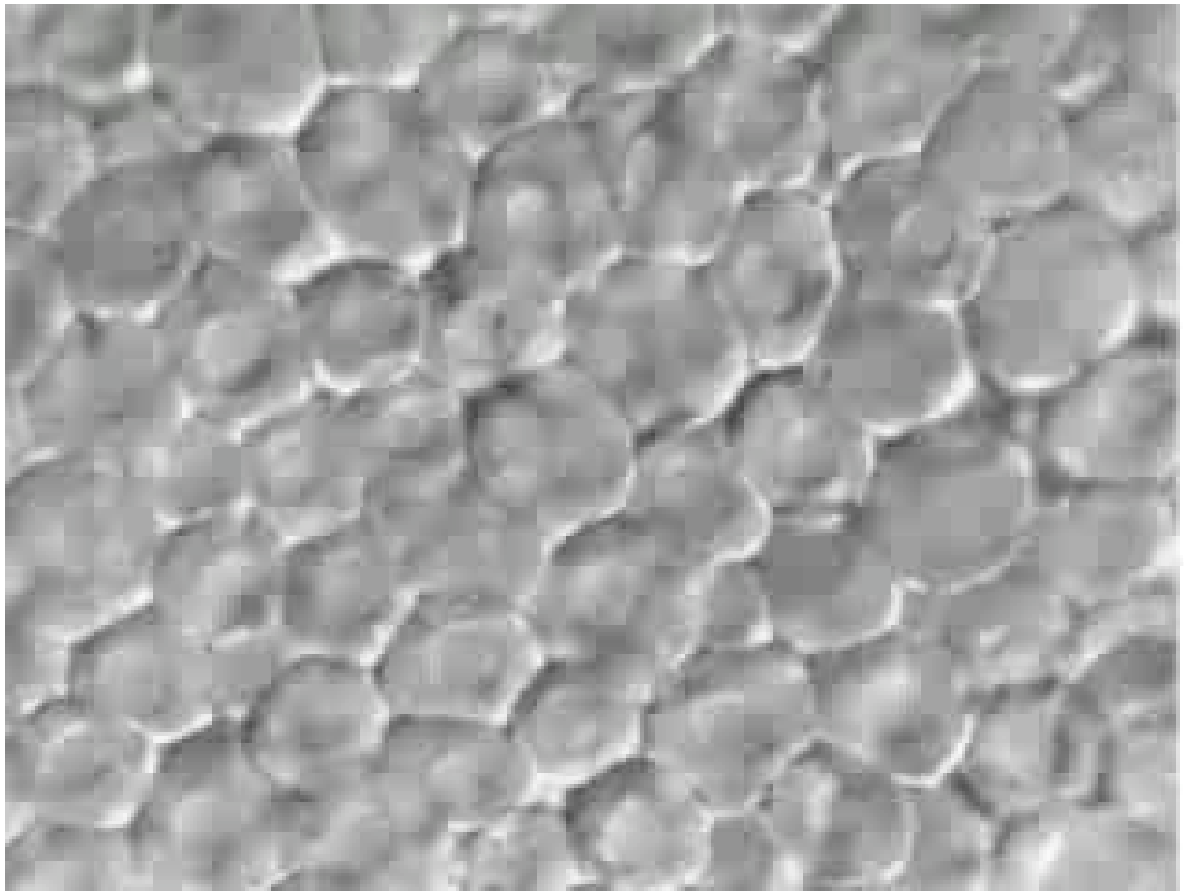
Zebrafish

Movie: **Zebrafish** development, P.Z. Myers, University of Minnesota
(source: YouTube)

CWI

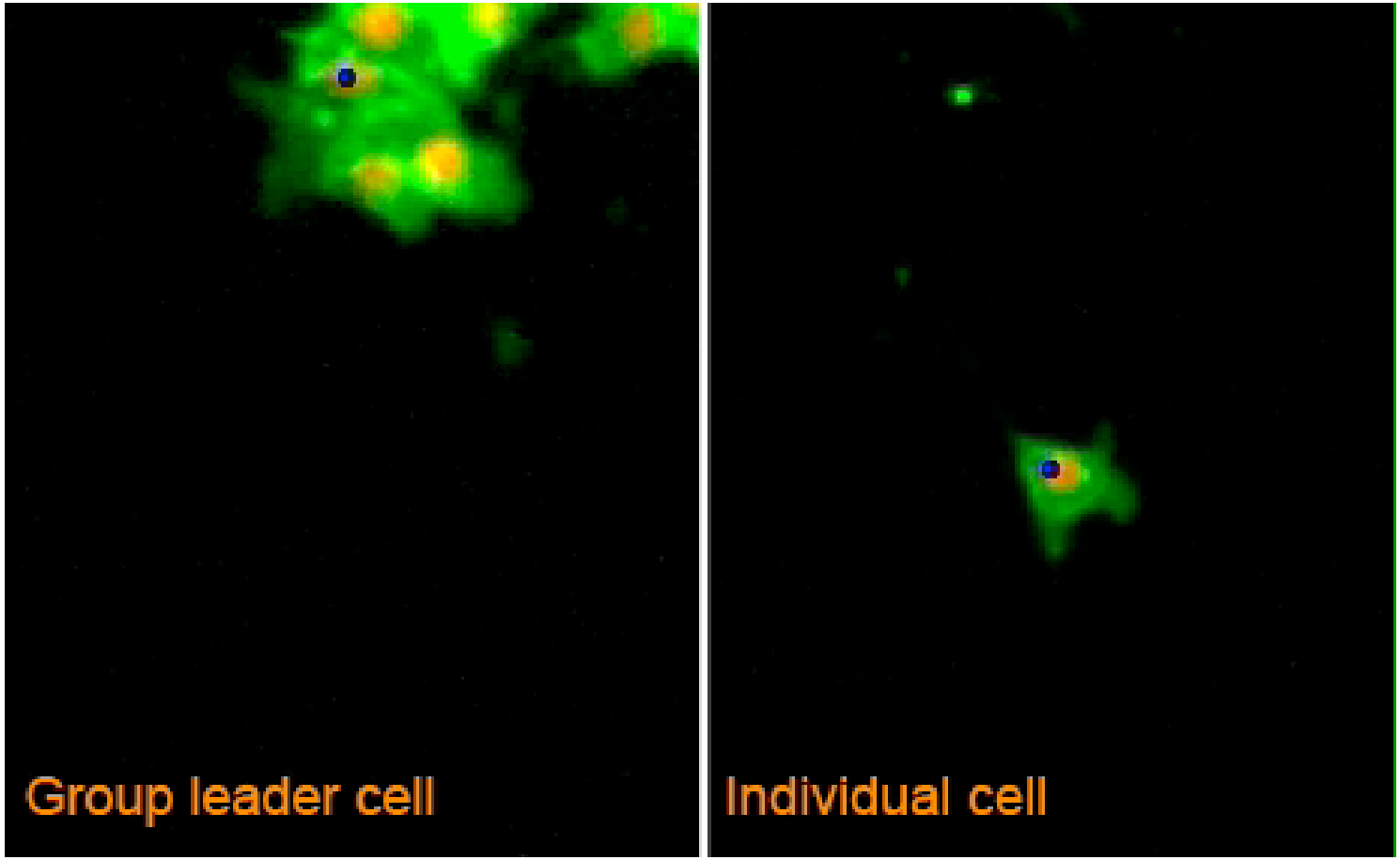
Netherlands Consortium for Systems Biology

Individual cells seemingly act independently...



Zebrafish blastoderm (embryonic tissue), P.Z. Myers, University of Minnesota (source: YouTube)

... but cells respond to each other



Contact-inhibition in cultured frog neural crest cells. Carmona-Fontaine *et al.* Nature 2008.

CWI

Netherlands Consortium for Systems Biology

How does genetics regulate development?

1. How does **genetics** regulate cell behaviors?
2. How do collective cell behaviors follow from **behaviors of individual cells**?

CWI

Netherlands Consortium for Systems Biology

How does genetics regulate development?

1. How does **genetics** regulate cell behaviors?
2. How do collective cell behaviors follow from **behaviors of individual cells**?

CWI

Netherlands Consortium for Systems Biology

How does genetics regulate development?

1. How does **genetics** regulate cell behaviors?
2. How do collective cell behaviors follow from **behaviors of individual cells**?

↓

- Experiments in cell cultures and *in vivo*
- Cell-based, computational modeling

CWI

Netherlands Consortium for Systems Biology

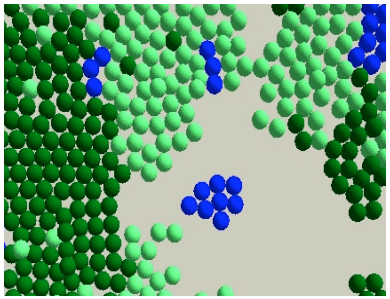
Cell-based Modeling

See *e.g.* Merks *et al. Phys. A* 2005

- Genetic and metabolic networks primarily regulate *individual cells*
  - Response to extracellular signals, secretion of signaling and extracellular matrix proteins, cell migration, cell adhesion, *etc.*
- To understand how genetics regulates multicellular phenomena, we must ask:
  - how genetics drives cell behavior (*i.e.* networks)
  - how cell behavior produces multicellular patterns
  - how the cells (and their regulatory networks) respond to the multicellular environment
- **"Middle-out approach" (Brenner, cited in Denis Noble 2006, *The Music of Life)***
  - the cell in the middle

Cell-based modeling techniques

- Individual/agent-based models
  - Cells represented as points, or spheres, or ellipsoids



Sun *et al.* J. Roy. Soc. Interface 2007

E.g., epitheliome (Smallwood, Sheffield)

- Many cell-based mechanisms depend on membrane movements and cell polarity
- Represent cells by *collections* of particles
  - Scales:
    - 1) pseudopods; 2) single cells; 3) tissues

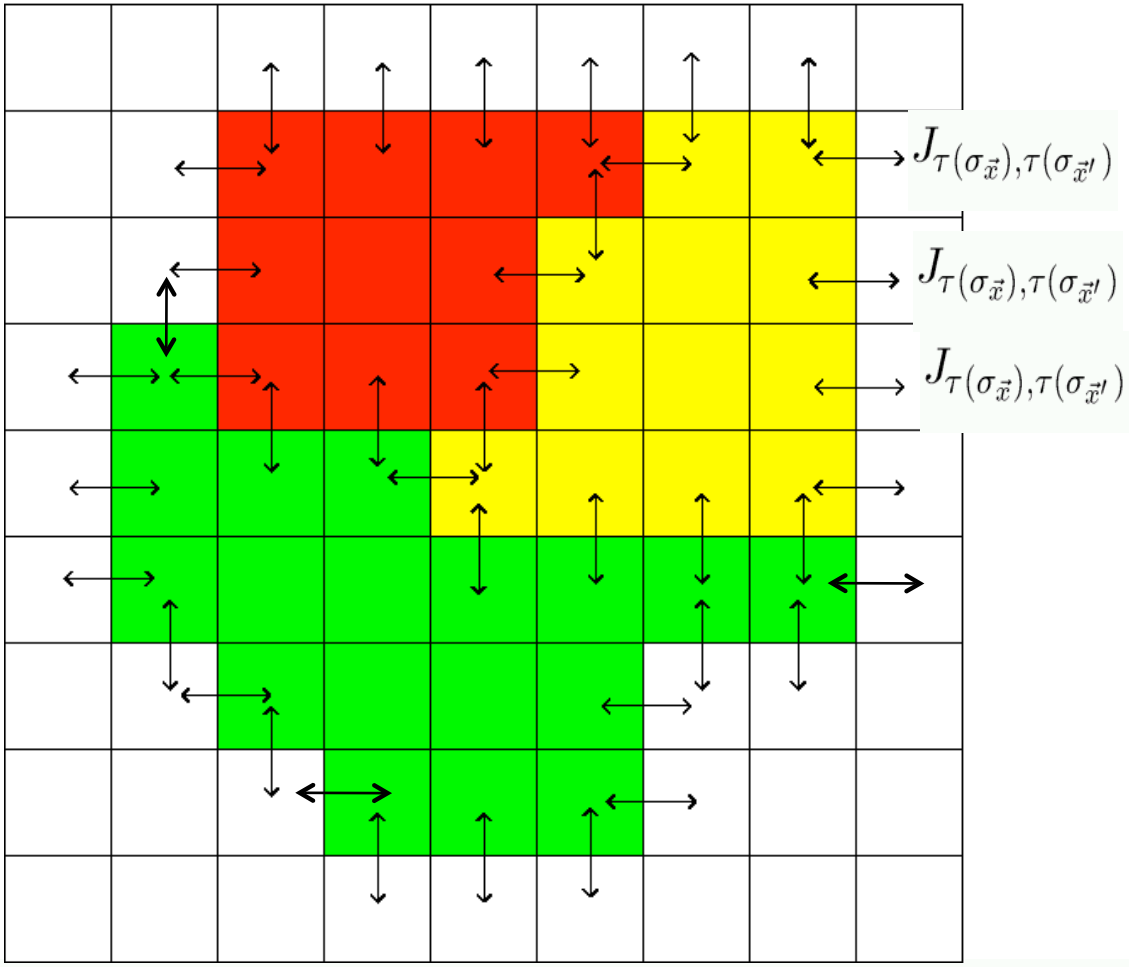
Cell-based modeling methodology

Cellular Potts Model

(Graner and Glazier, 1992; Glazier and Graner, 1993)

- *Cellular automata* model: cells live on grid
- **A cluster** of CA sites represents the *biological* cell
- Metropolis algorithm:
  - Generalized energy represents balance of forces in cell community:
    - cell size, cell adhesion, chemotaxis, *etc.*
  - Cells attempt to copy their state into neighboring sites: accepted if copy reduces energy

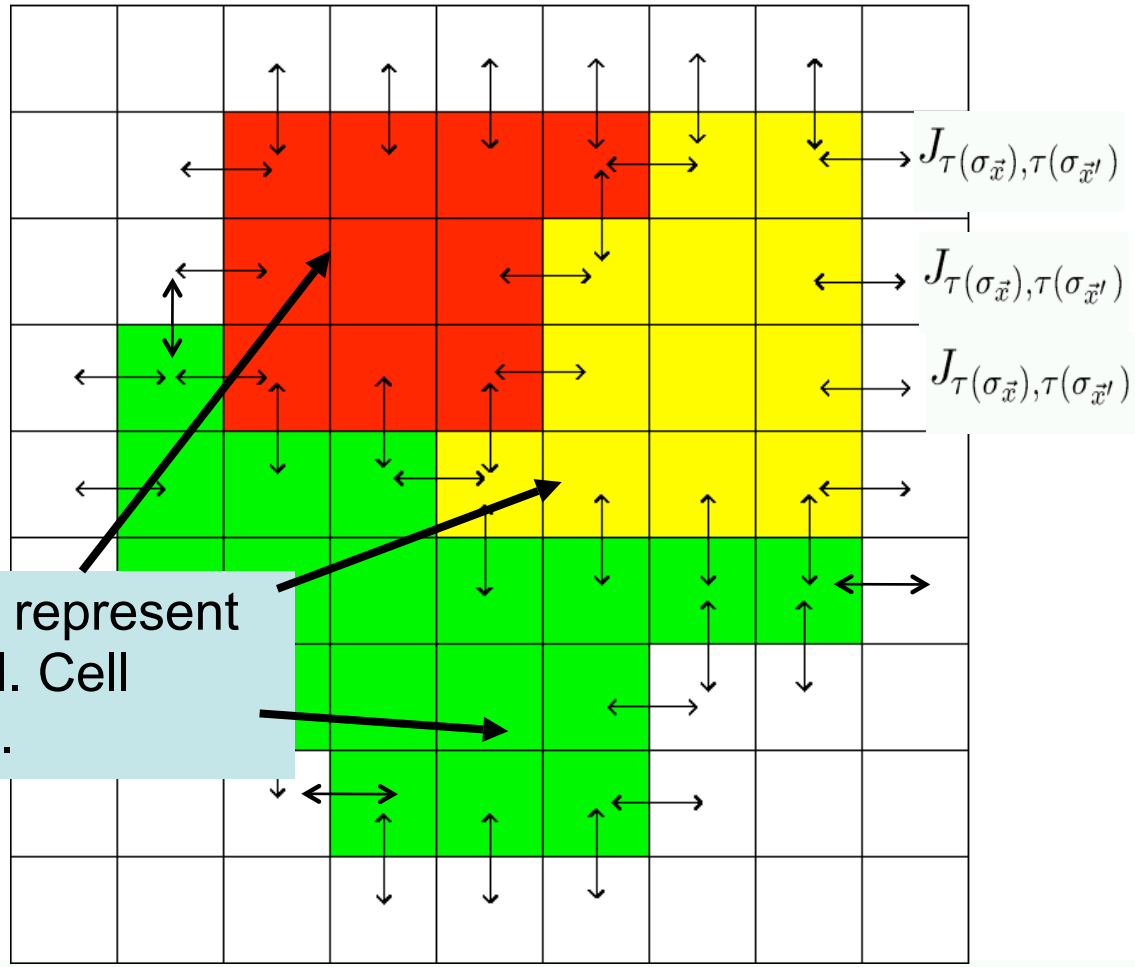
Cellular Potts Model



$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model

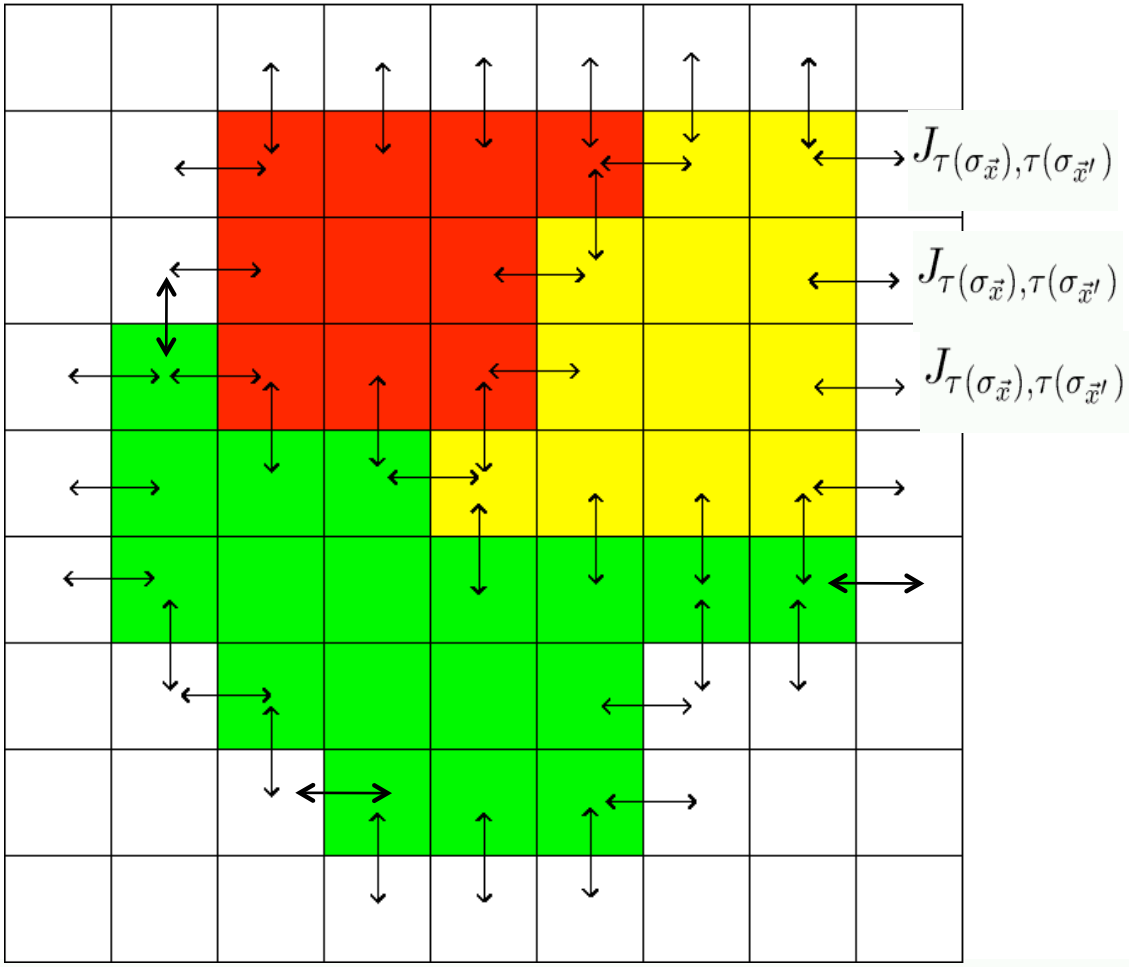


Multiple grid sites represent one biological cell. Cell identity index is σ.

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1-\delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma}(a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

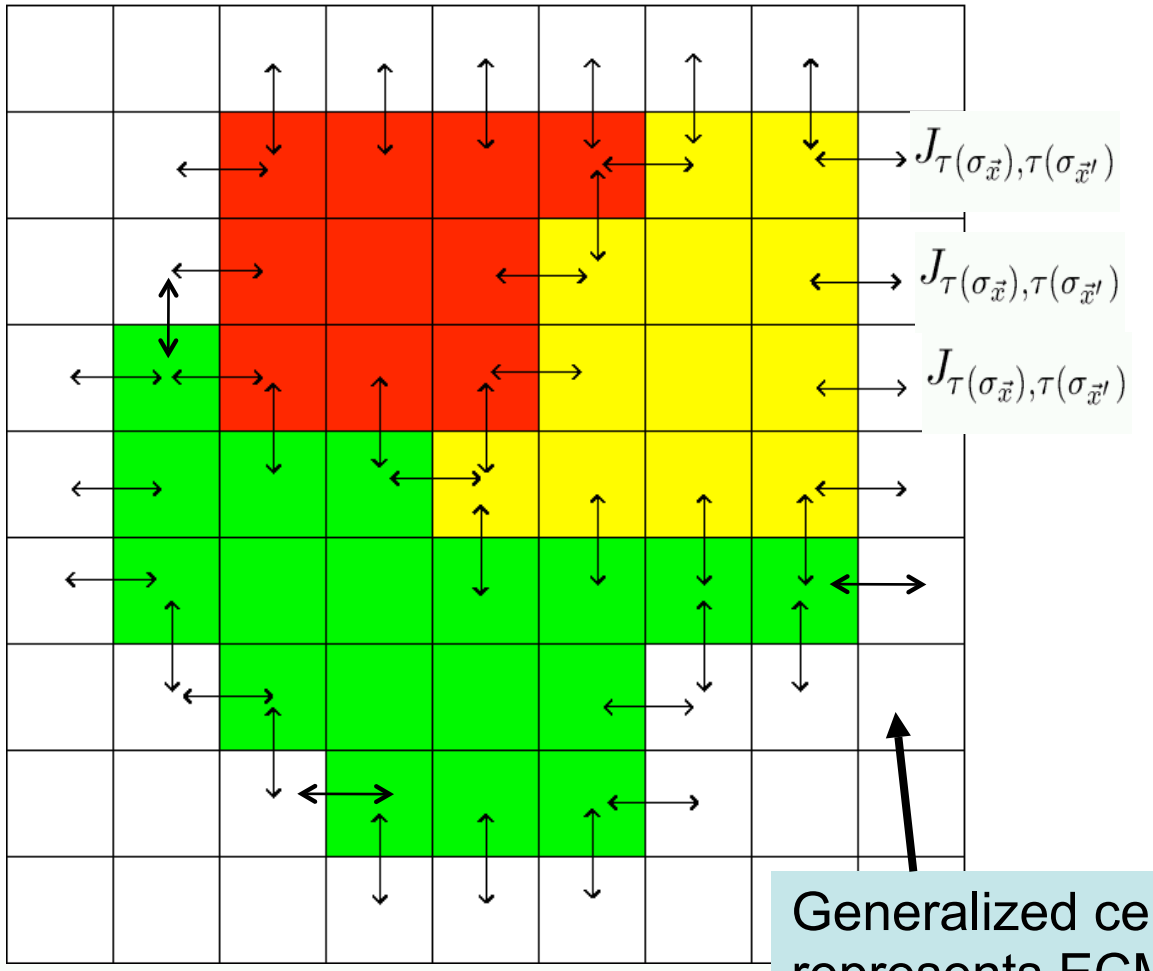
Cellular Potts Model



$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model

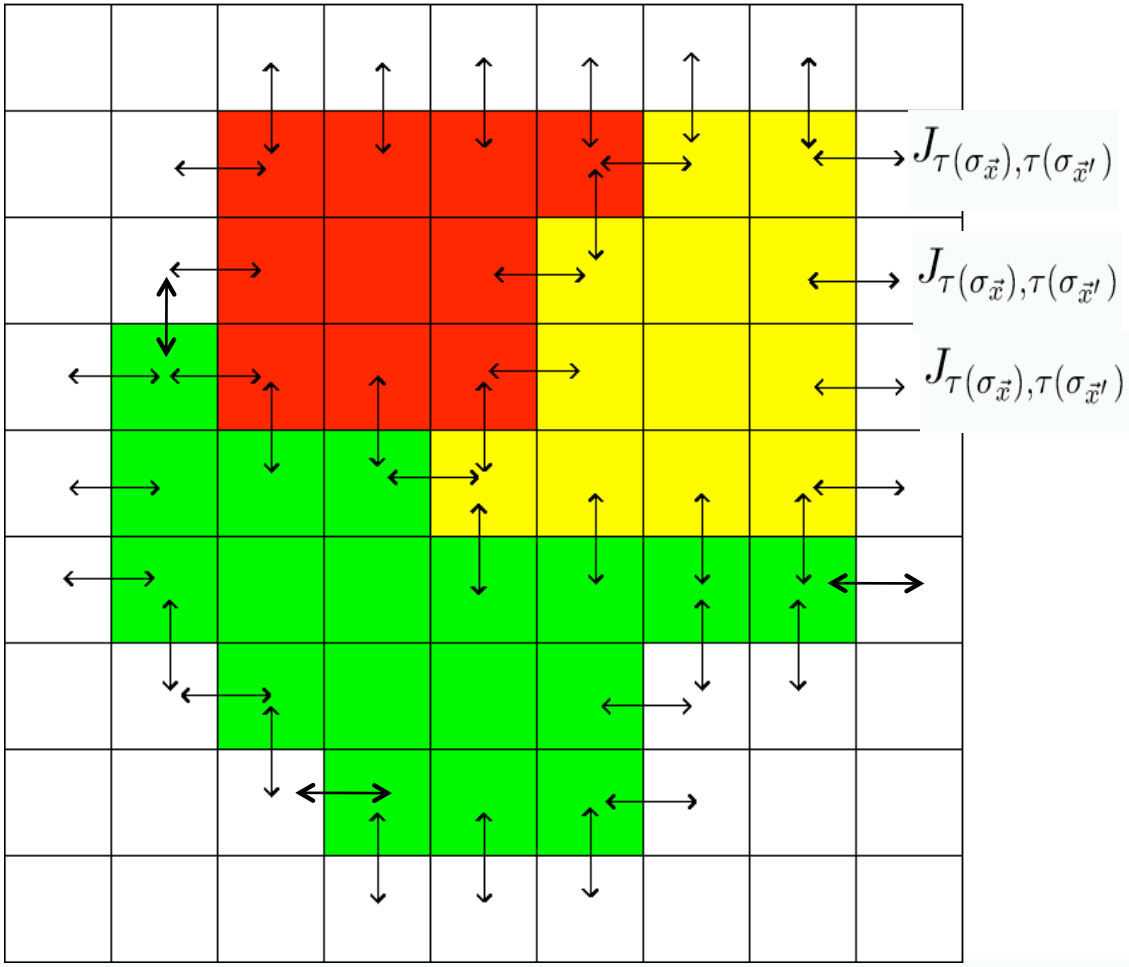


Generalized cell type 0 represents ECM or “medium”.

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1-\delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma}(a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model

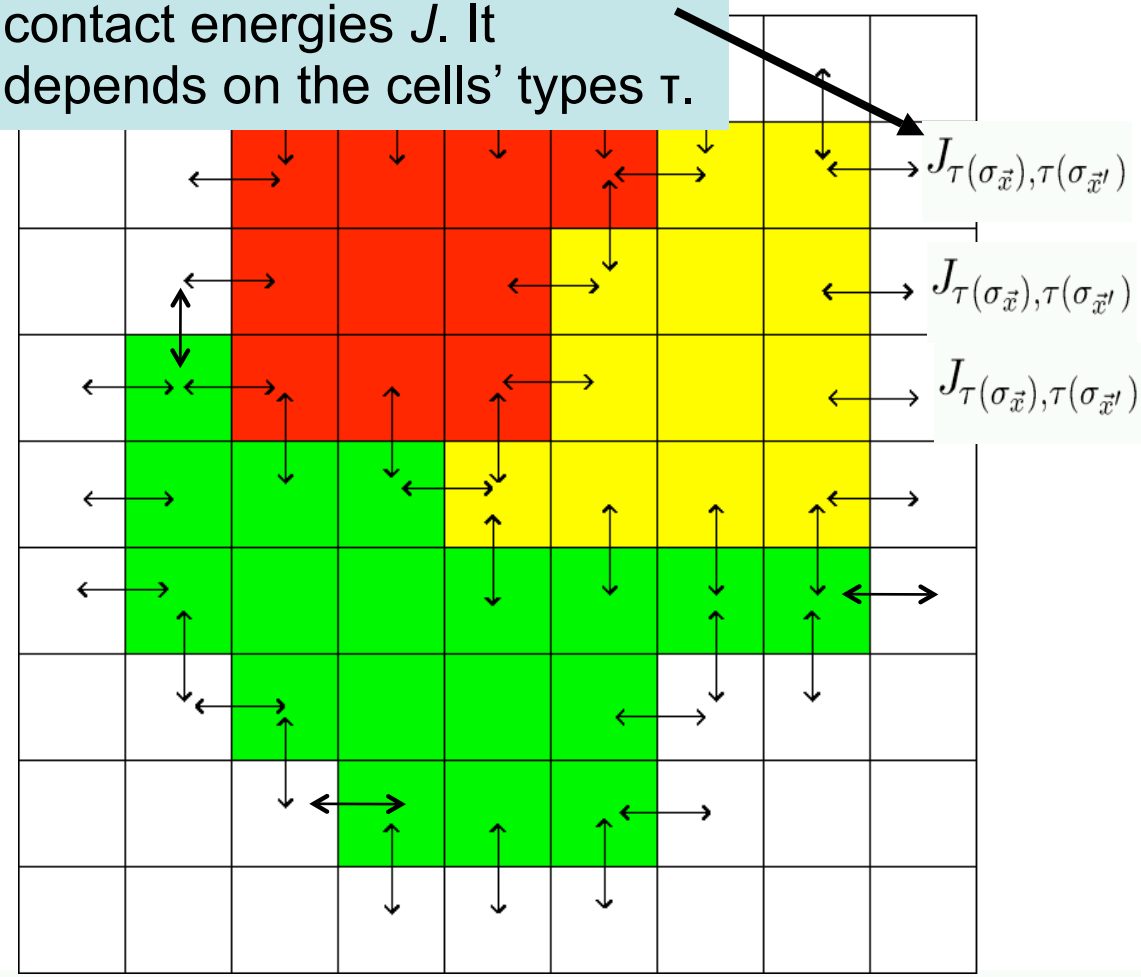


$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model

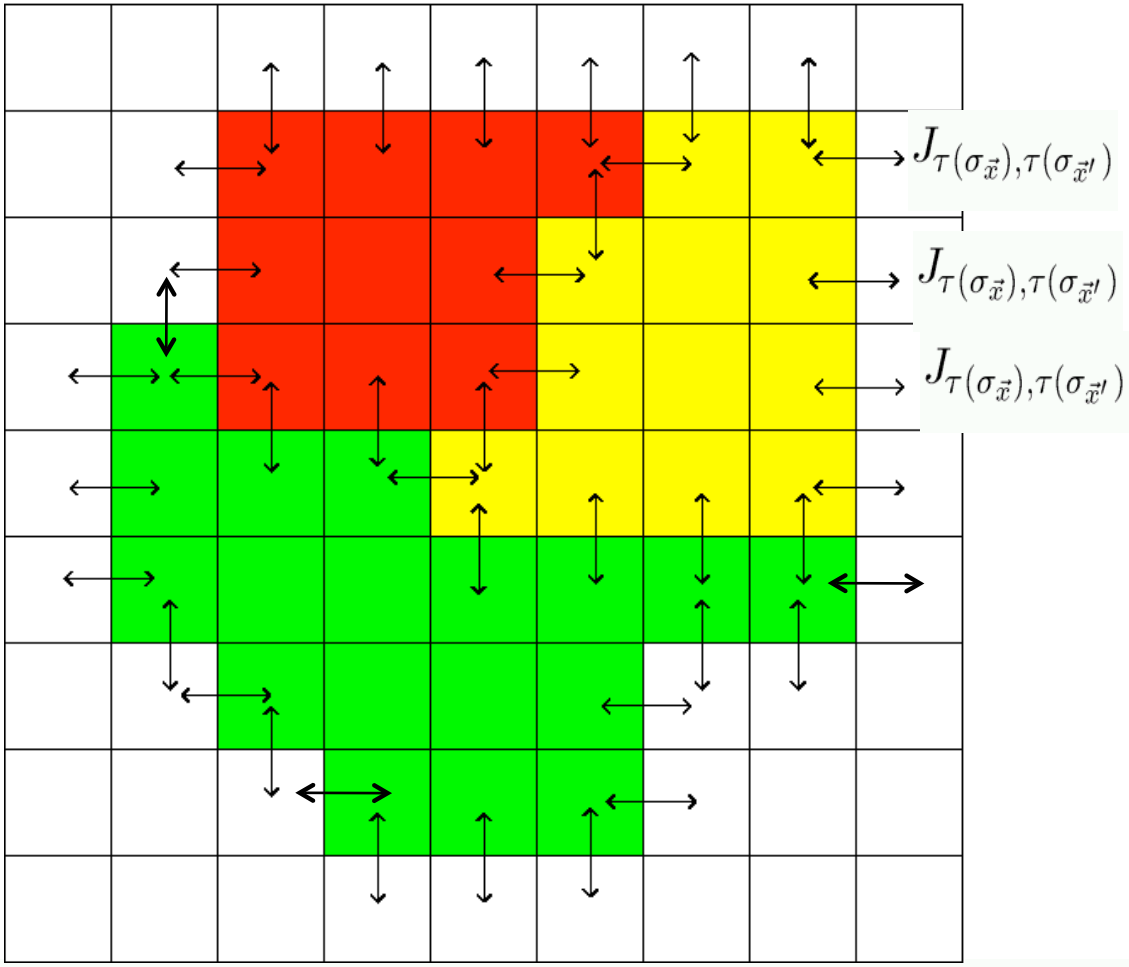
Cell adhesion given by contact energies *J*. It depends on the cells' types τ.



$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1-\delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma}(a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

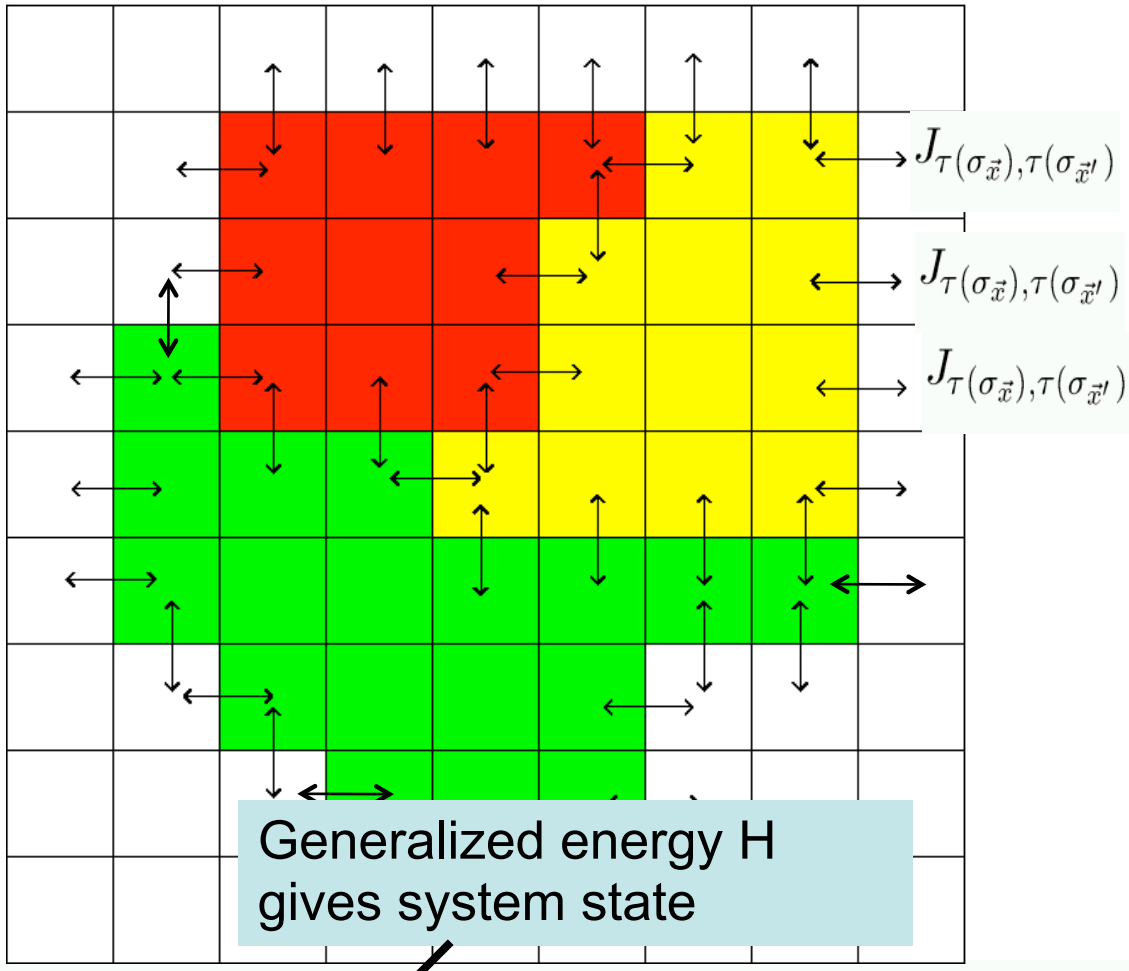
Cellular Potts Model



$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model



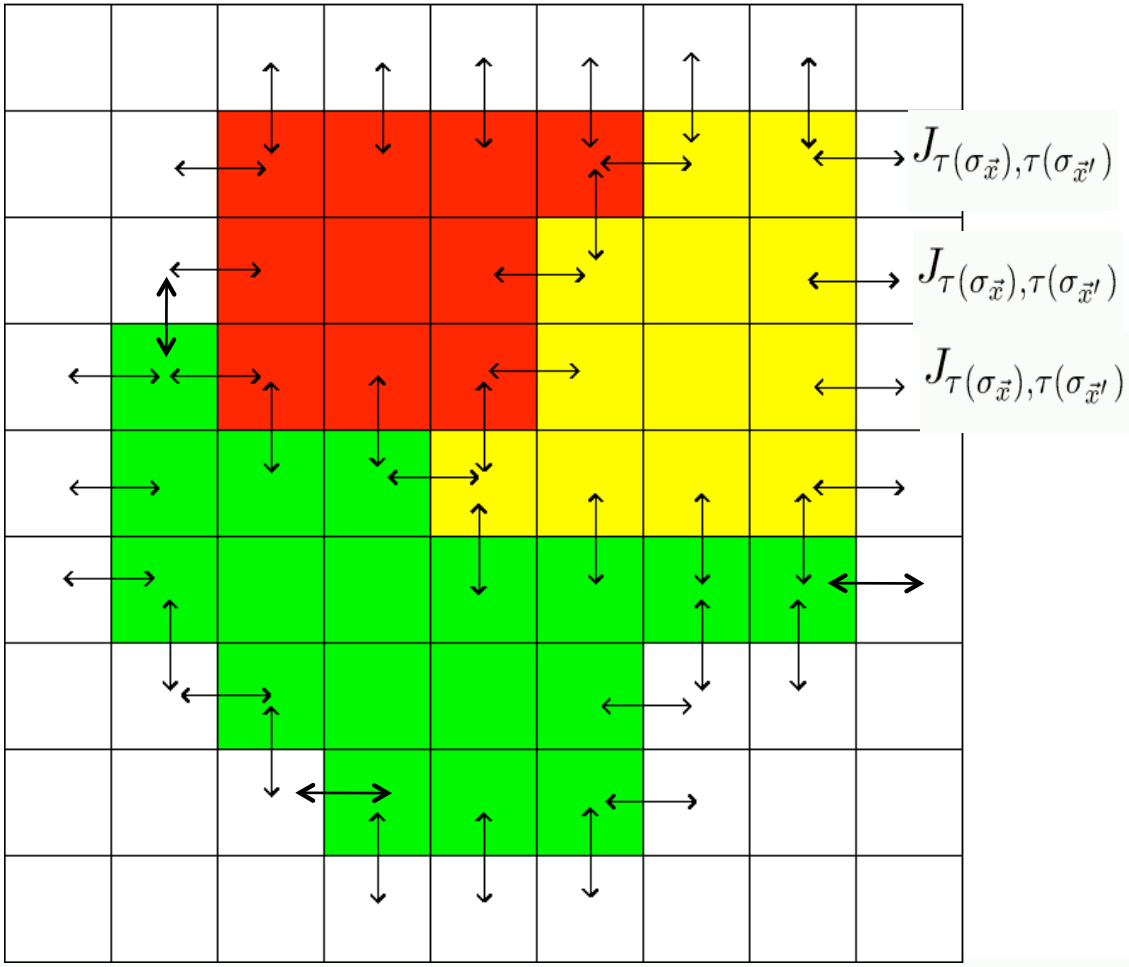
Generalized energy H gives system state

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1-\delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma}(a_\sigma - A_\sigma)^2$$

Cell adhesion

Volume conservation

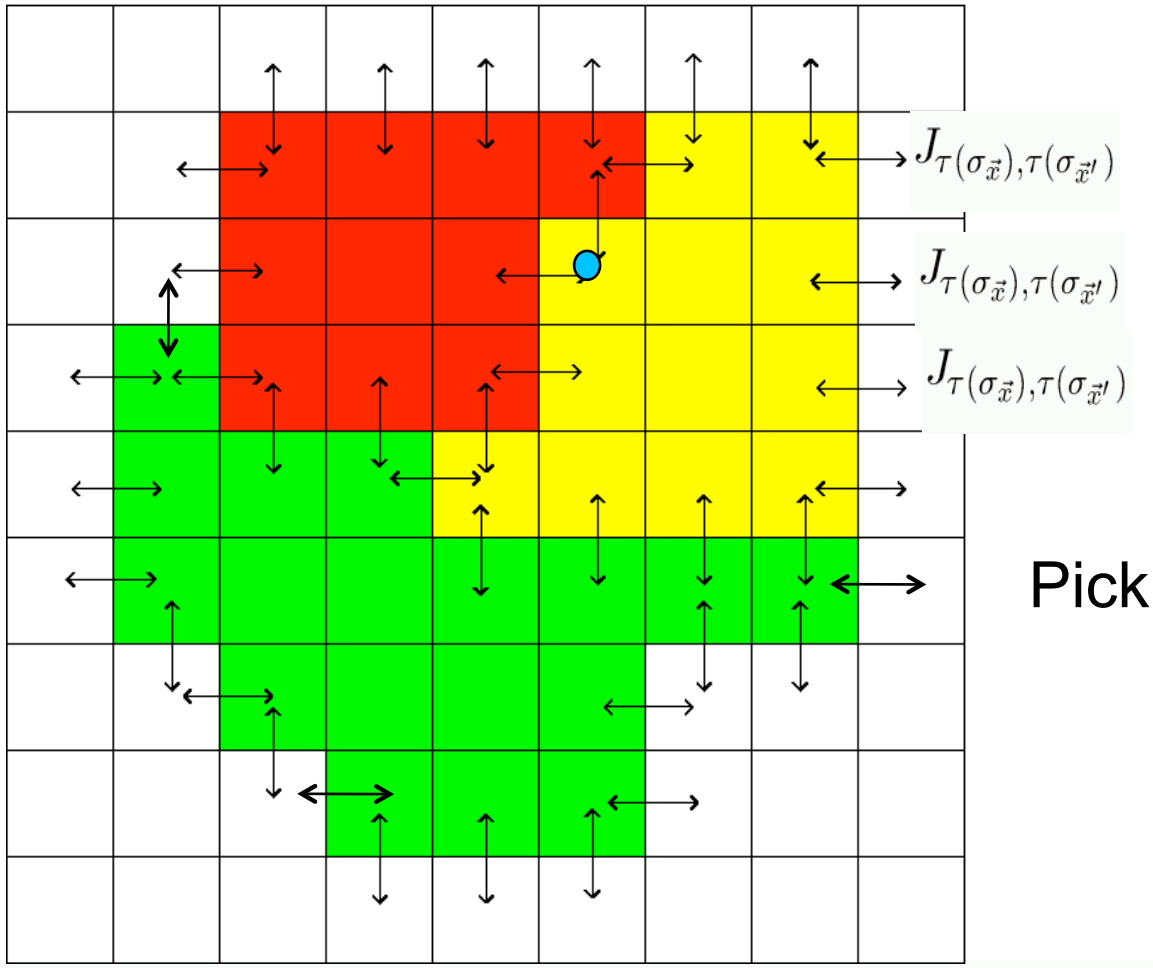
Cellular Potts Model



$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model

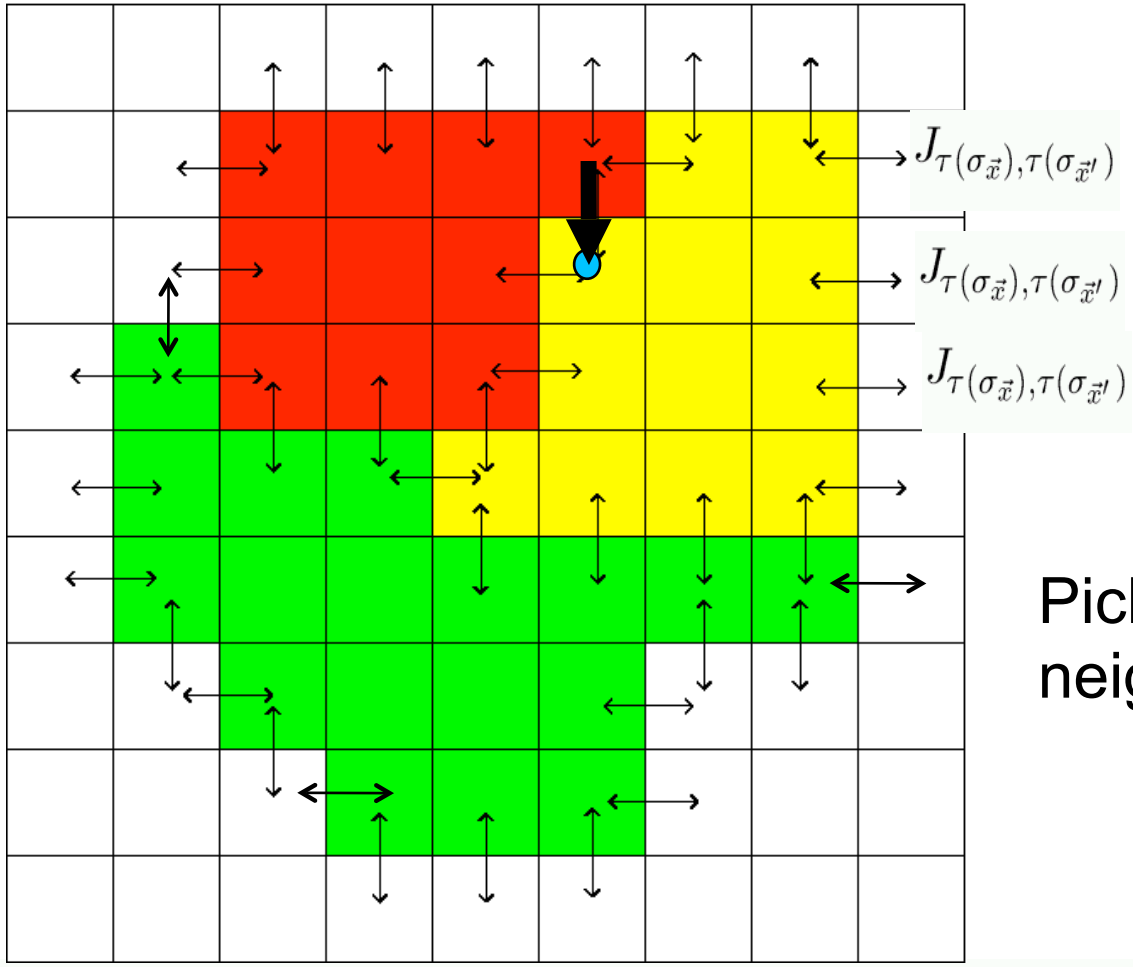


Pick random site

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1-\delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma}(a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model

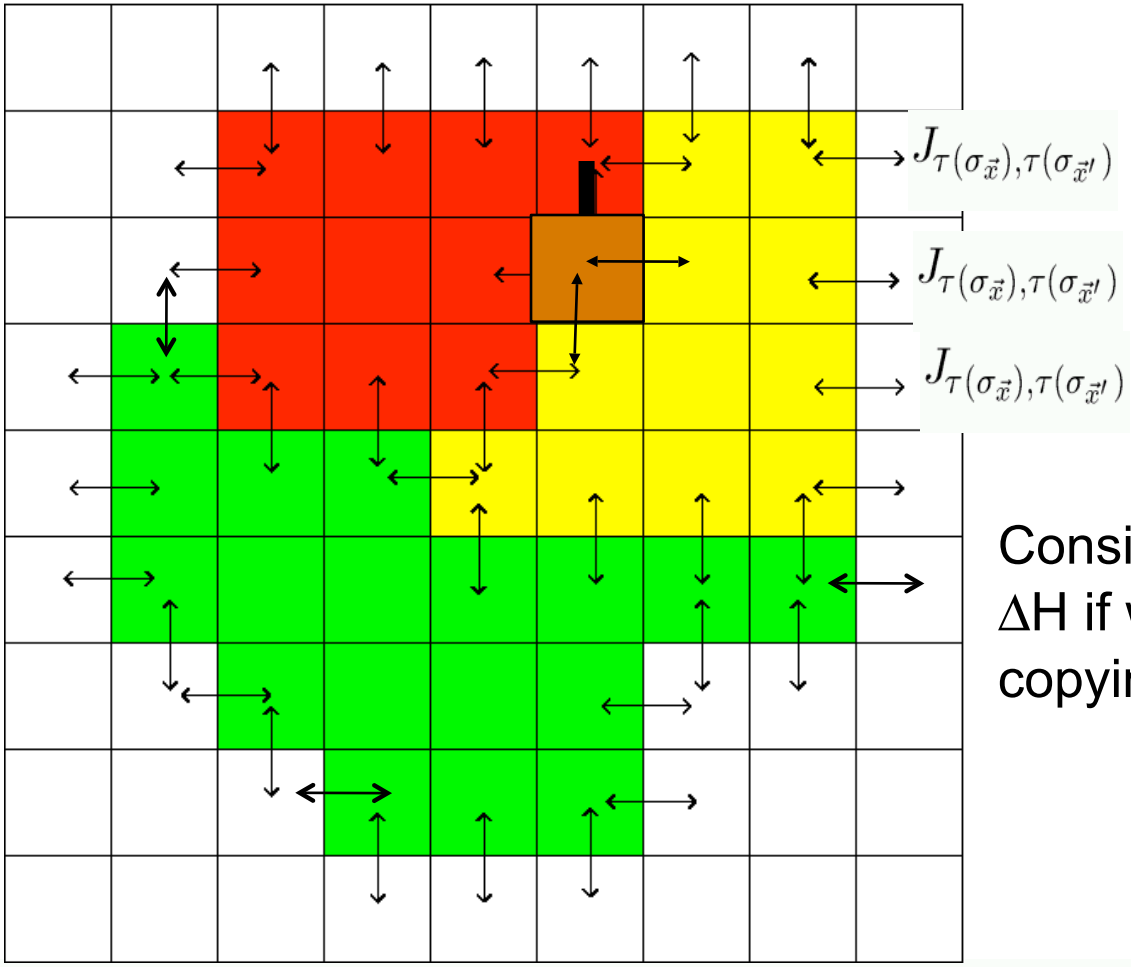


Pick random neighbor

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model

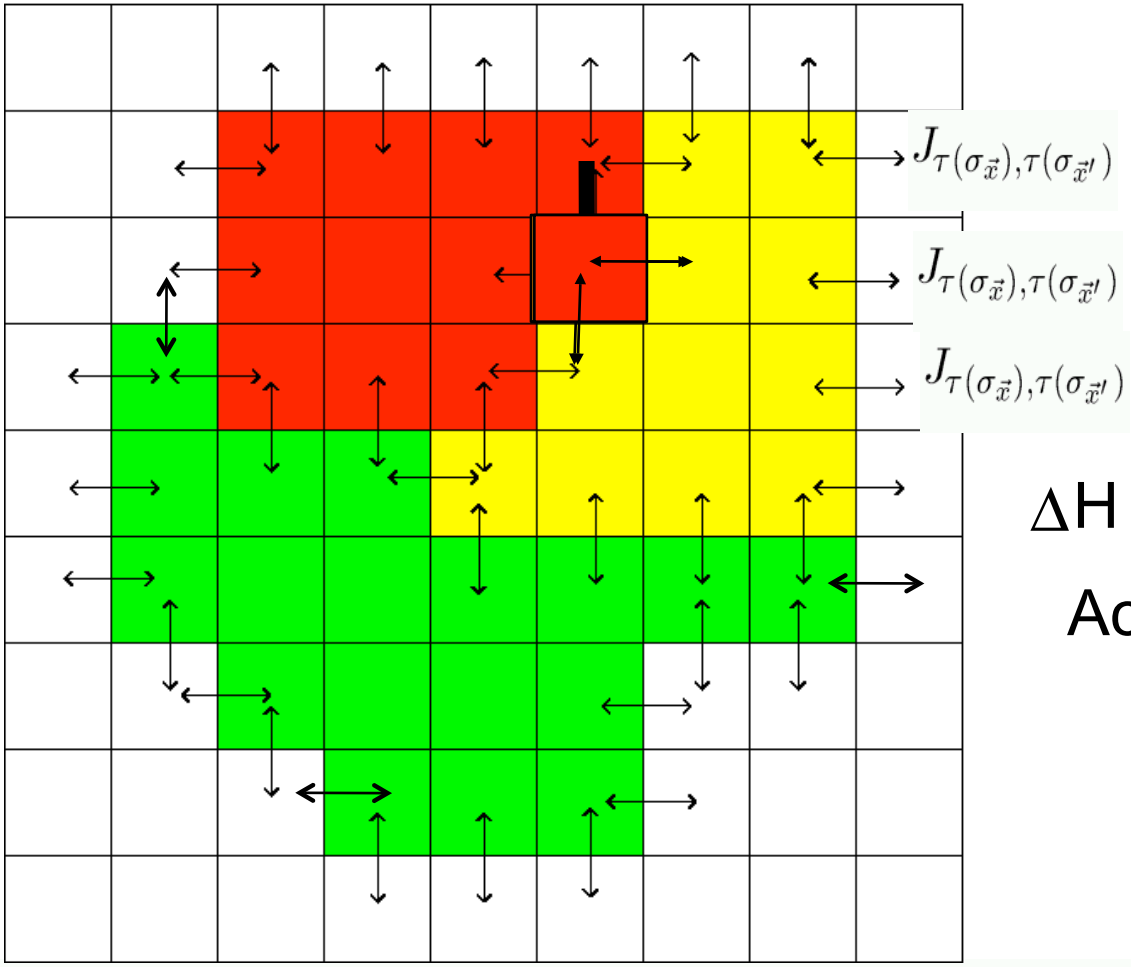


Consider energy change ΔH if we accepted this copying

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1-\delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma}(a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model



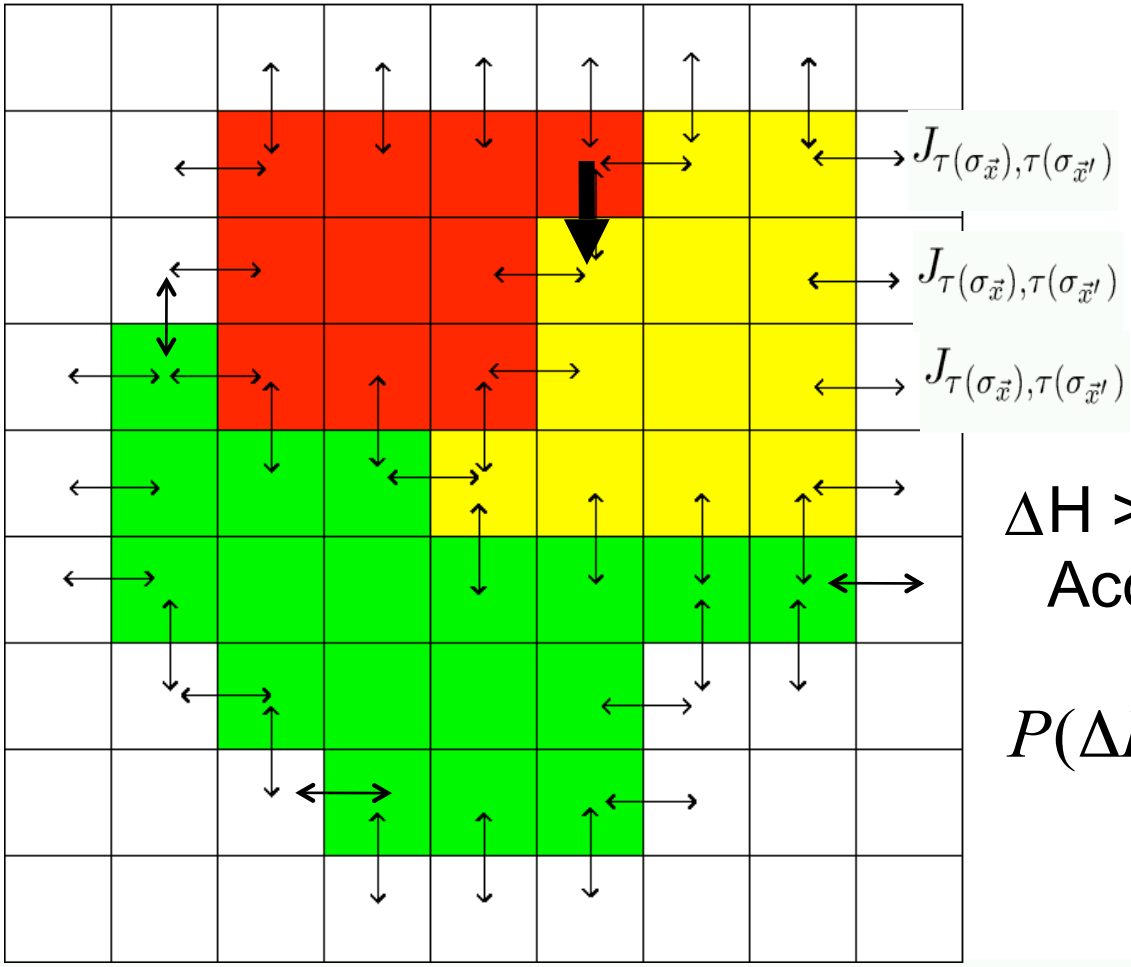
$\Delta H < 0$?

Accept always

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma}(a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model



$\Delta H > 0$?

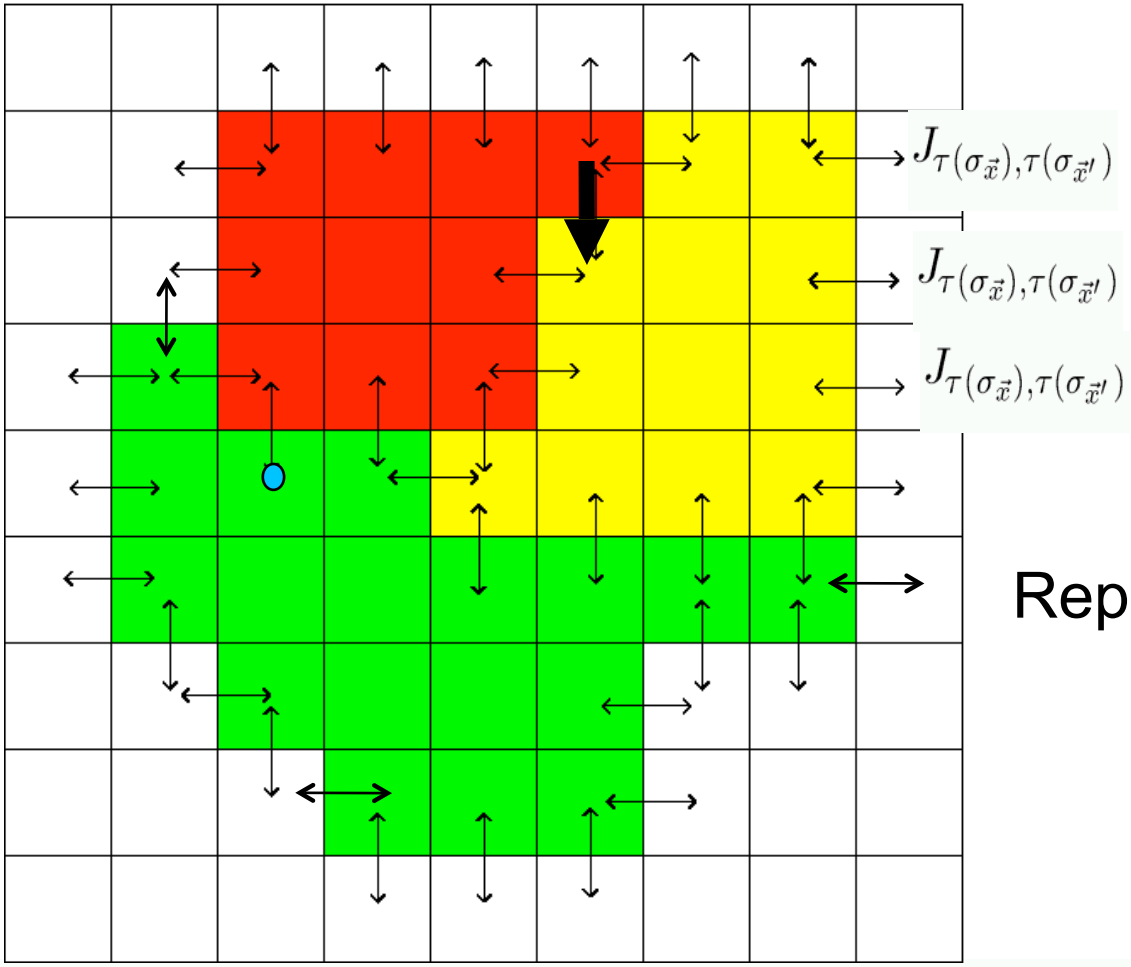
Accept with

$$P(\Delta H) = e^{\frac{-\Delta H + H_0}{T}}$$

$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2$$

Cell adhesion Volume conservation

Cellular Potts Model

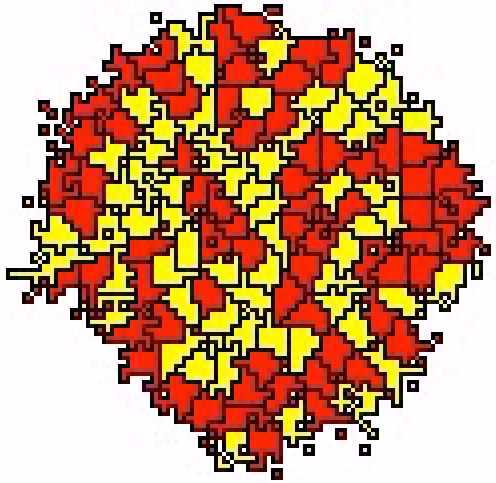


Repeat

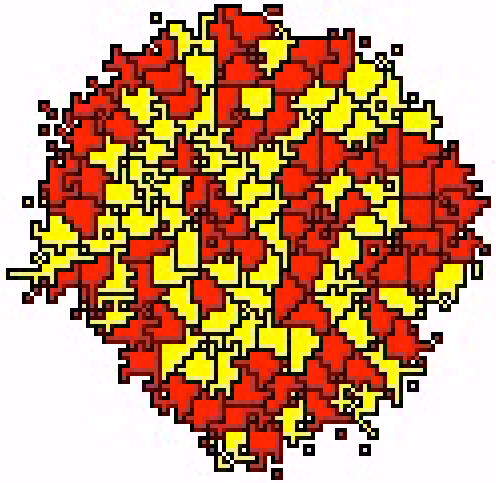
$$H = \sum_{\vec{x},\vec{x}'} J_{\tau(\sigma_{\vec{x}}),\tau(\sigma_{\vec{x}'})}(1 - \delta_{\sigma_{\vec{x}},\sigma_{\vec{x}'}}) + \lambda \sum_{\sigma} (a_\sigma - A_\sigma)^2$$

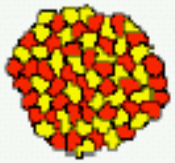
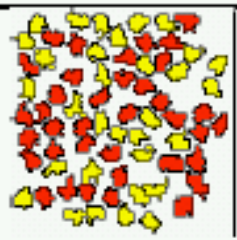
Cell adhesion Volume conservation

Homotypic adhesion
(yellow adheres to yellow, red to red)



Homotypic adhesion
(yellow adheres to yellow, red to red)



Initial configuration	
Sorting $J_{yellow,\ yellow} = J_{red,red} < J_{yellow,\ red}$	
Mixing $J_{yellow,\ red} > J_{yellow,\ yellow} = J_{red,red}$	
Engulfment $J_{yellow,\ red} < J_{yellow,\ medium}$ $J_{red,medium} < J_{yellow,\ medium}$	
No cell-cell adhesion $J_{cell,\ cell} > 2\ J_{cell,\ medium}$	

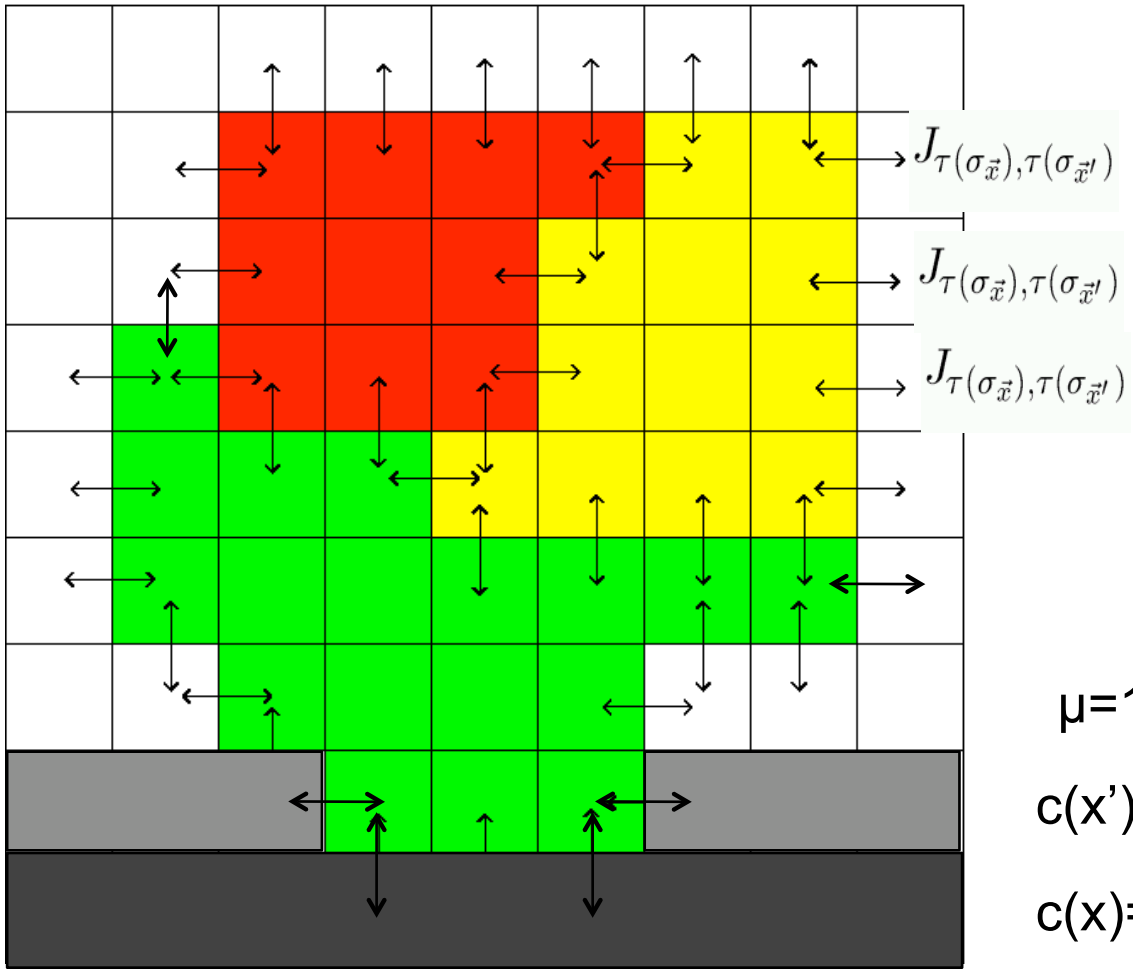
Chemotaxis

- Modify the energy term to bias pseudopod extensions according to chemical gradient (algorithm by Savill and Hogeweg *1997)*

$$\Delta H -= \mu(c(\vec{x}) - c(\vec{x}'))$$

Chemotaxis

Pseudopods extend more likely up chemical gradient



μ=100

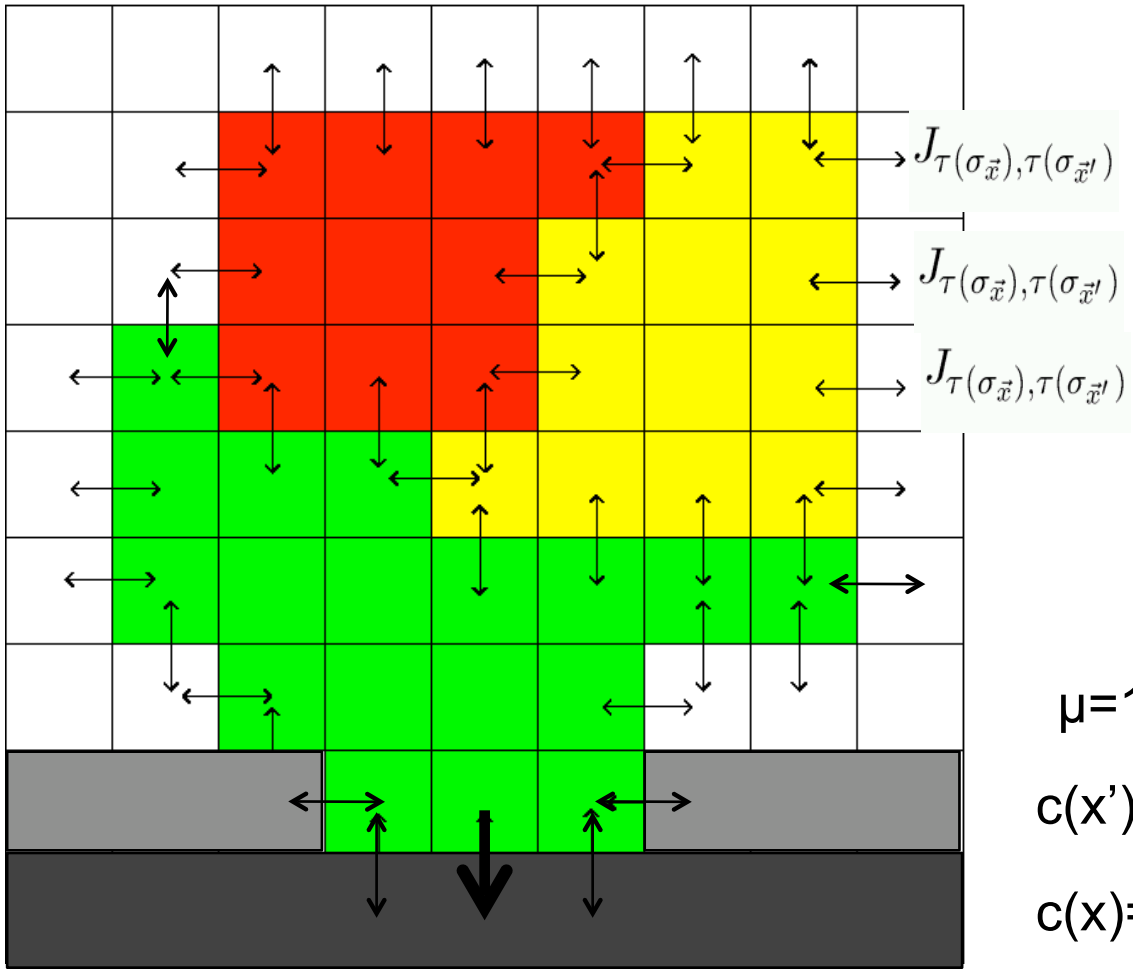
c(x')=0.5

c(x)=1

ΔH -= 50

Chemotaxis

Pseudopods extend more likely up chemical gradient



μ=100

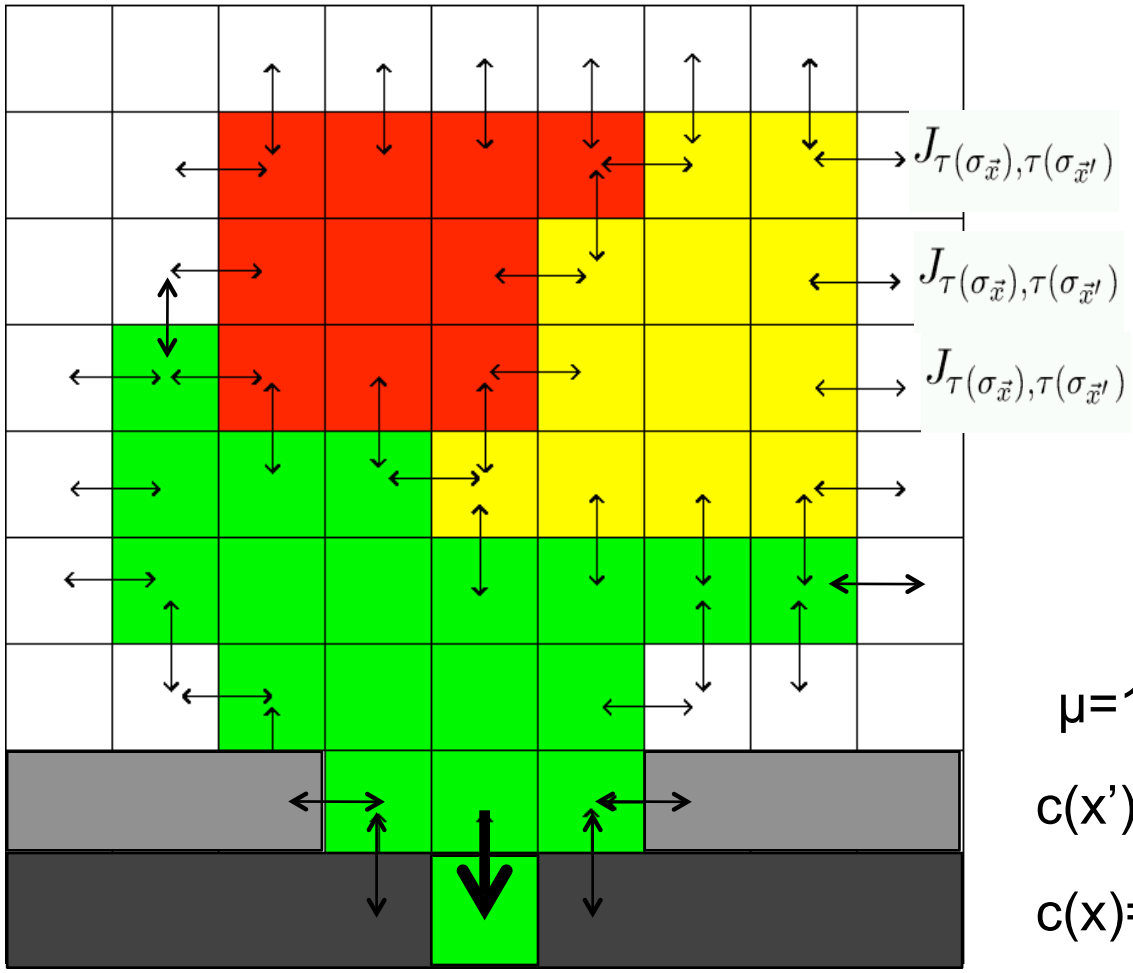
c(x')=0.5

c(x)=1

ΔH -= 50

Chemotaxis

Pseudopods extend more likely up chemical gradient



μ=100

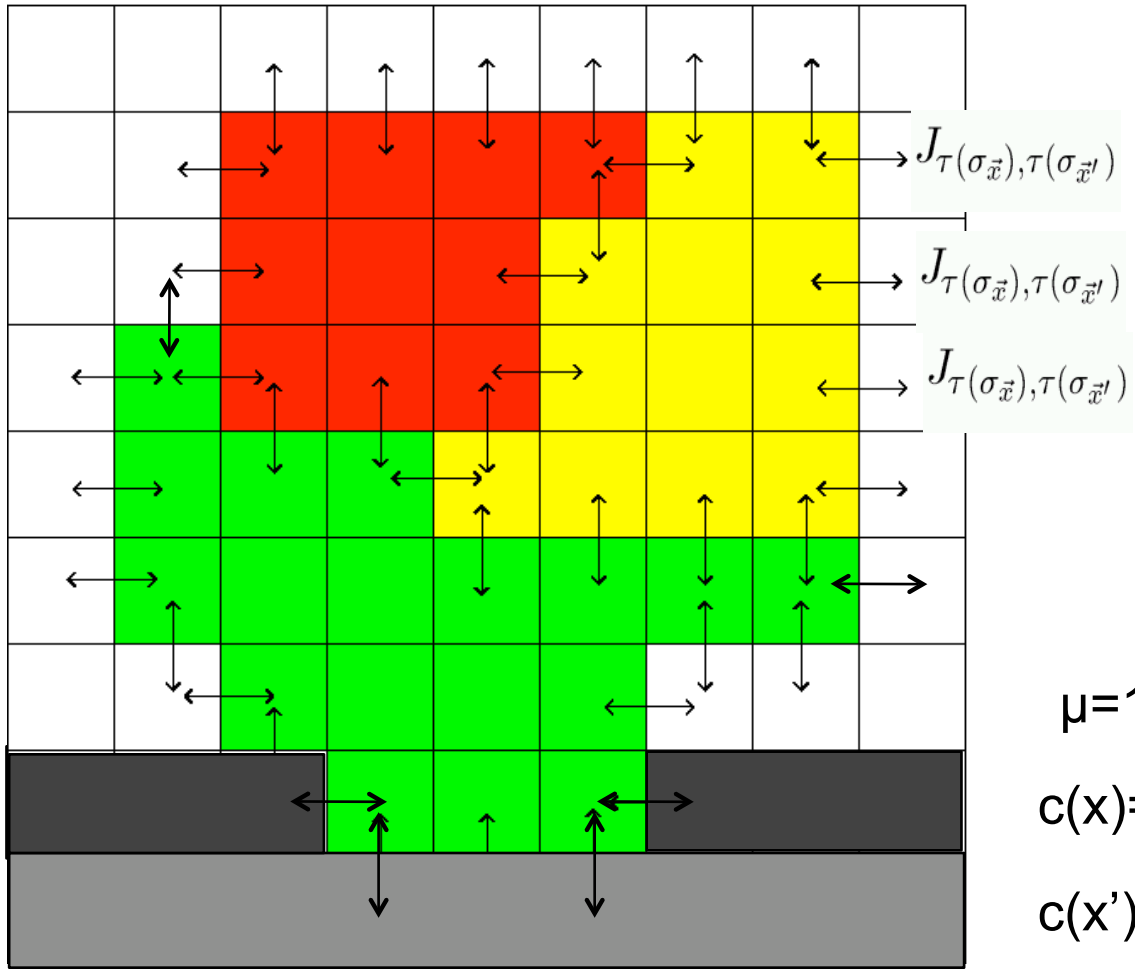
c(x')=0.5

c(x)=1

ΔH -= 50

Chemotaxis

Pseudopods retract more likely up chemical gradient



μ=100

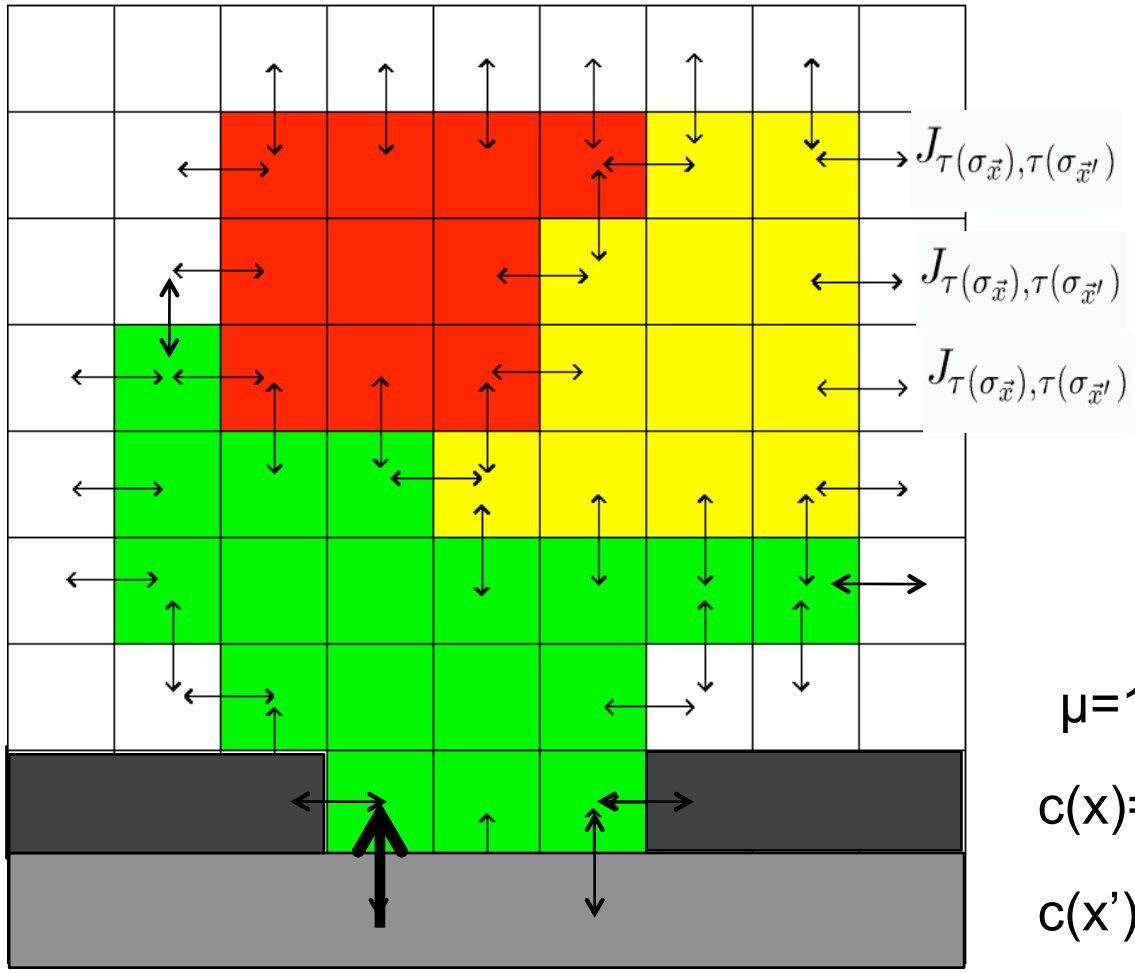
c(x)=1.0

c(x')=0.5

ΔH -= 50

Chemotaxis

Pseudopods retract more likely up chemical gradient



μ=100

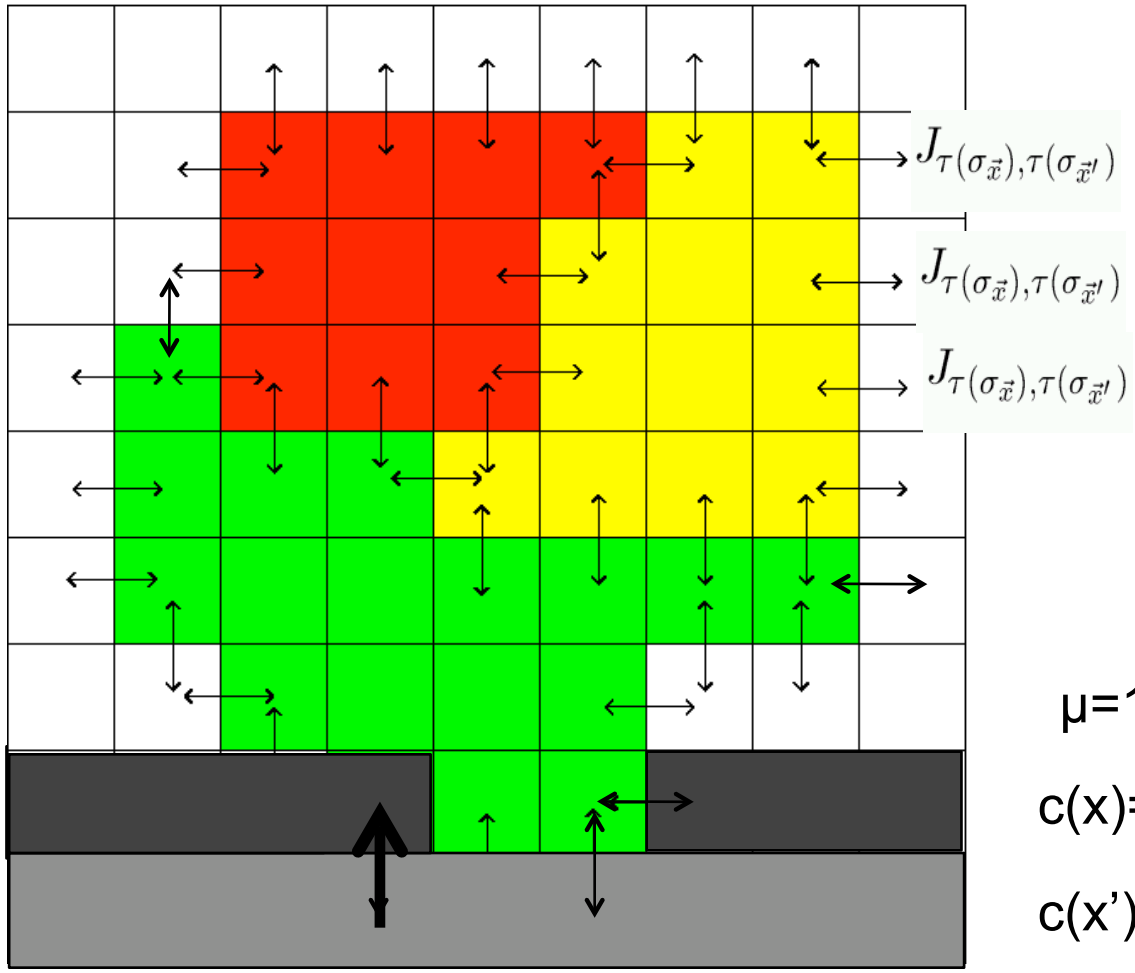
c(x)=1.0

c(x')=0.5

ΔH -= 50

Chemotaxis

Pseudopods retract more likely up chemical gradient



μ=100

c(x)=1.0

c(x')=0.5

ΔH -= 50

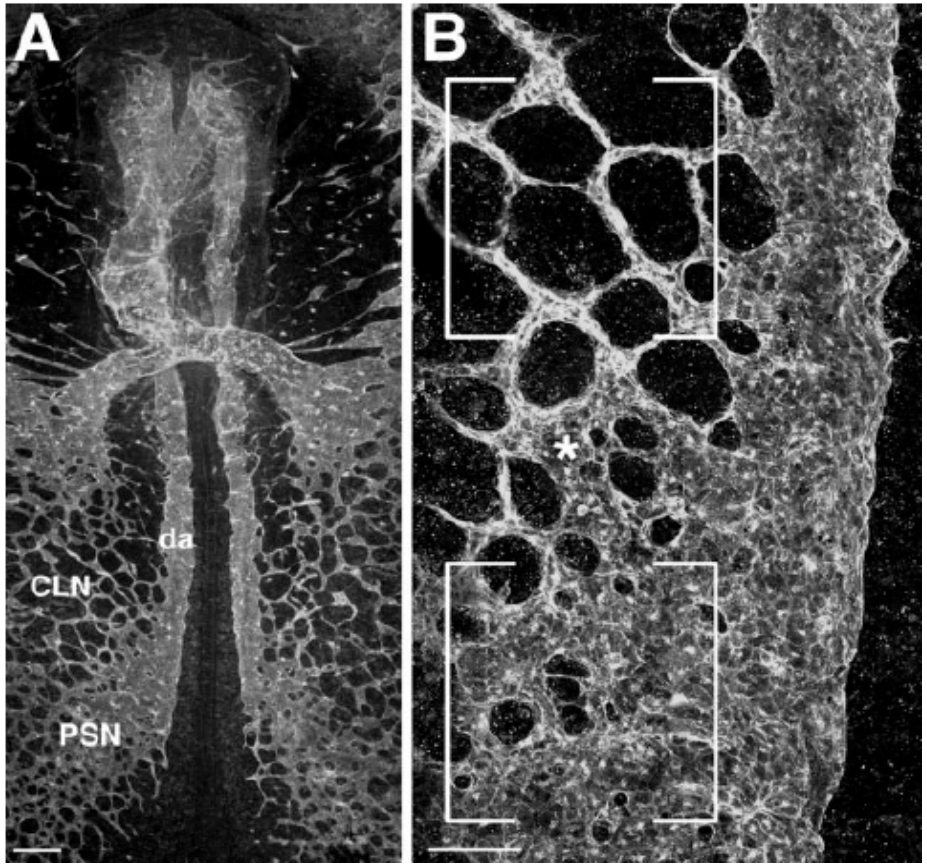
CWI

Netherlands Consortium for Systems Biology

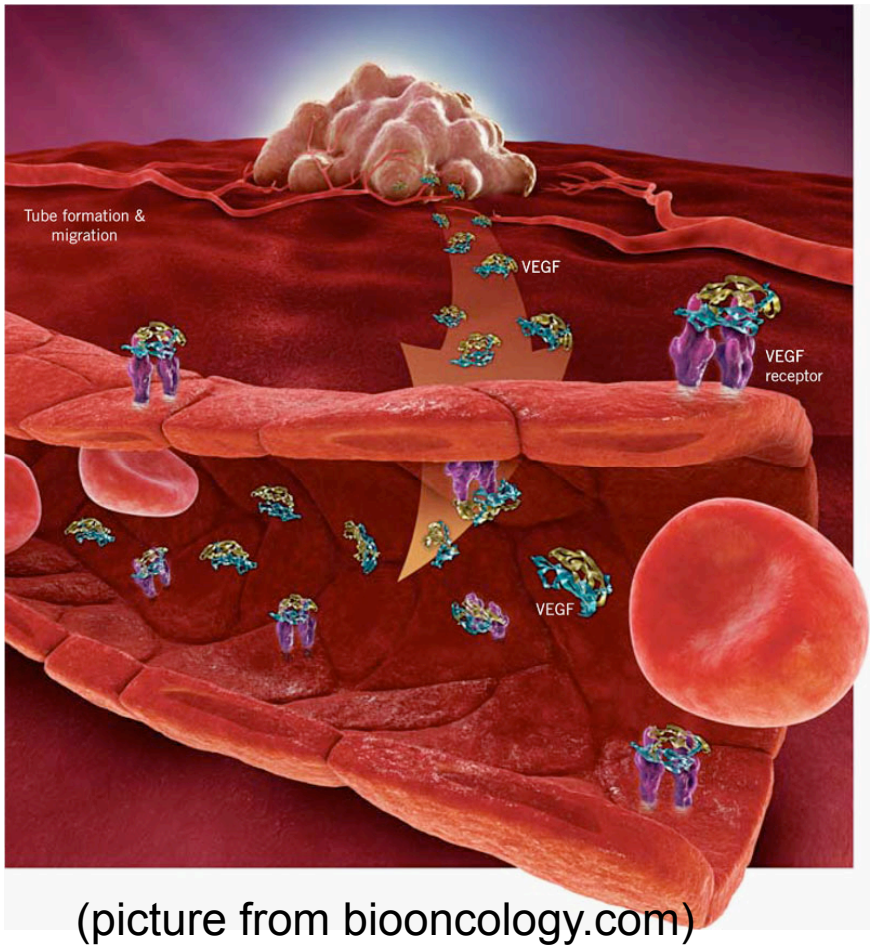
Blood vessel growth

Vasculogenesis
de novo assembly of endothelial cells

Angiogenesis sprouting or splitting of existing blood vessels



LaRue *et al.* (2003), *Dev. Dyn.* **228**:21.



(picture from biooncology.com)

Why model blood vessel growth?

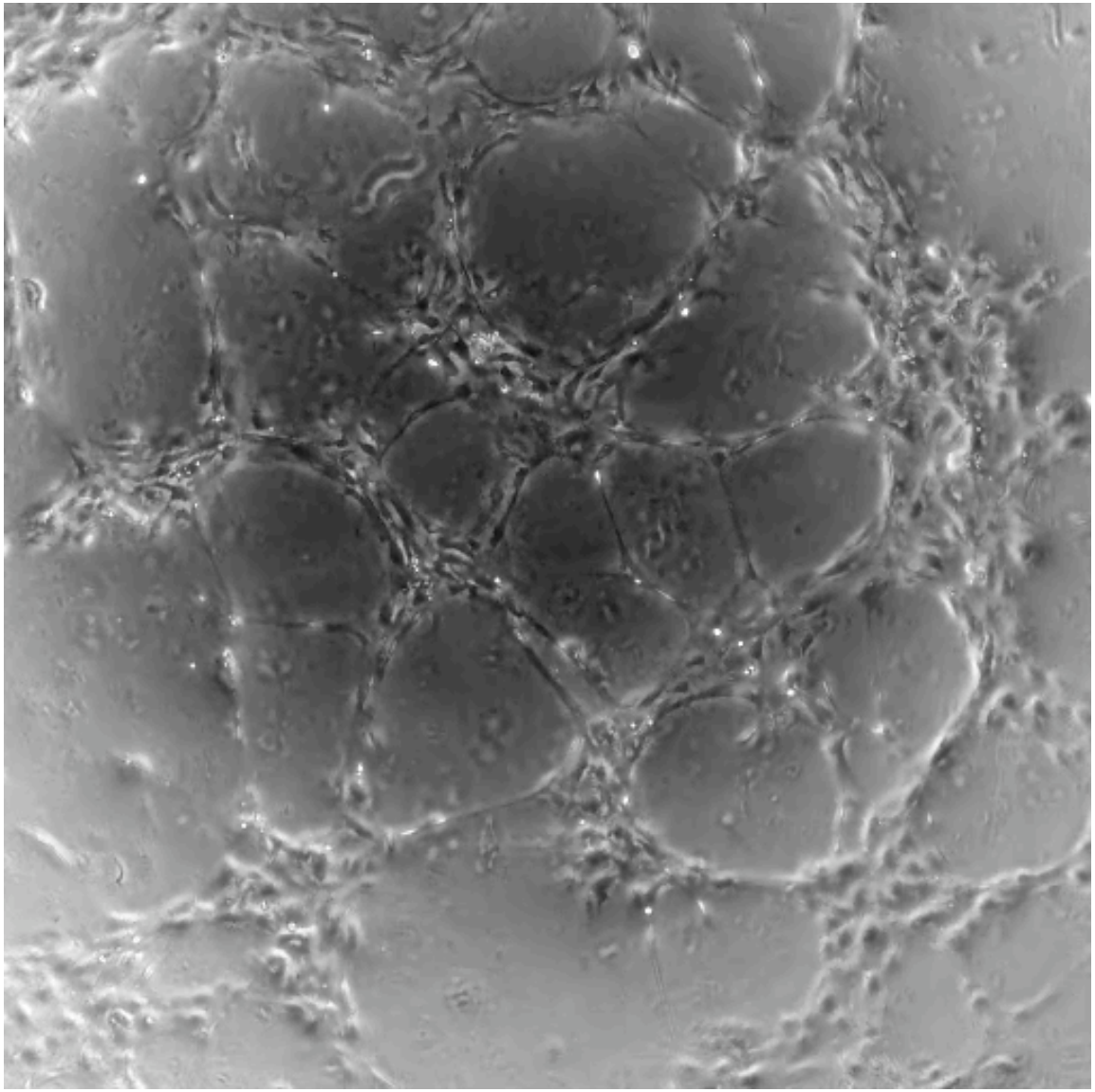
- *How* does endothelial cells' behavior drive blood vessel growth?
  - Distinguish *intrinsic* patterning abilities of endothelial cells from *extrinsic* patterning cues (*i.e.* signals from organs or tumors)
- Interplay between genetics and physics in biological development
- Are angiogenesis and vasculogenesis two sides of the same coin?
- **Tissue engineering:** Predict how scaffold and engineered tissue optimally guide endothelial cells to form blood vessels
- **Tumor growth:** controlling biophysical factors of tumor angiogenesis

CWI

Netherlands Consortium for Systems Biology

Simplified experimental system

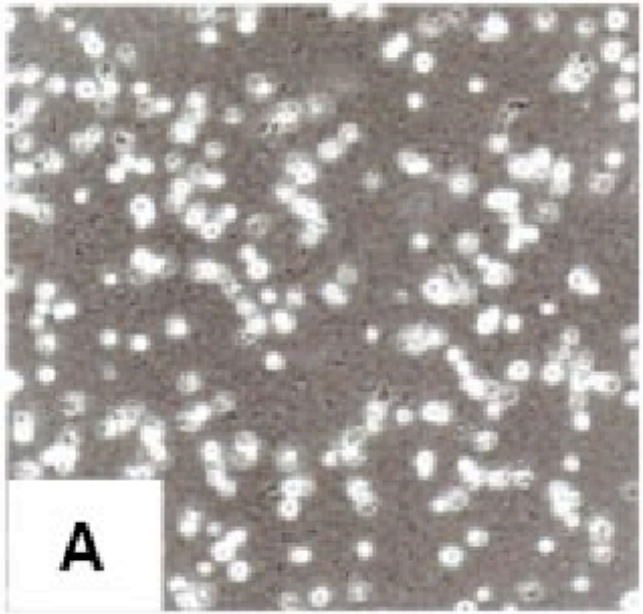
Human umbilical vein endothelial cells (HUVEC) in *Matrigel*

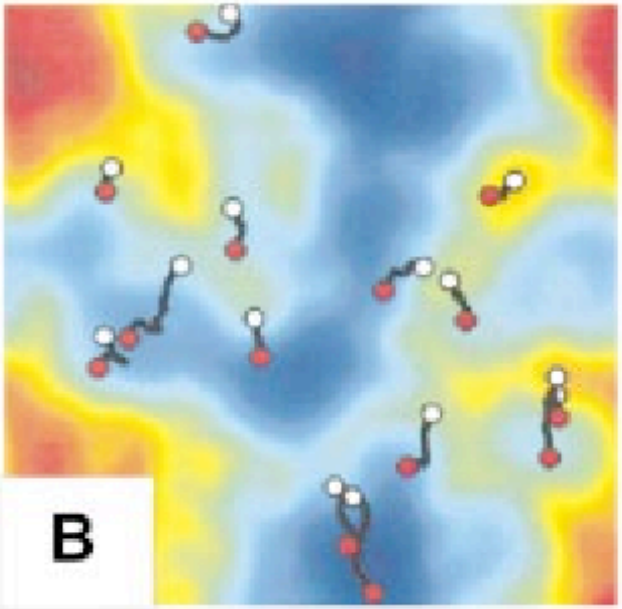


Movie: courtesy **Luigi Preziosi, *Politecnico di Torino, Italy***

Hypothesis: Chemotaxis
(Gamba *et al.* 2003; Serini *et al.*, 2003)

Observation: cells migrate to higher concentrations of cells





Partial-differential equation model

Gamba et al. (PRL 90 (41): 118101)

$$\frac{\partial n}{\partial t} + \nabla \cdot (n\vec{v}) = 0 \quad (1)$$

$$\frac{\partial \vec{v}}{\partial t} + \vec{v} \cdot \nabla \vec{v} = \mu \nabla c \quad (2)$$

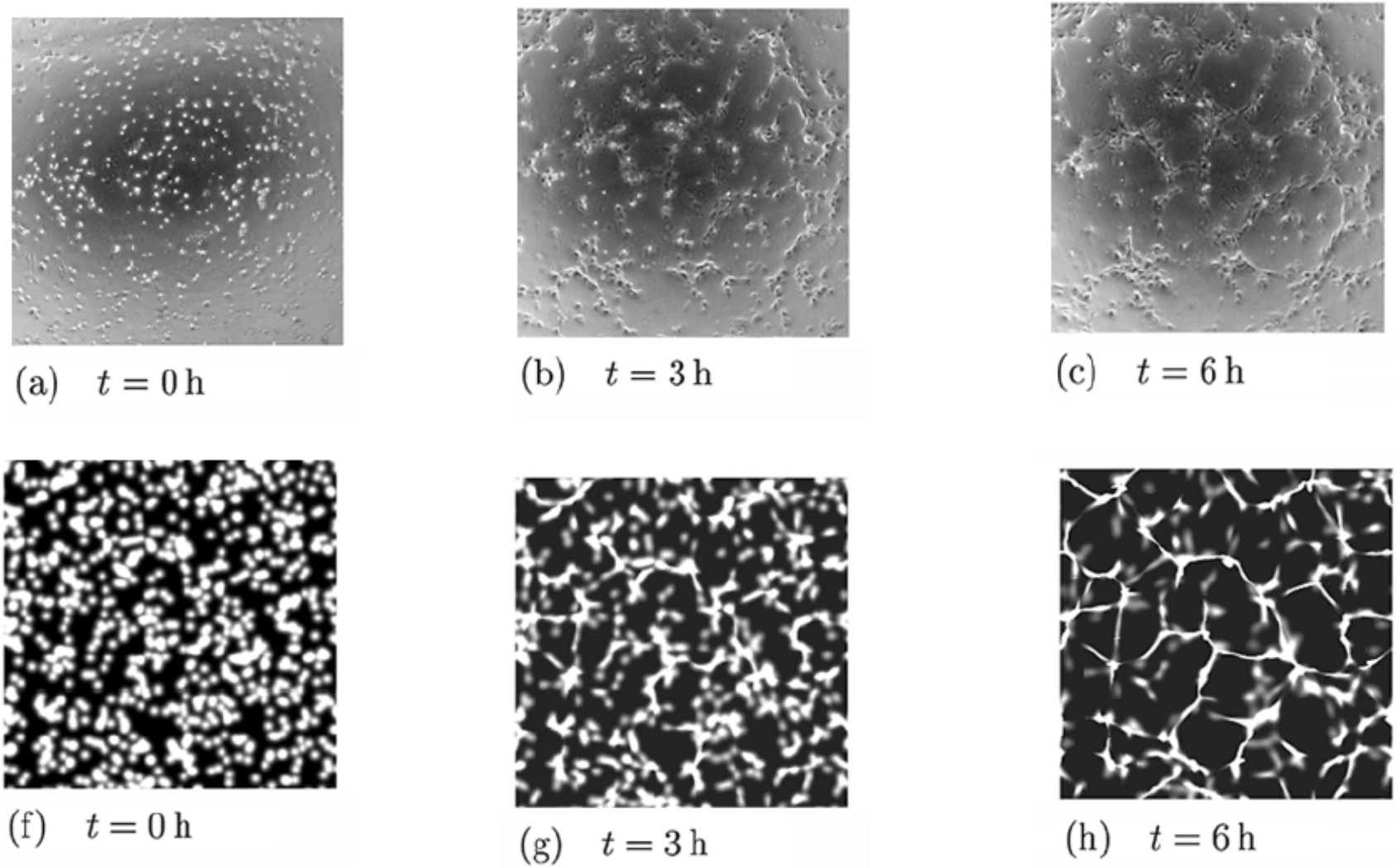
$$\frac{\partial c}{\partial t} = D\nabla^2 c + \alpha n - \tau^{-1} c \quad (3)$$

"Translation" of equations:

(1) Cell density *n* moves according to velocity field ***v***

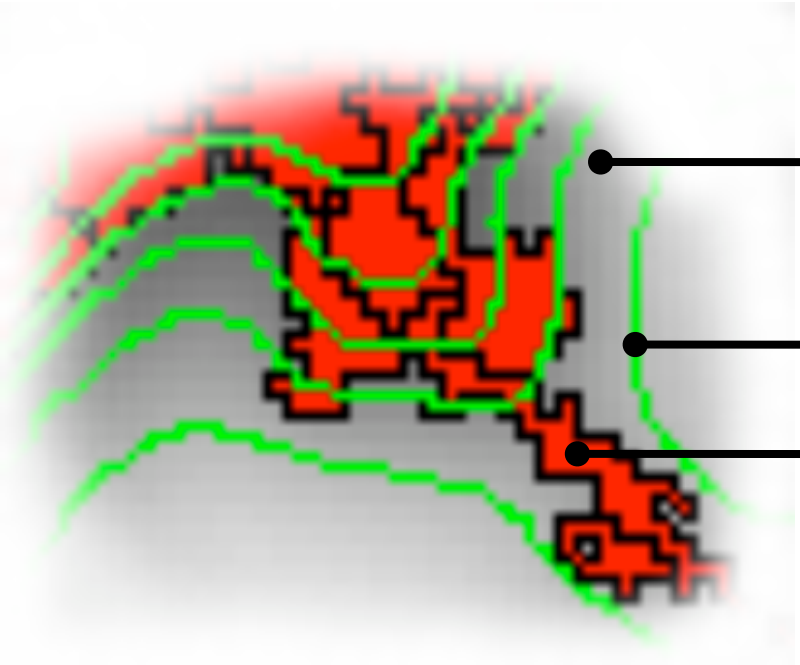
(2) Chemical gradient **accelerates** cells

(3) Chemoattractant *c* diffuses, is secreted by cells, and degrades



Ambrosi & Gamba, *B. Math. Biol.* 2004

Cell-based model of endothelial cell behavior



Grey-scale indicates chemoattractant concentration

chemoattractant concentration isoline

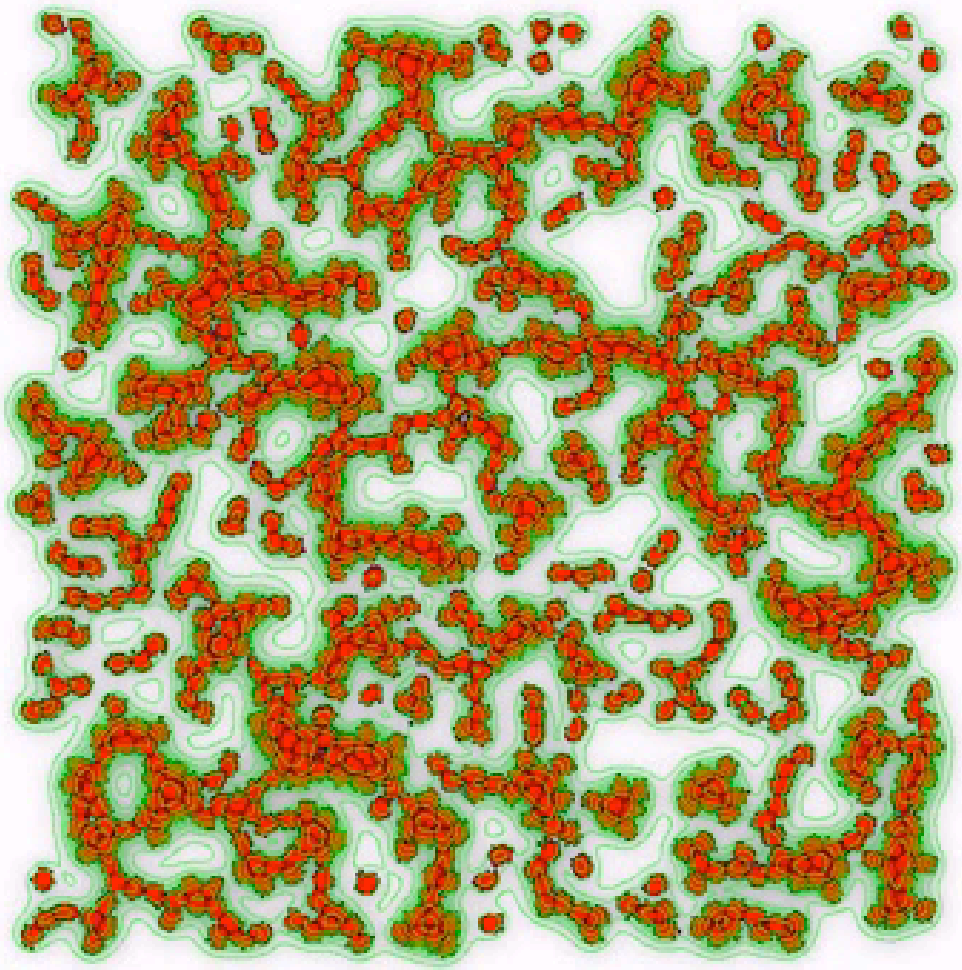
Endothelial cell

- **Endothelial cells**
- Probe environment with filopodia
- Secrete chemoattractants
- Move up chemoattractant gradients

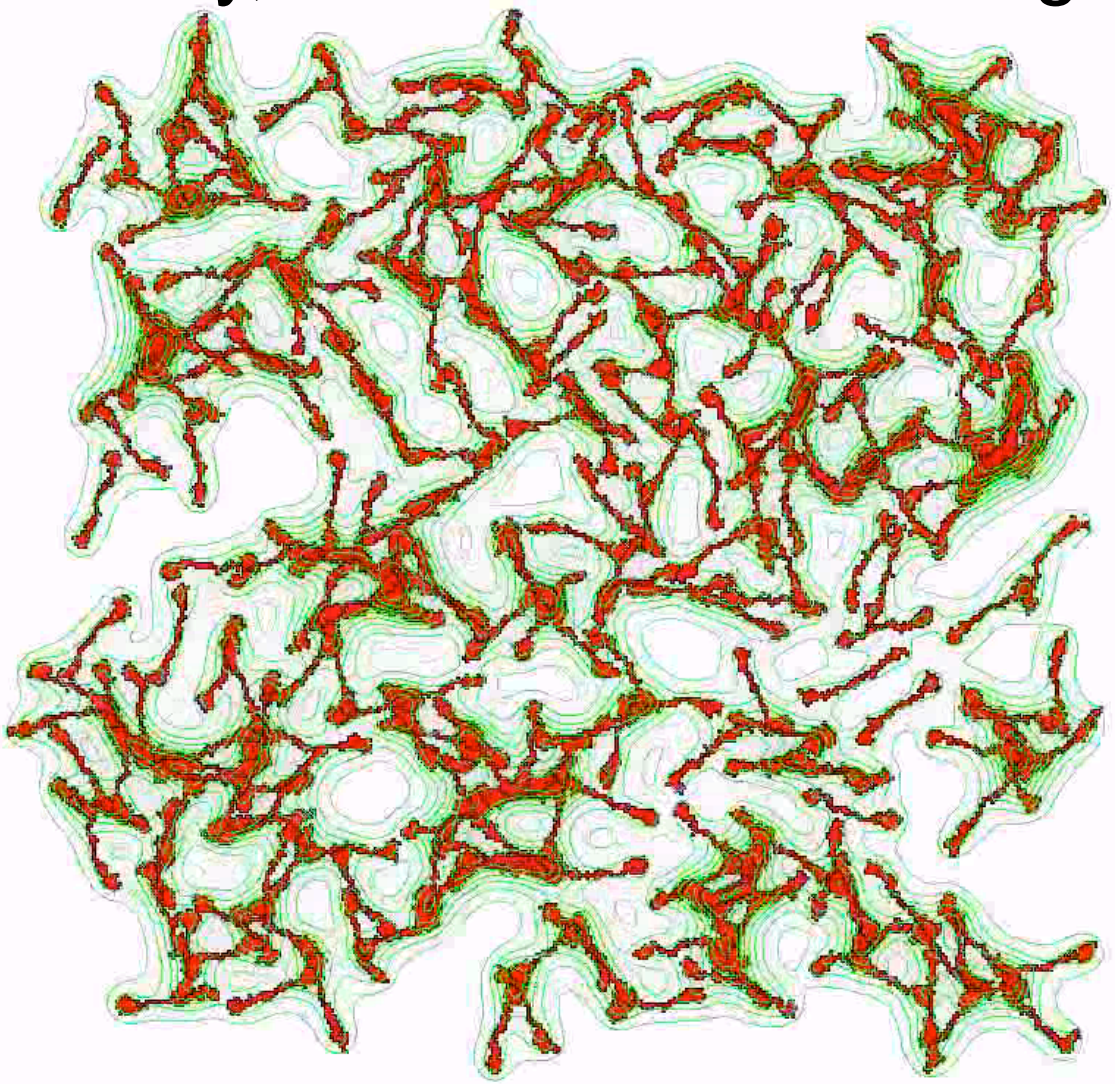
- **Chemoattractant**
- Diffuses through extracellular matrix
- Rapidly degrades in ECM

CWI

Netherlands Consortium for
Systems Biology



- In reality, endothelial cells elongate...



$L \approx 100\ \mu\text{m}$ $A \approx 400\ \mu\text{m}^2$

With Sergey Brodsky, Stuart A. Newman, Michael S. Goligorsky and James Glazier
Merks *et al.* *Dev. Biol.* 2006

CWI

Netherlands Consortium for Systems Biology

Cell shape correlates with patterning *in vivo*

Control

+soluble VEGFR-1

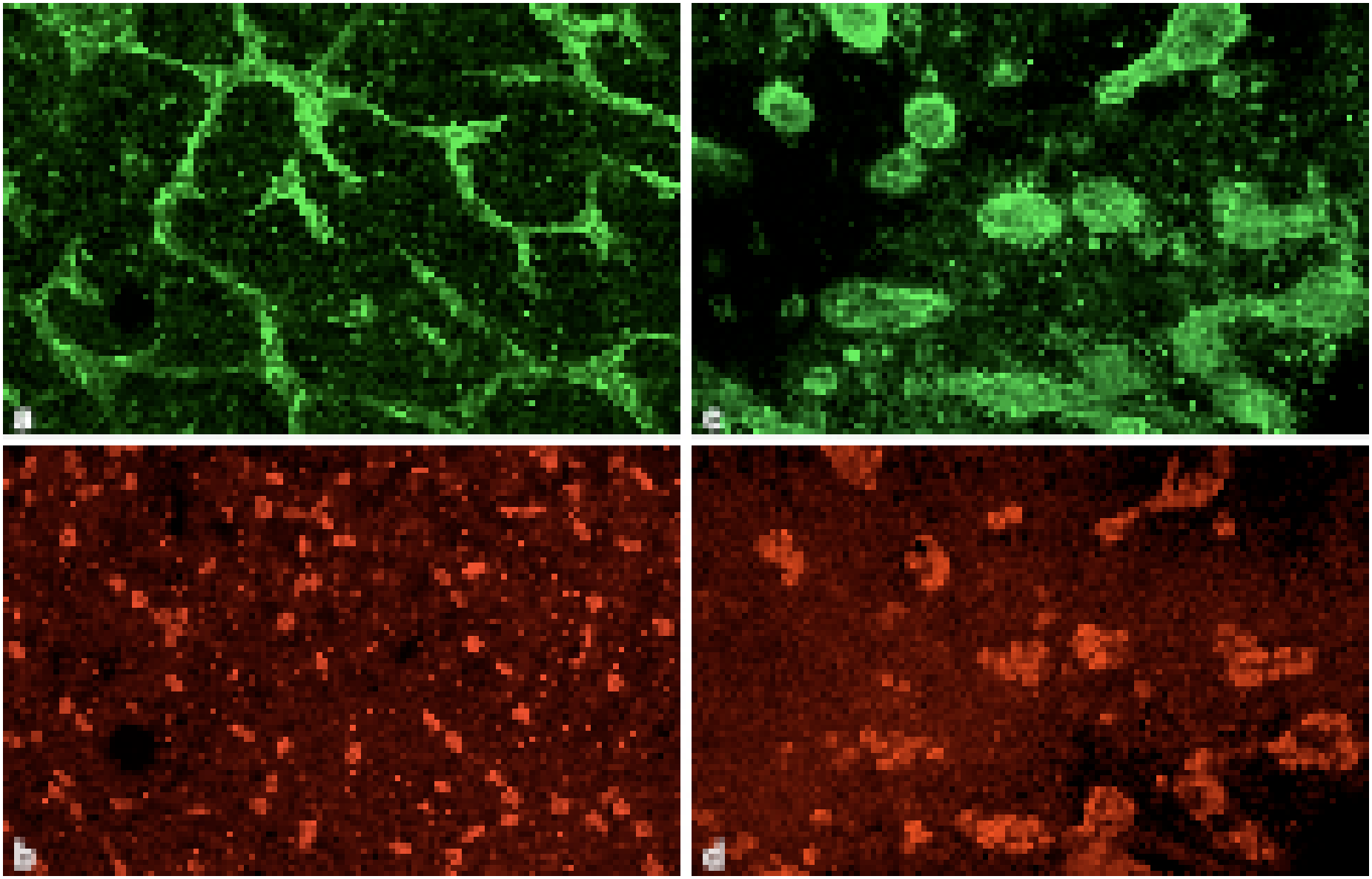


Figure courtesy of Charles D. Little, KUMC (from Drake *et al.* 2000)

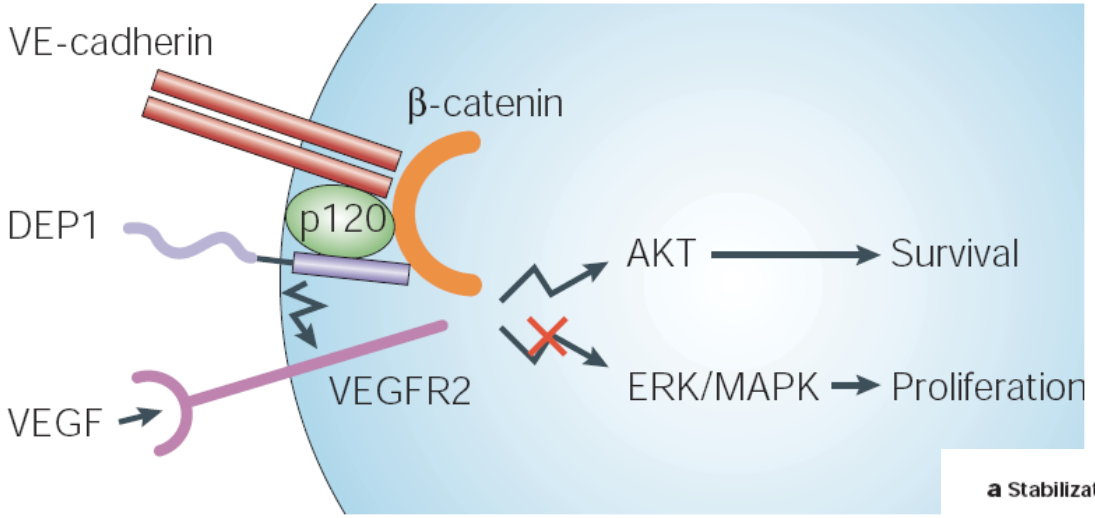
CWI

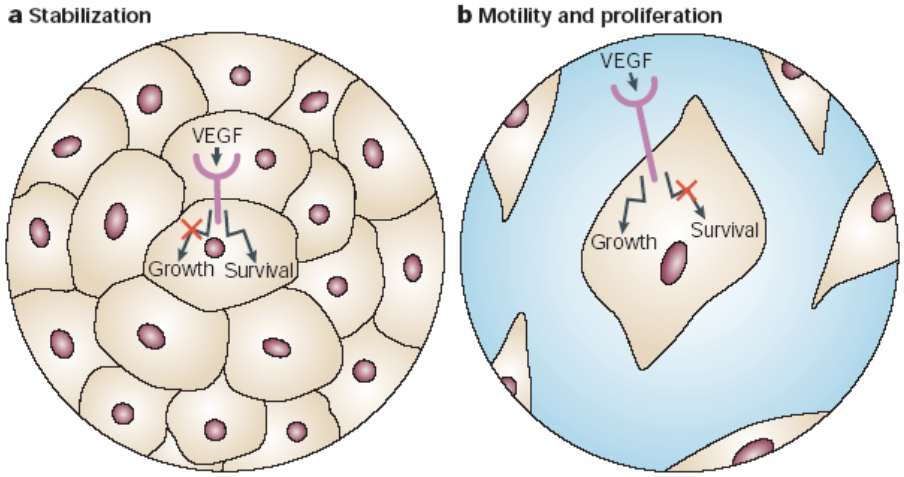
Netherlands Consortium for Systems Biology

Contact-inhibited motility

Cell contact suppresses response to chemoattractant?

Context-dependent effect of VEGF
(VEGF = growth factor stimulating blood vessel growth)

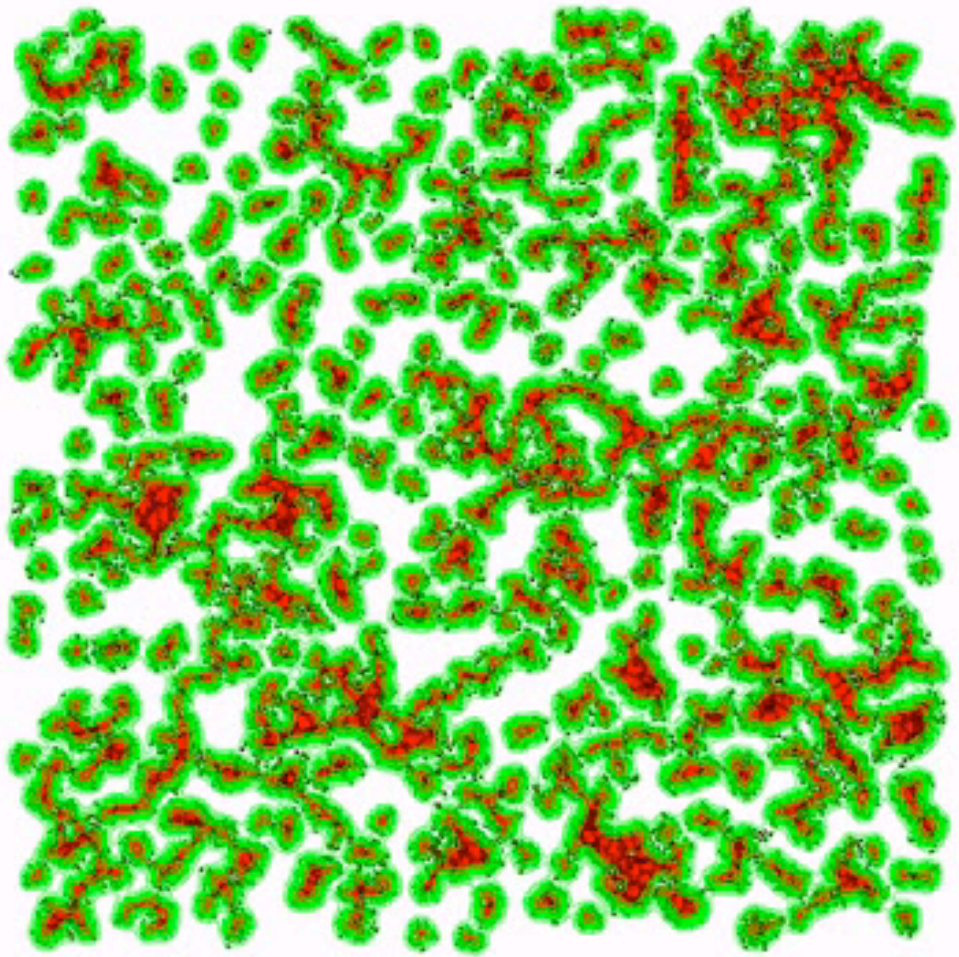




Dejana, *Nat. Rev. MCB, 2004*

Contact-inhibited motility

with Erica Perryn, Abbas Shirinifard and James Glazier
Indiana University Bloomington and Kansas University Medical Center



Merks, Perryn, Shirinifard and Glazier, *PLoS Comp. Biol.,* 2008

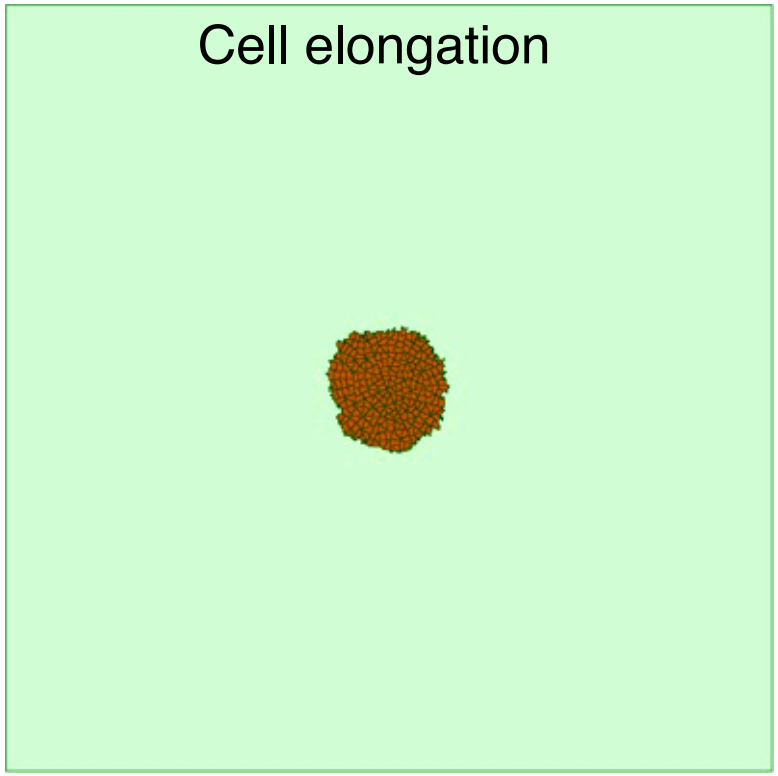
Merks & Glazier (2006). *Nonlinearity* 19, C1-C10

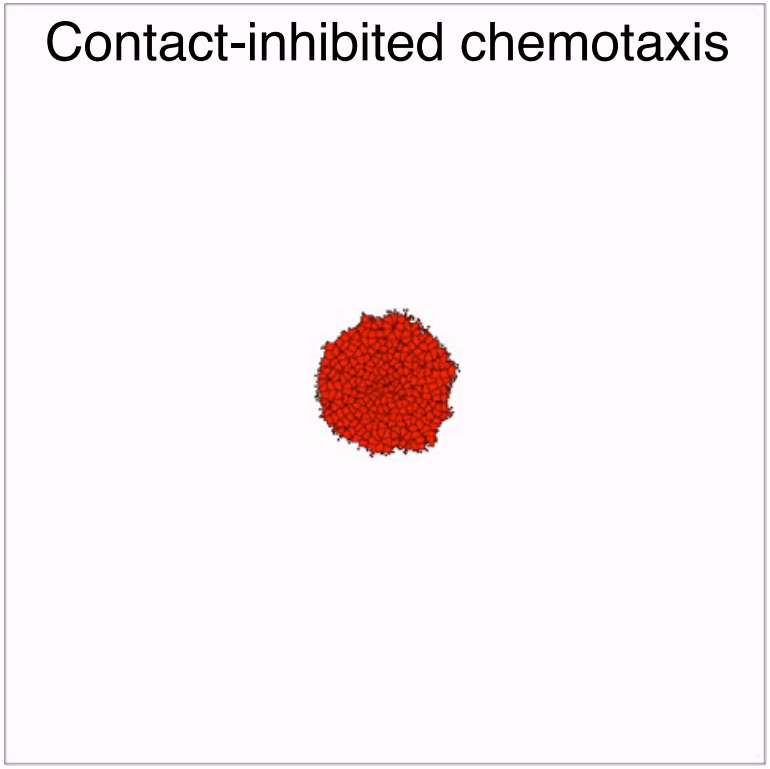
CWI

Netherlands Consortium for Systems Biology

Vasculogenesis and angiogenesis: two sides of the same coin?

- Same mechanisms also drive “sprouting”



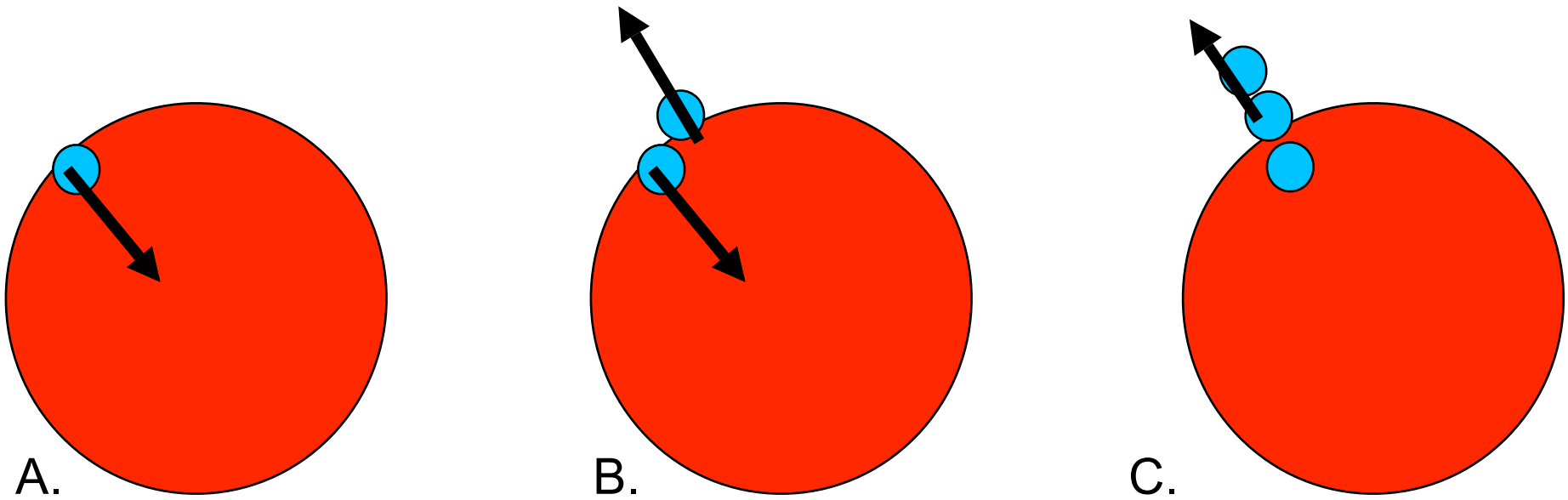


Merks & Glazier (2006). *Nonlinearity* 19, C1-C10

Merks, Perryn, Shirinifard and Glazier, *PLoS Comp. Biol.,* 2008, e1000163

Contact-inhibited chemotaxis

Buckling instability?



A. Only peripheral cells chemotact to center

B. Invading surface cells displace interior cells

Only possible with contact inhibition

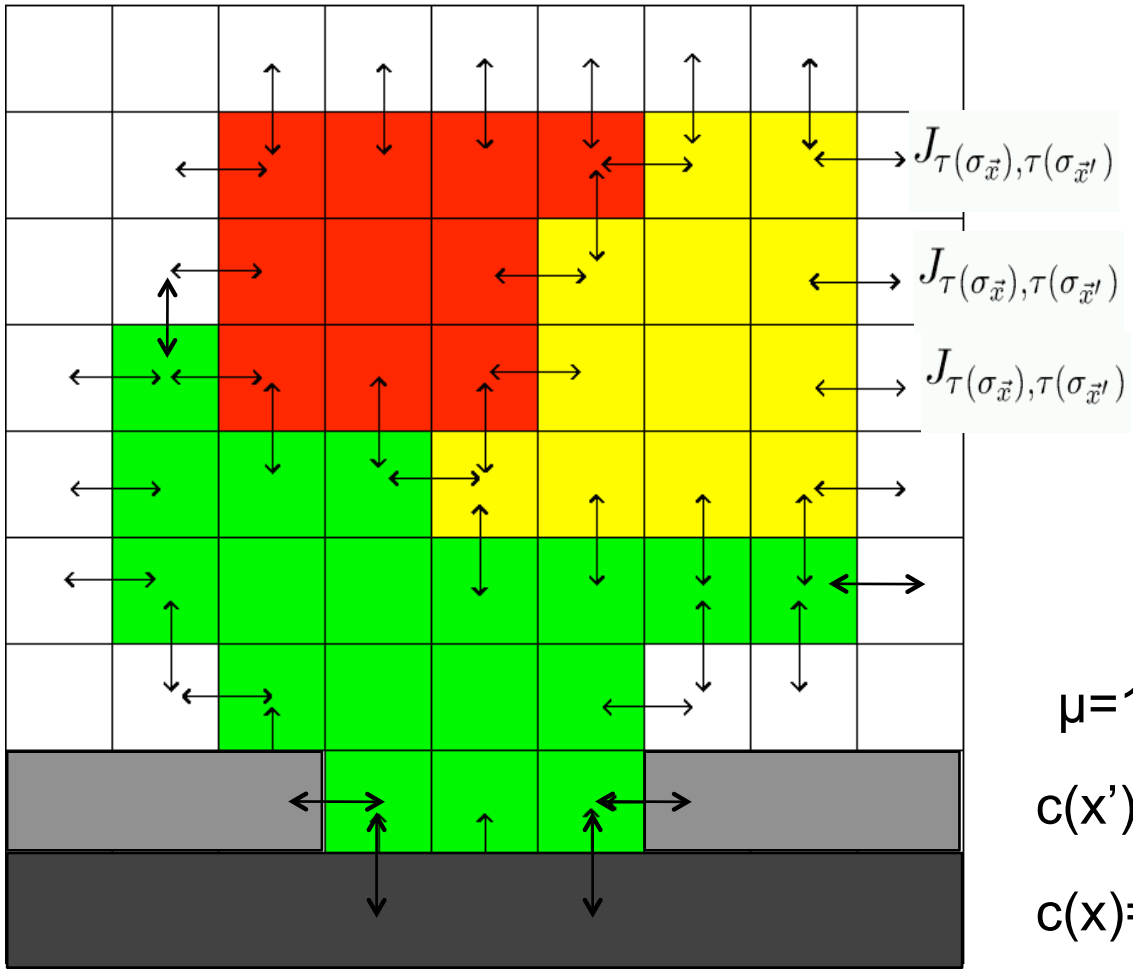
C. Resulting pressure pushes peripheral cells further outwards

CWI

Netherlands Consortium for Systems Biology

Chemotactic pushing required for buckling instability?

Eliminate pushing: **extension-only chemotaxis**



μ=100

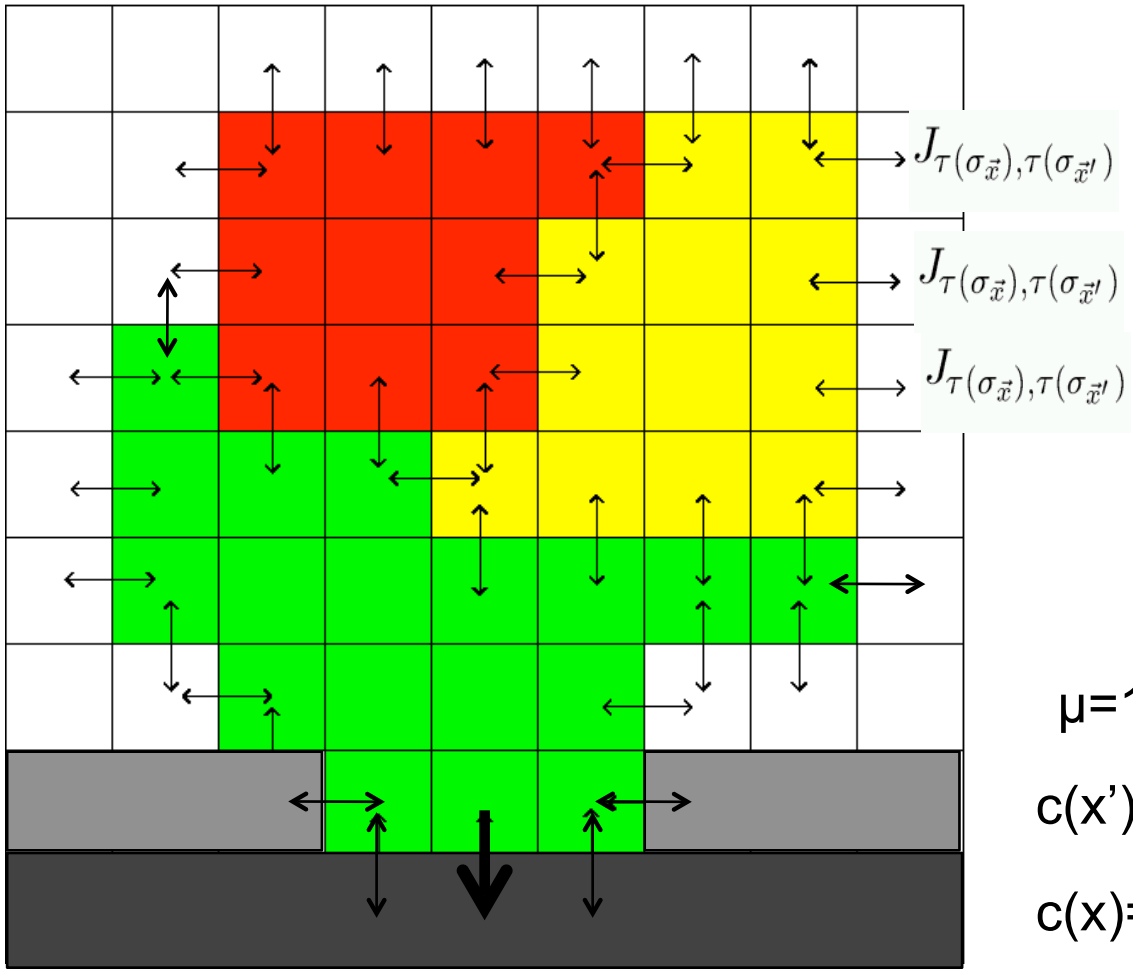
c(x')=0.5

c(x)=1

ΔH -= 50

Chemotactic pushing required for buckling instability?

Eliminate pushing: **extension-only chemotaxis**



μ=100

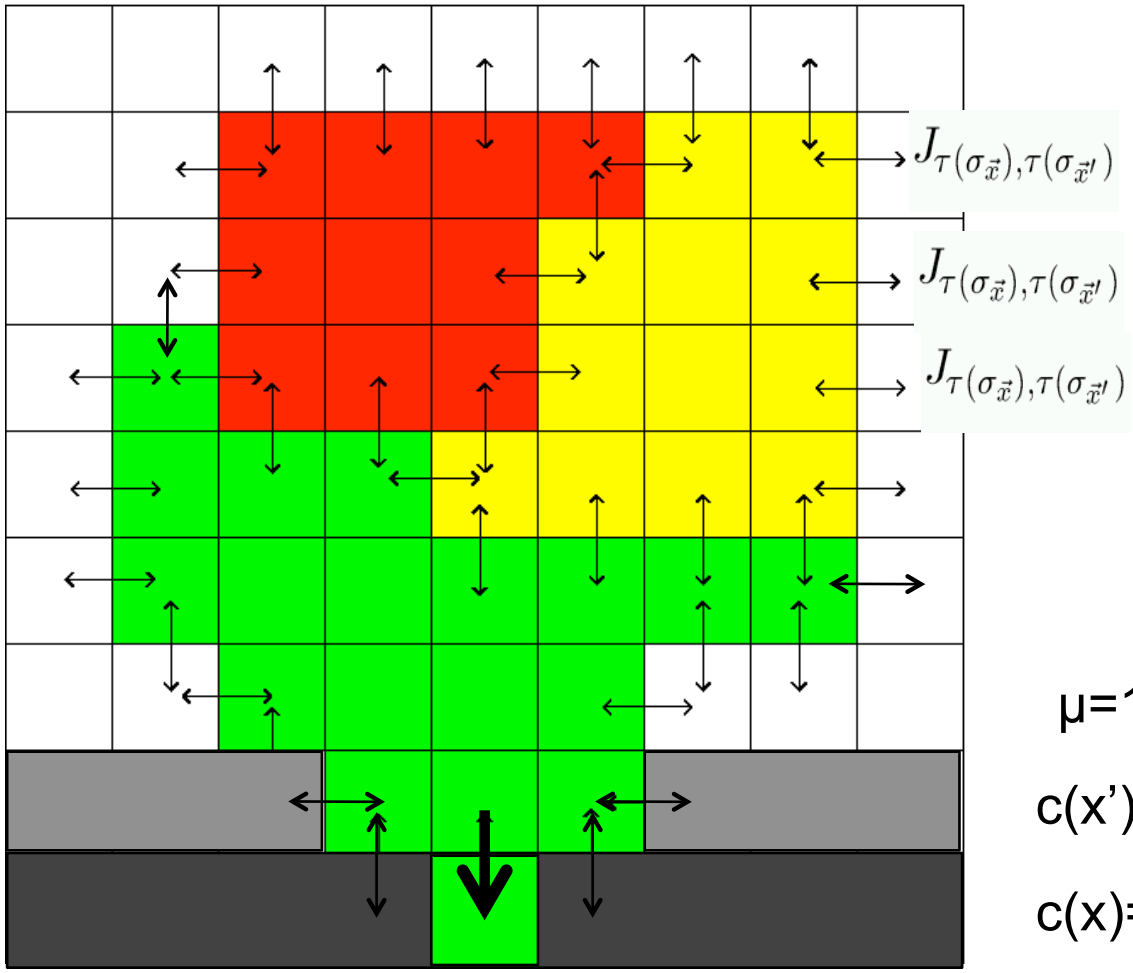
c(x')=0.5

c(x)=1

ΔH -= 50

Chemotactic pushing required for buckling instability?

Eliminate pushing: **extension-only chemotaxis**



μ=100

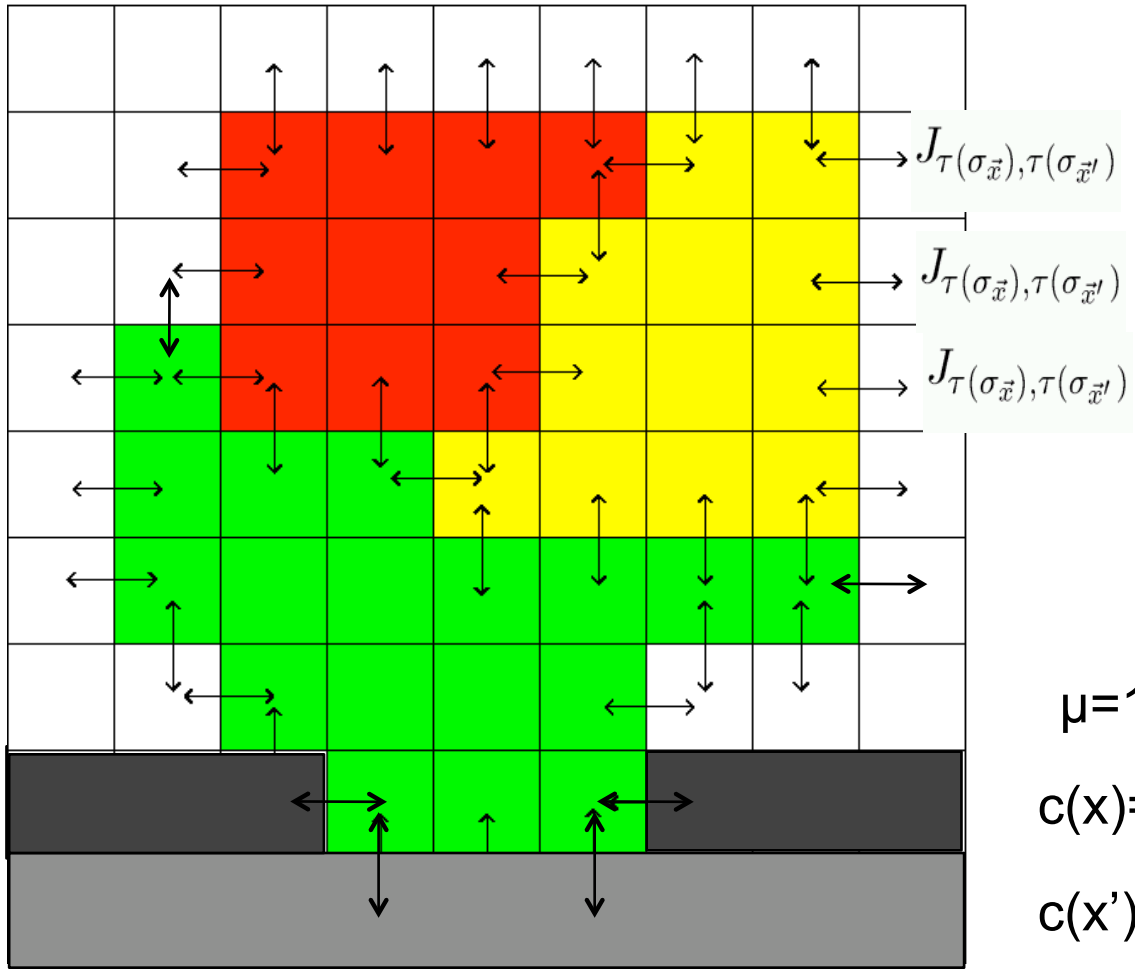
c(x')=0.5

c(x)=1

ΔH -= 50

Extension-only chemotaxis

Pseudopod retraction energetically **neutral**



μ=100

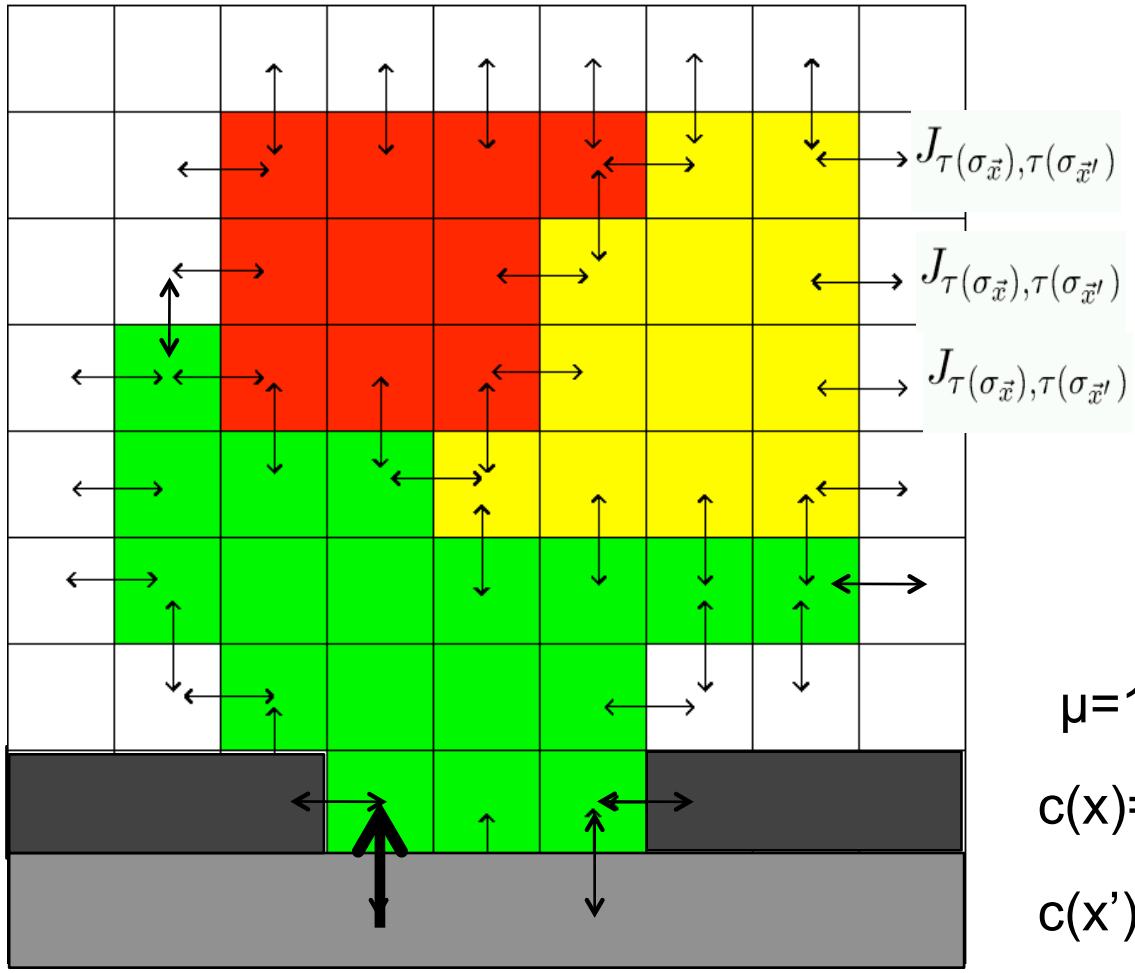
c(x)=1.0

c(x')=0.5

ΔH -= 0

Extension-only chemotaxis

Pseudopod retraction energetically **neutral**



μ=100

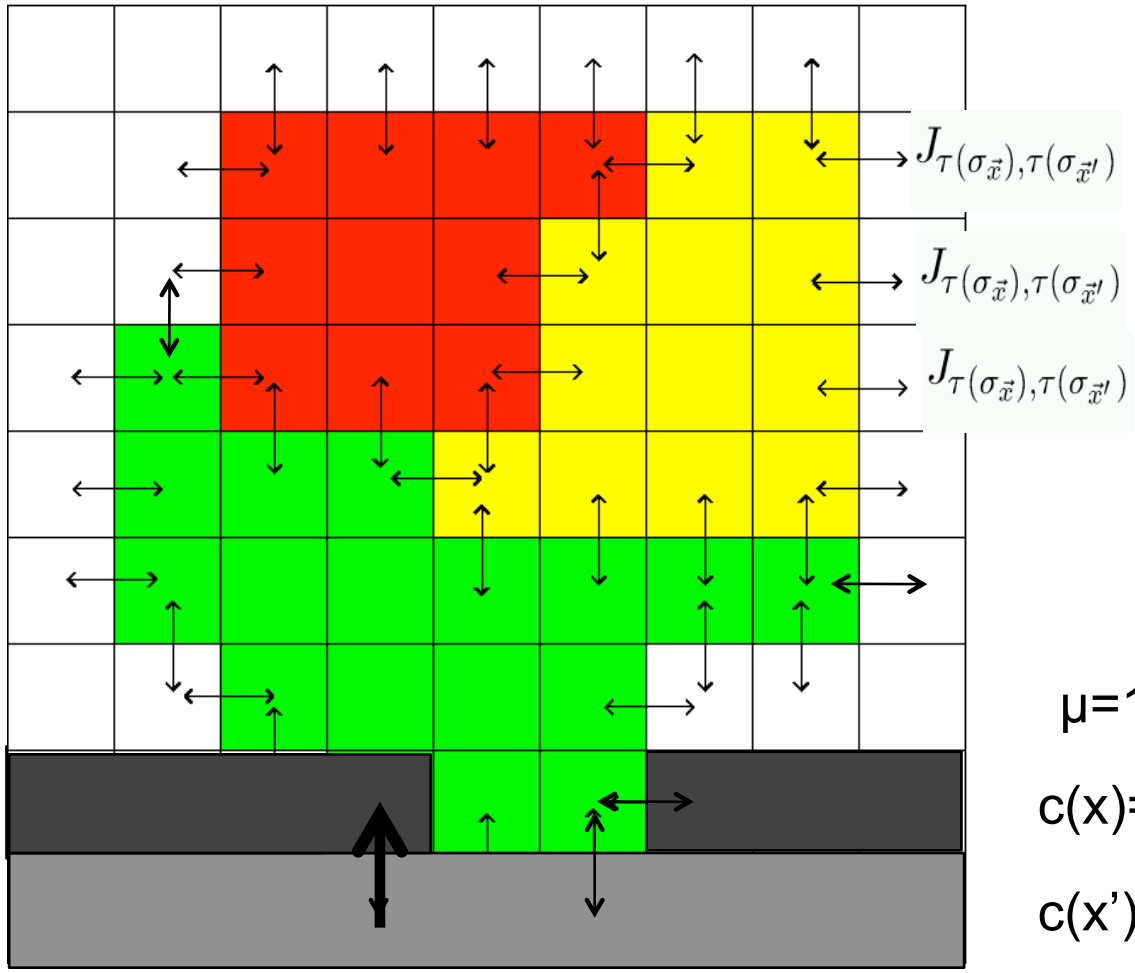
c(x)=1.0

c(x')=0.5

ΔH -= 0

Extension-only chemotaxis

Pseudopod retraction energetically **neutral**



μ=100

c(x)=1.0

c(x')=0.5

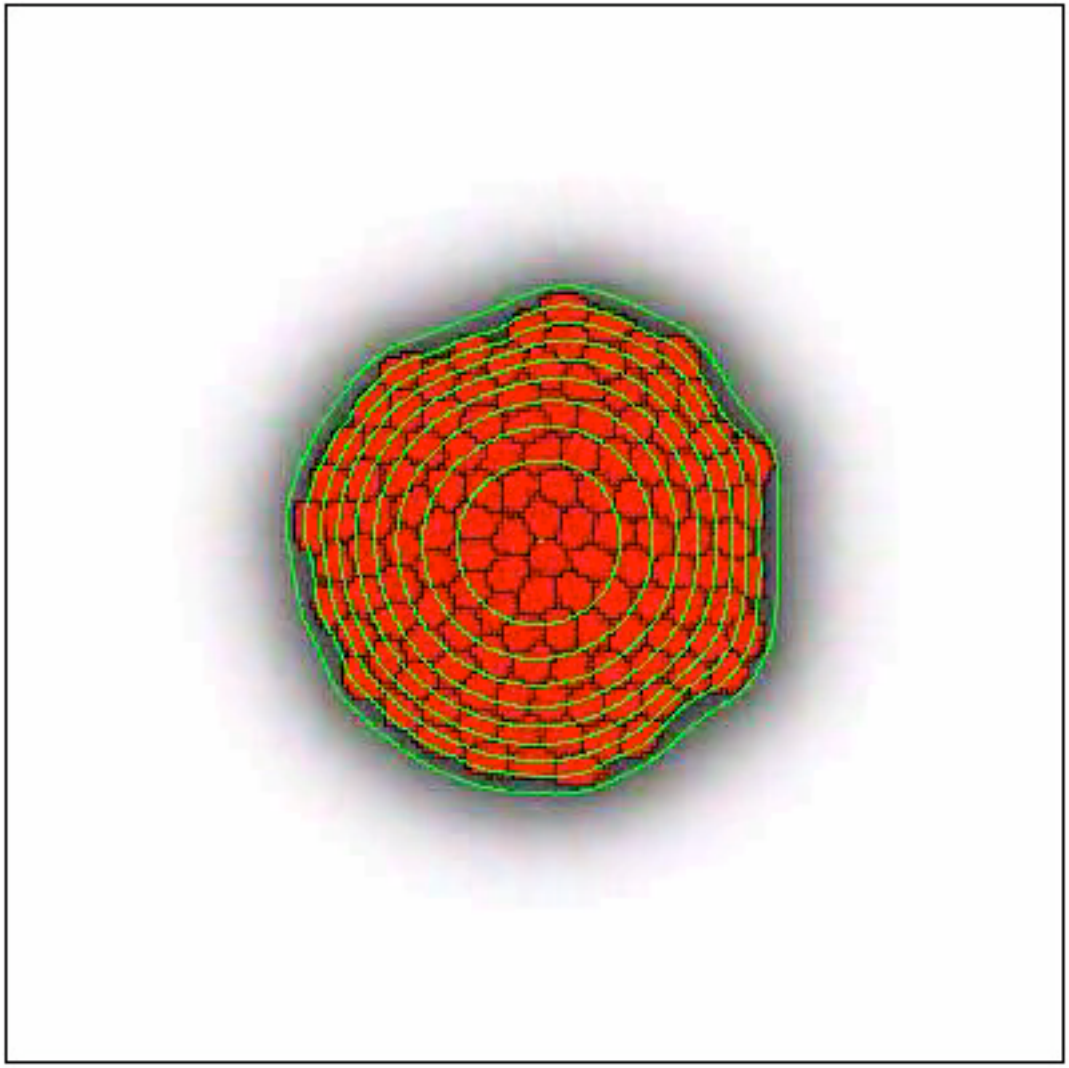
ΔH -= 0

CWI

Netherlands Consortium for Systems Biology

Clusters do not sprout with extension-only chemotaxis

Buckling instability responsible for sprouting?

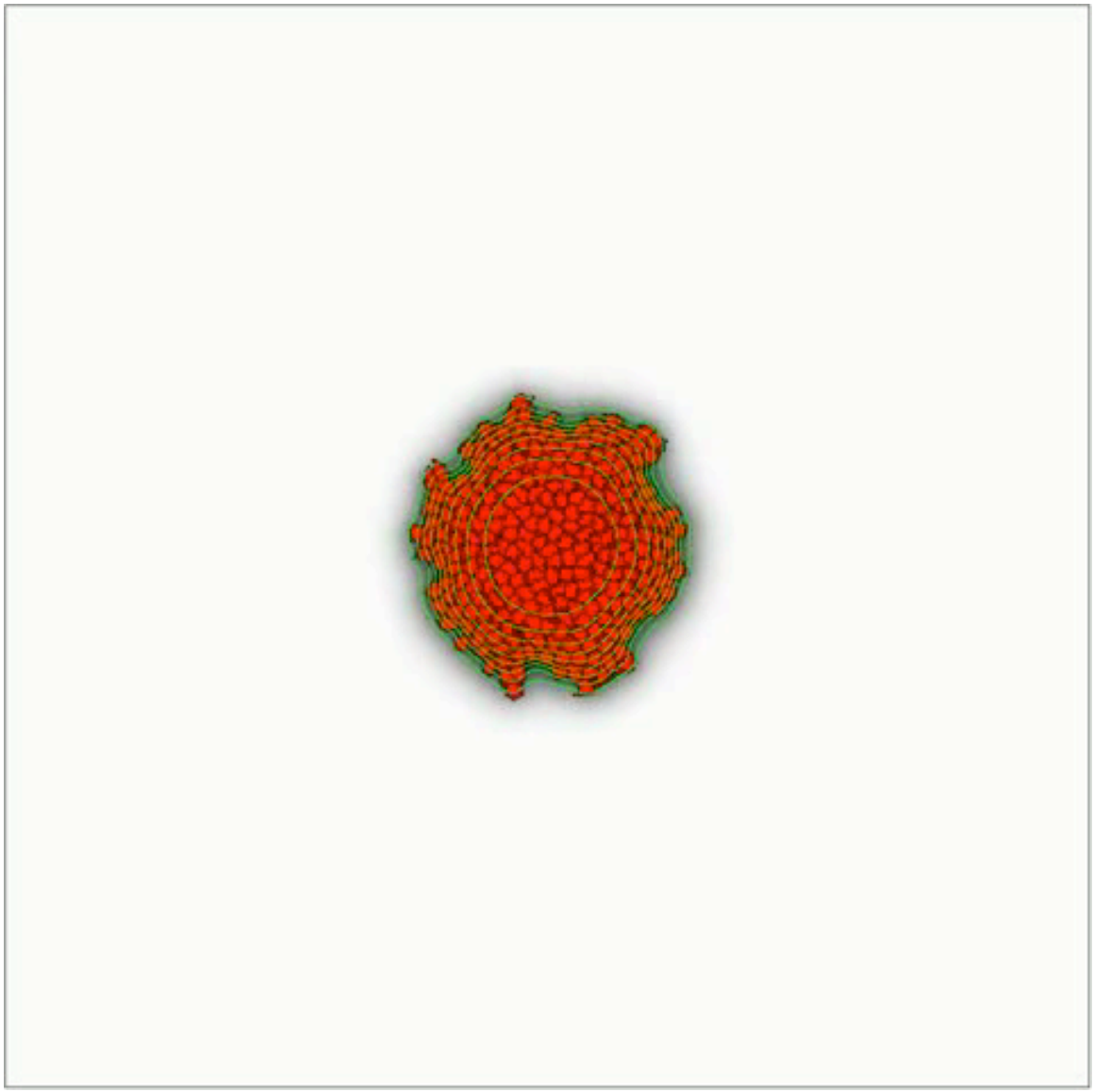


T=50

CWI

Netherlands Consortium for Systems Biology

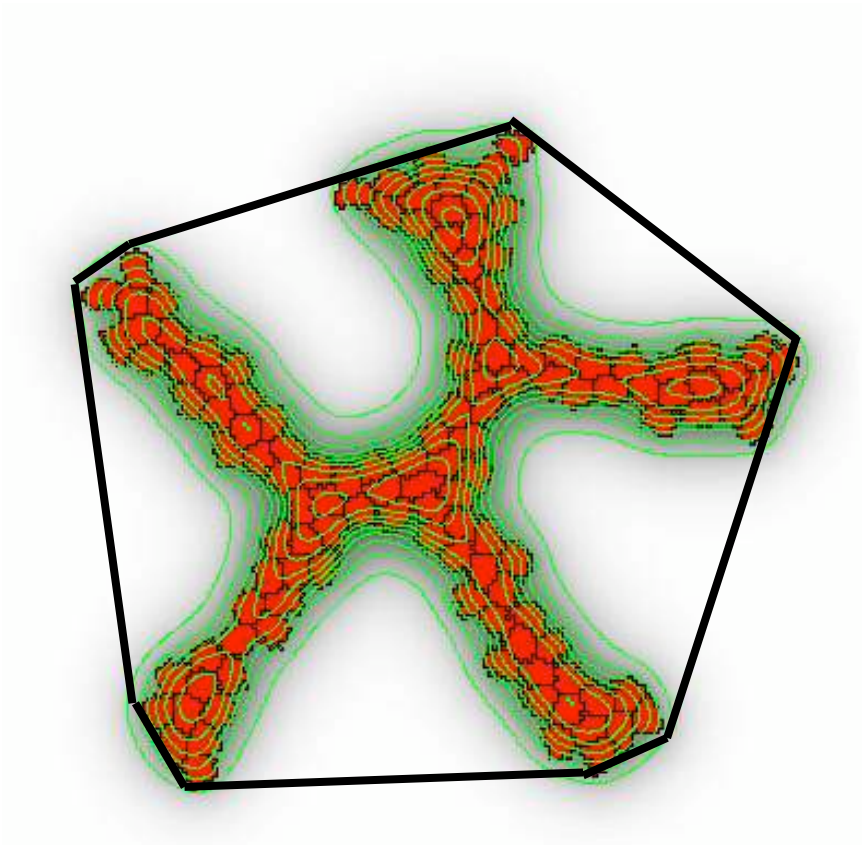
Clusters do sprout if cells move more actively



T=200

Morphology measure: compactness

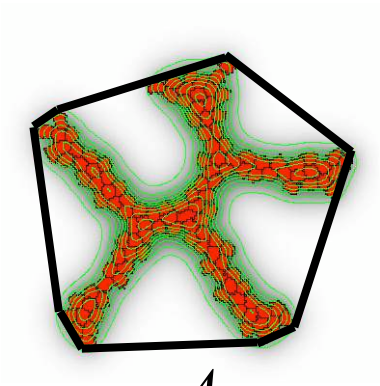
$$C = \frac{A_{object}}{A_{convex\,hull}}$$



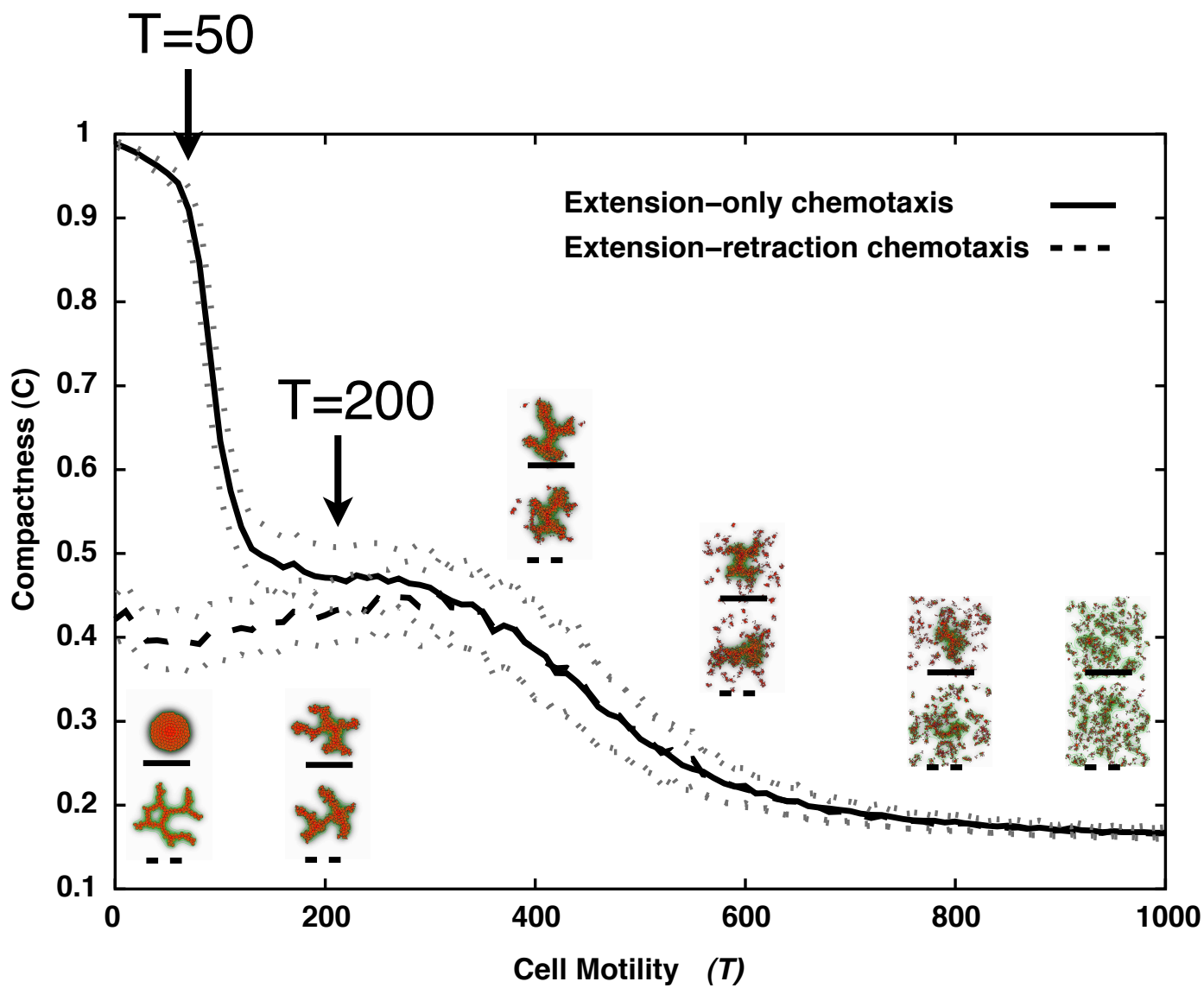
CWI

Netherlands Consortium for Systems Biology

Clusters sprout with extension-only chemotaxis at high cell motility

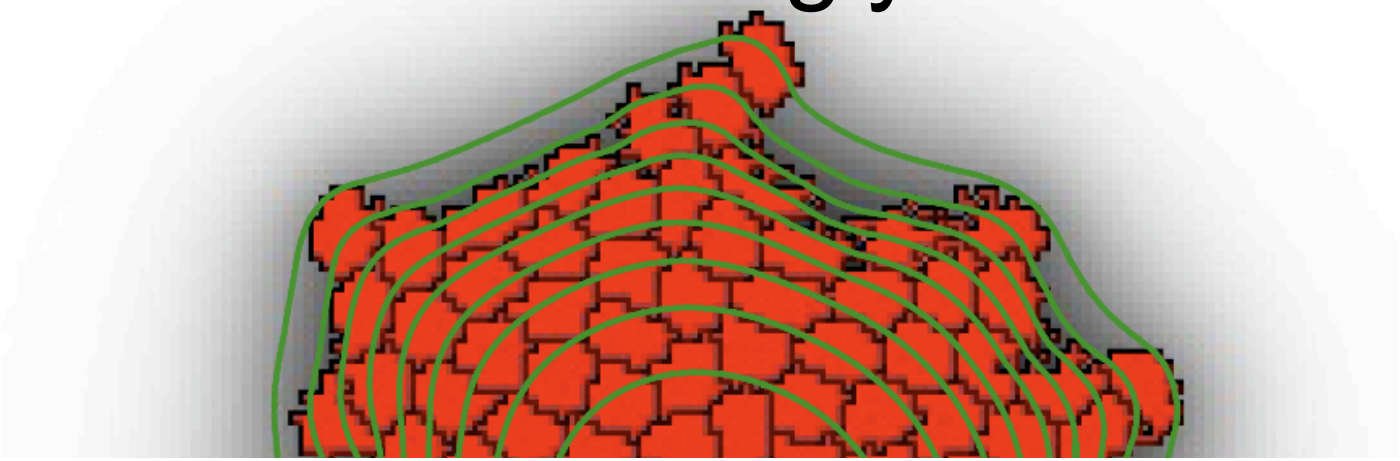


$$C = \frac{A_{object}}{A_{convex\,hull}}$$



Merks *et al. PLoS Comp. Biol.* 2008, e1000163

Chemoattractant *inhibits* pseudopod extension most strongly at concavities

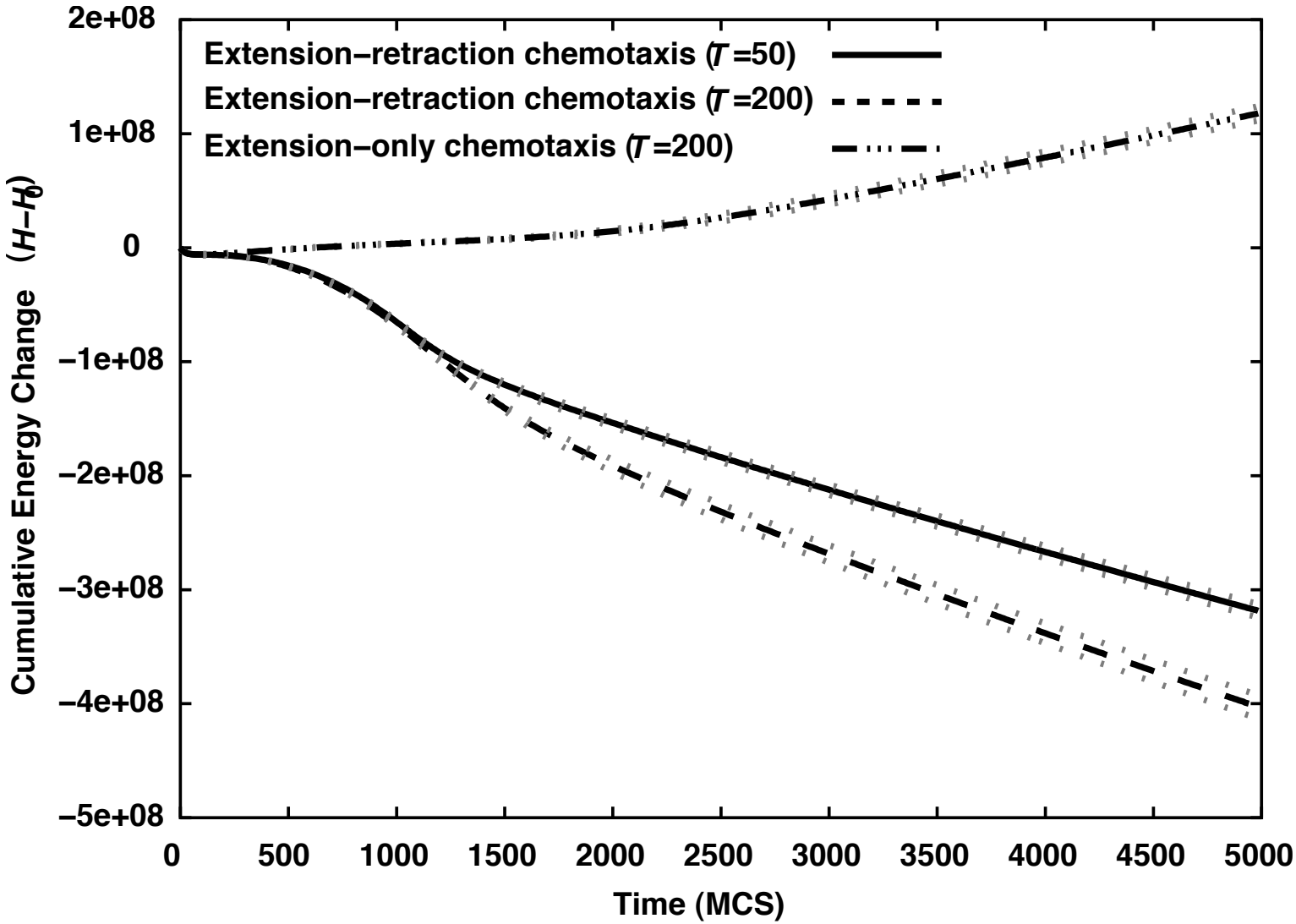


- At high motility (T): many pseudopod extensions, which the chemical gradients counteracts
- Gradient is most shallow at convexities
- Cells at sprout tips move faster than those between branches
- Note: chemoattractant here **also** acts as an *inhibitor* of cell motility!

CWI

Netherlands Consortium for Systems Biology

Dissipative sprouting mechanism



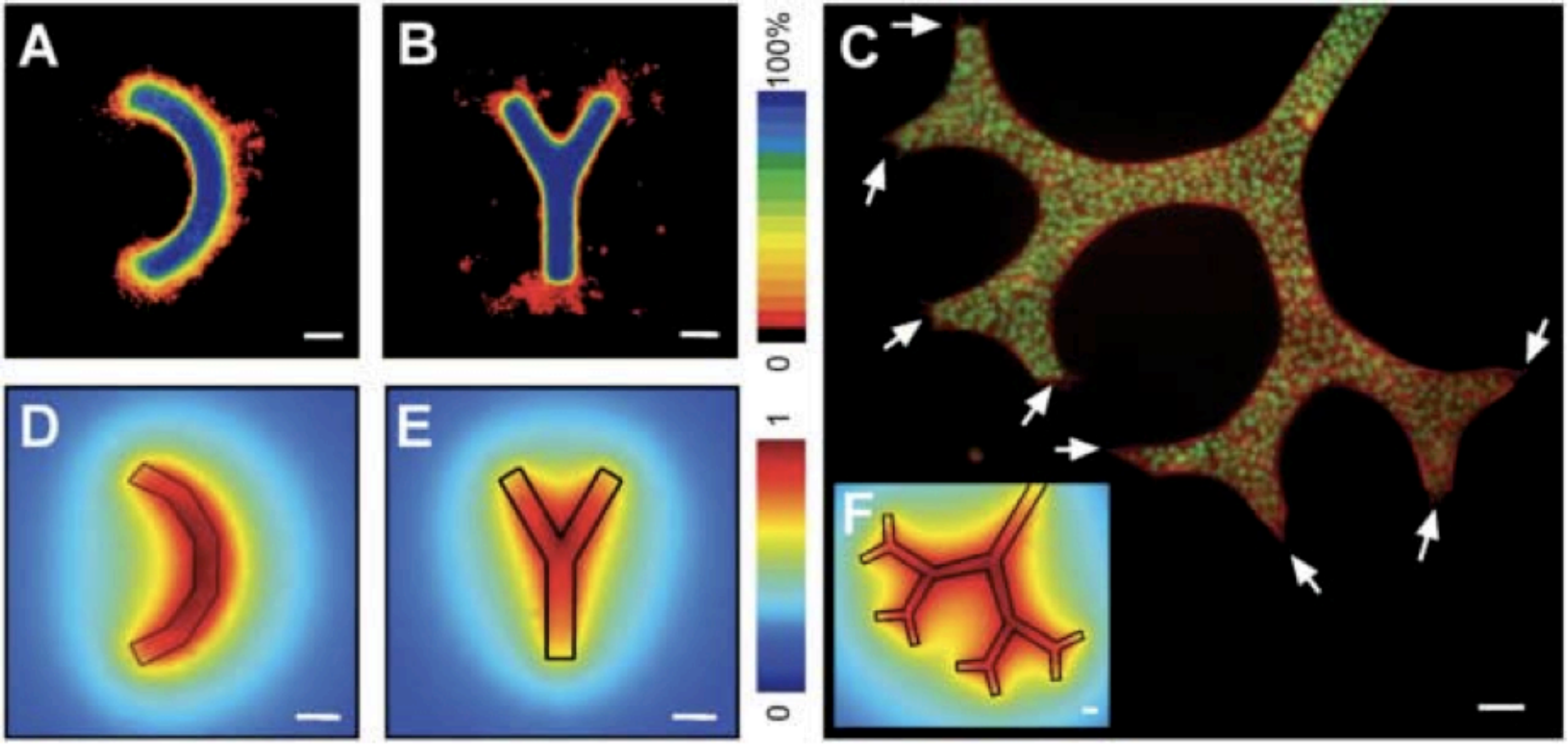
Merks *et al. PLoS Comp. Biol.* 2008, e1000163

CWI

Netherlands Consortium for Systems Biology

General mechanism for branching?

Autocrine TGF-β inhibits sprouting in cultured, mammary epithelial tubes: **Nelson *et al. Science* 2006**

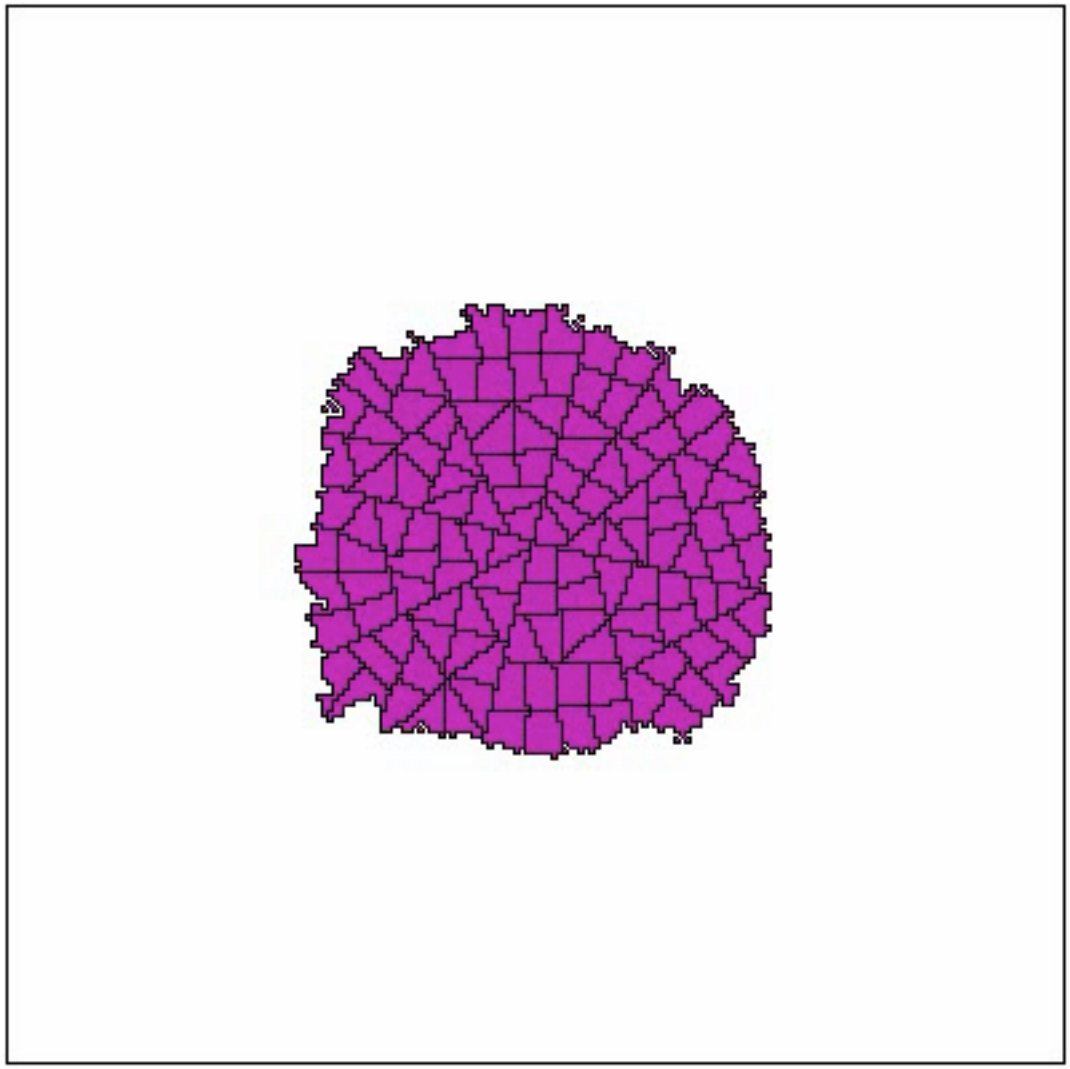


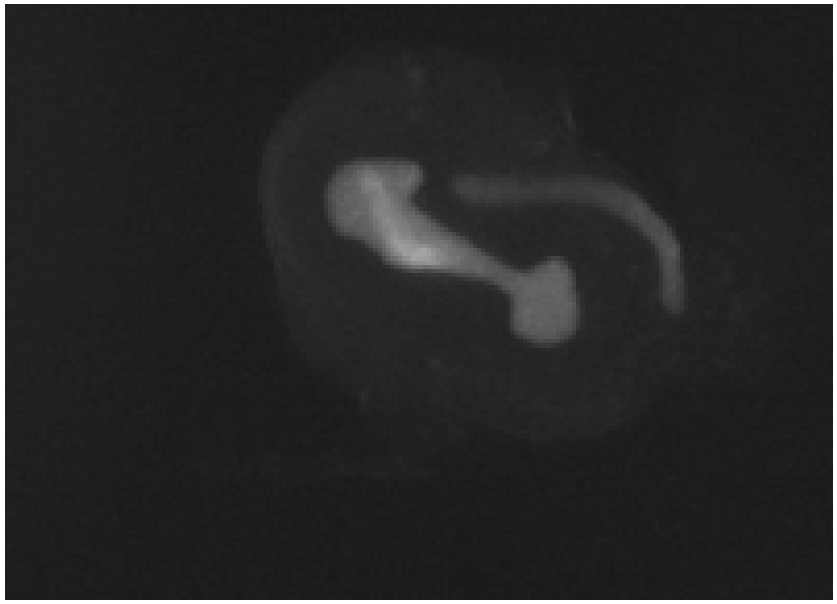
CWI

Netherlands Consortium for Systems Biology

Cells secrete sprouting inhibitor

Model for epithelial tube formation?

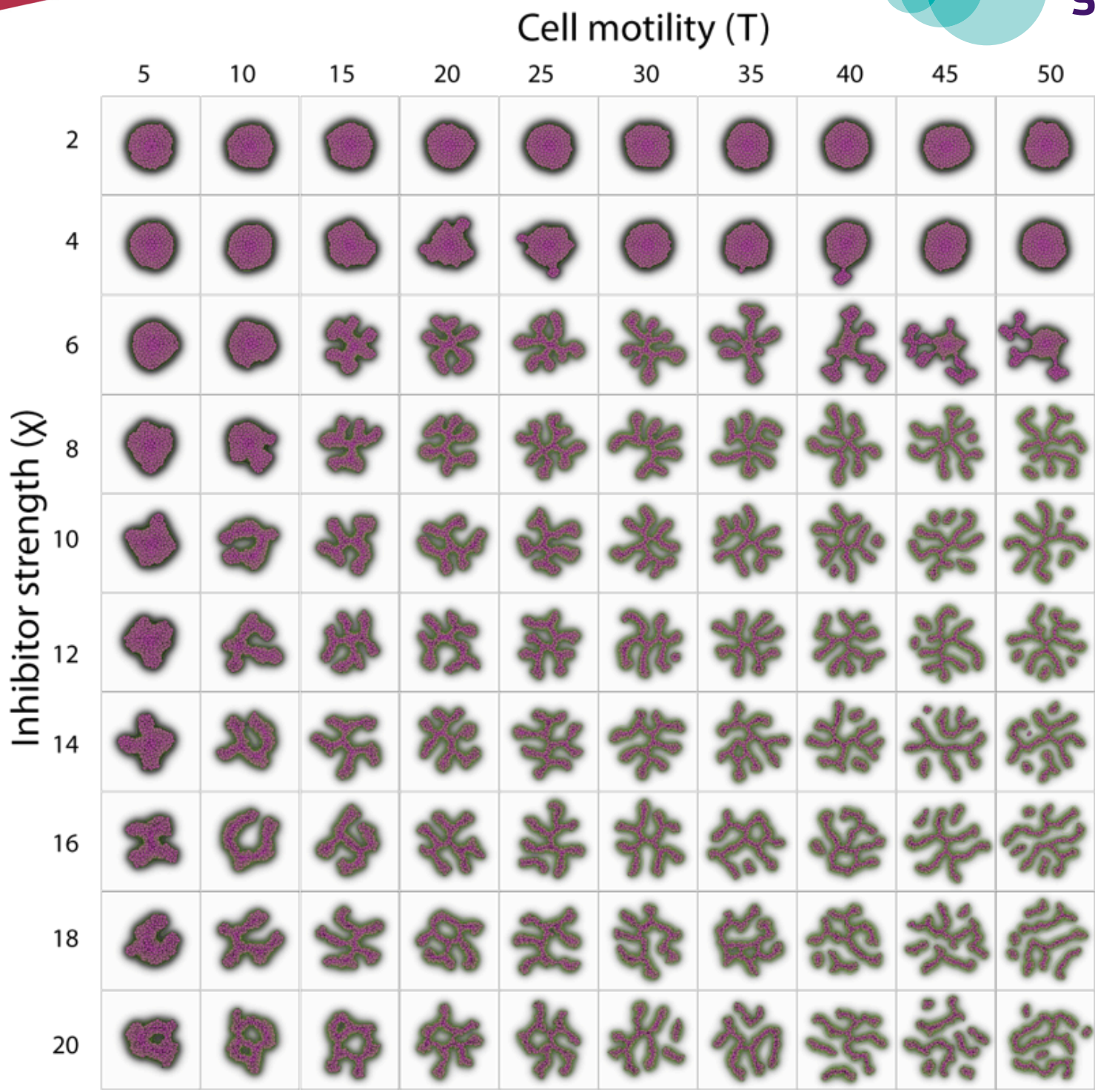




cf., Bud branching in the embryonic kidney
Watanabe and Constantini, *Dev. Biol.* 2004

CWI

Netherlands Consortium for
Systems Biology



Conclusions

- Cells secreting chemoattractant, and moving towards it:
  - produce “blobs” of cells
- Vascular-like network and sprouts with:
  - cell elongation
  - contact inhibition
- *De novo* and sprouting blood vessel growth: two sides of same coin?
- Buckling-like instability partly responsible for sprouting
- Dissipative mechanism for sprouting:
  - secreted chemical can act both as chemoattractant and as inhibitor of cell motility

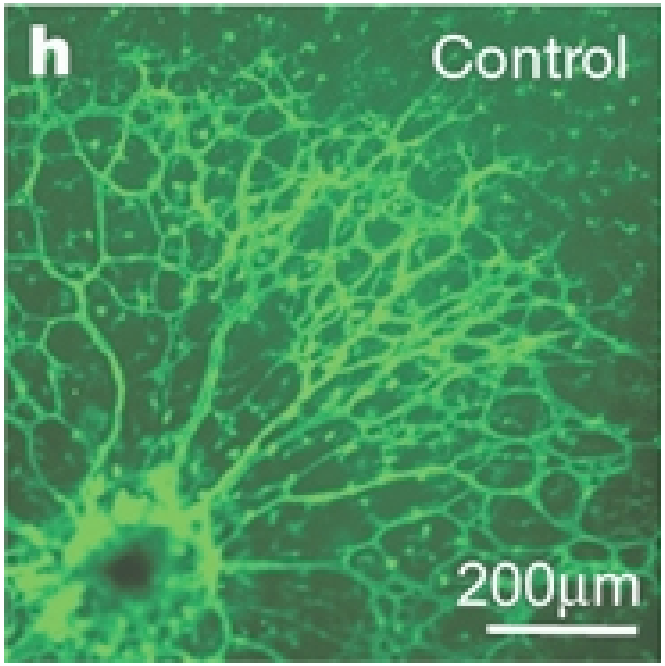
CWI

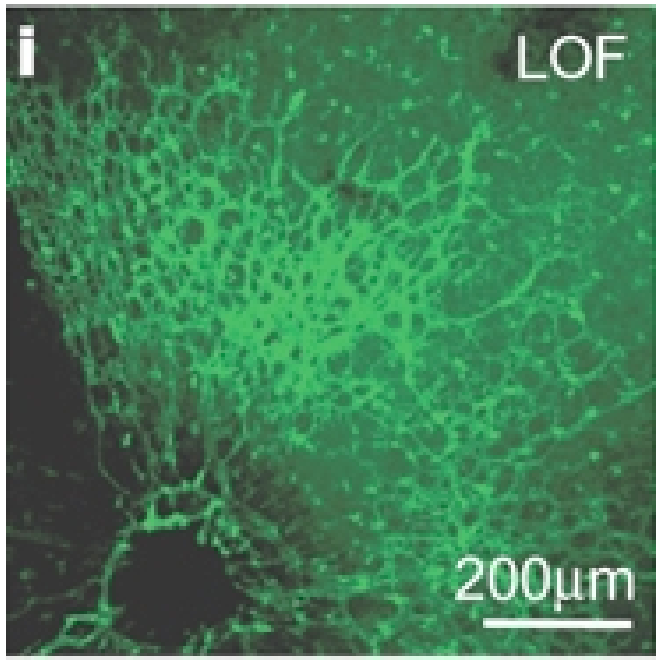
Netherlands Consortium for Systems Biology

Ongoing work (Margriet Palm)

Add subcellular dynamics

- Tip cell selection (Dll4-Notch1)





Hellström et al. Nature 2007

- More connections without tip cell selection

CWI

Netherlands Consortium for Systems Biology

Tip cell selection

Margriet Palm

- Tip and stalk cell properties:
  - Tip cells are more motile than stalk cells.
  - Stalk cells cohere more strongly than tip cells
  - Tip cells chemotact to secreted chemoattractant
- ODE model in each cell describe tip cell selection
  - Dll4-Notch network -> lateral inhibition of tip cell fate

Ongoing work (Josephine Daub)

Interactions between cells and extracellular matrix (ECM)

- All cells surrounded by extracellular materials
  - Collagen, fibrin, fibronectin, ...
  - Cells secrete and degrade ECM materials
  - ECM is mechanical support and medium for mechanical and chemical signalling
- Models help focus experimental research on relevant molecular aspects of subcellular dynamics

CWI

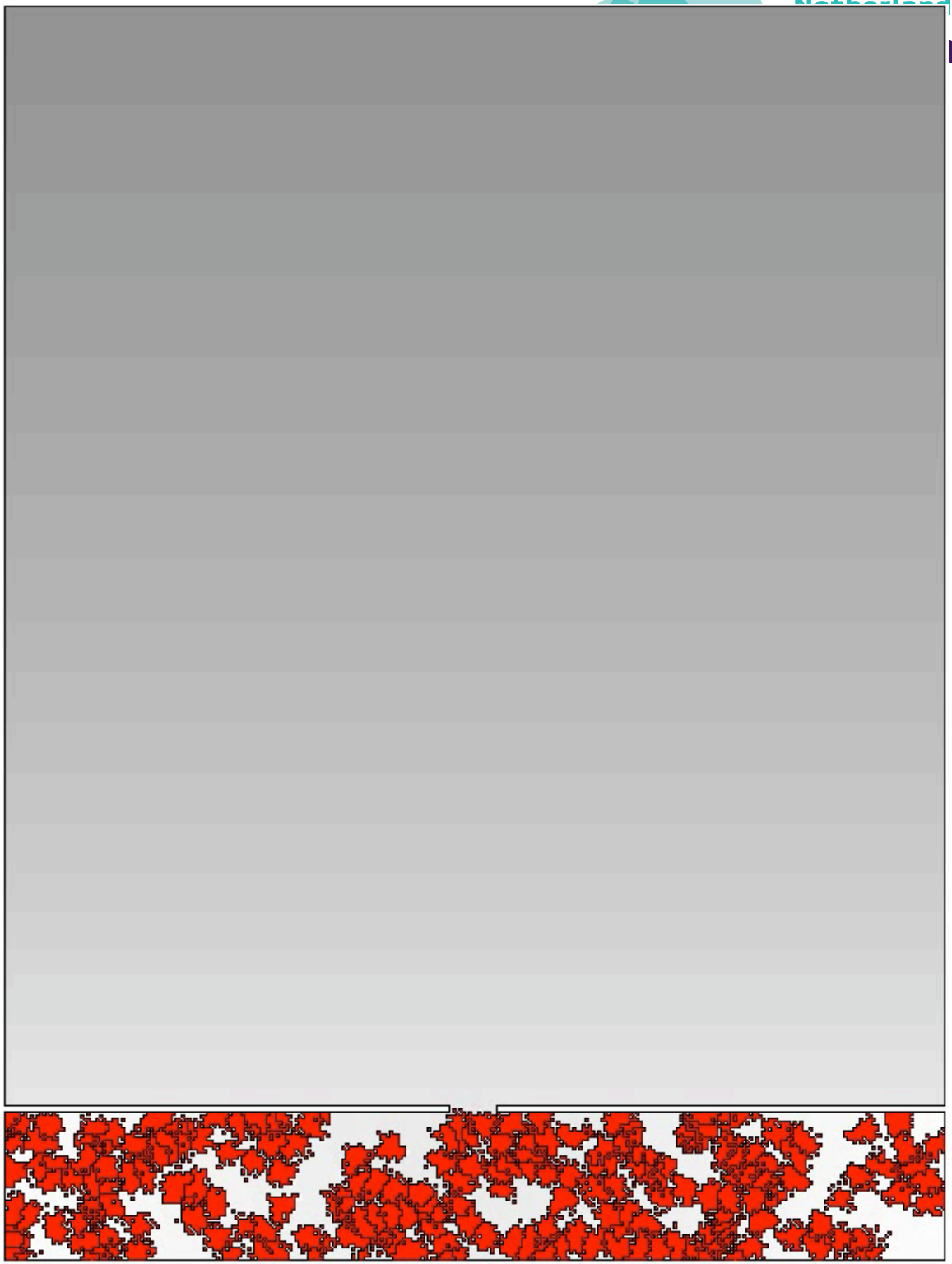
Netherlands Consortium for Systems Biology

ECs secrete collagen

ECs follow collagen paths

ECs stimulated by VEGF degrade collagen

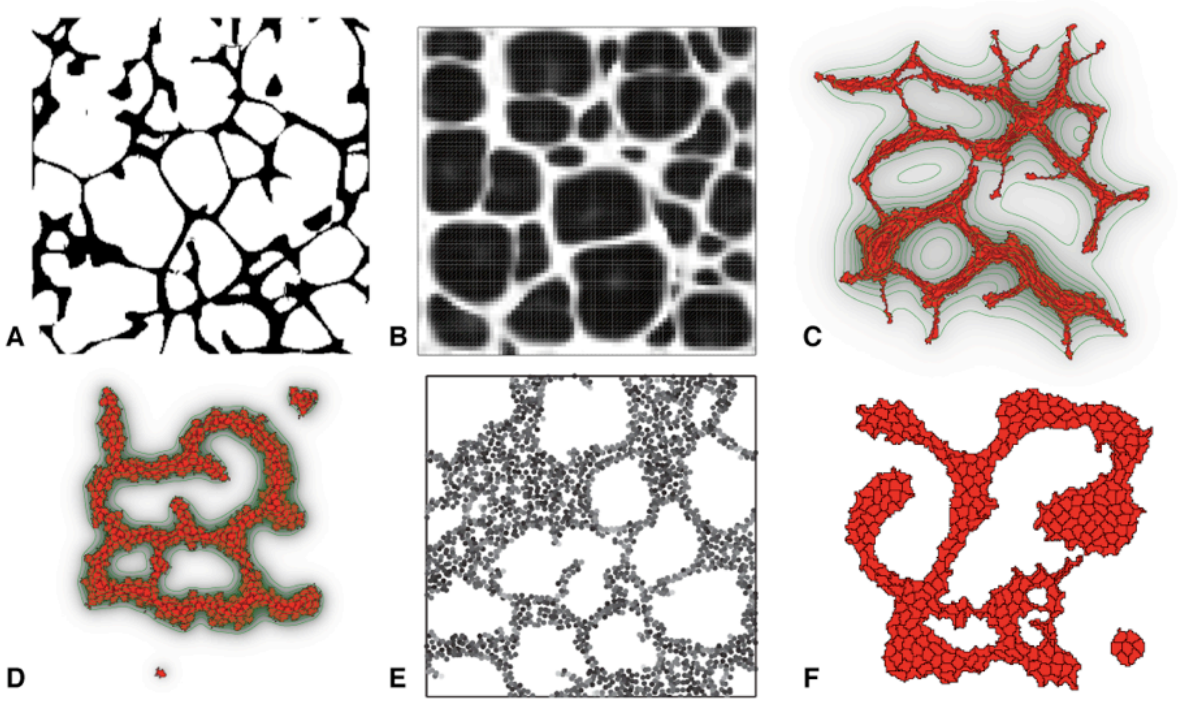
(Yin *et al.* 2008)



Ongoing work

Building *quantitative* cell-based models

- How to distinguish alternative models?
  - Alternative mechanisms produce similar results:

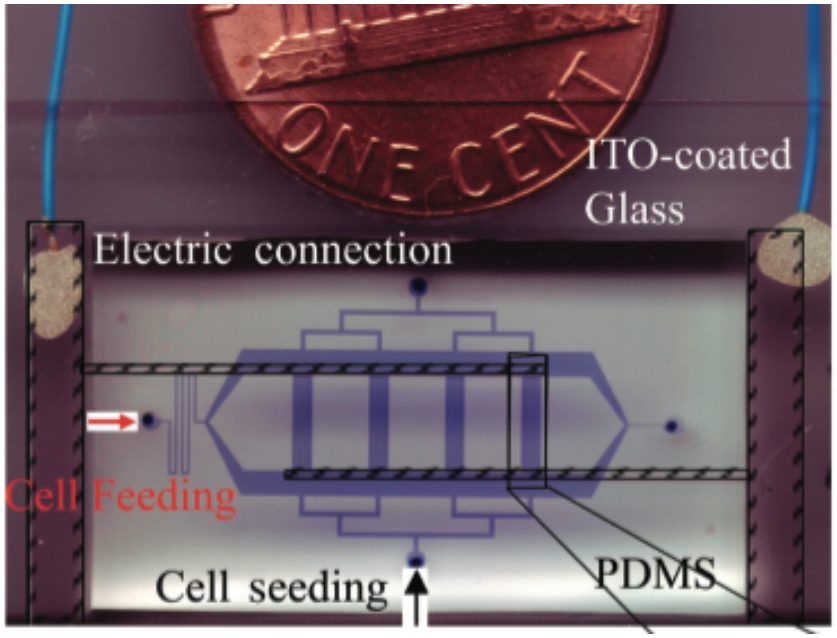


- Need to find subtle discrepancies between model and experiment

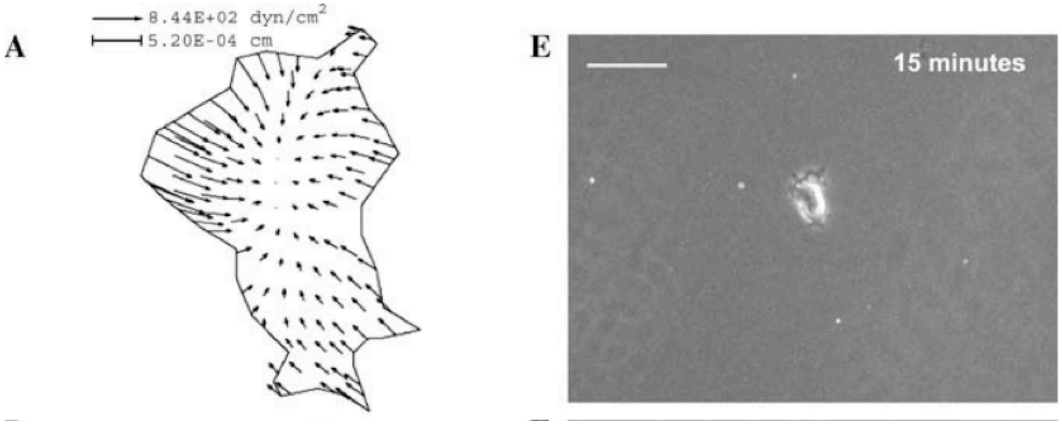
Merks and Koolwijk, *Math. Model. Natural Phenom.* 2009

Quantitative data will help

- New experimental techniques quantify cell behavior
  - microfluidics
  - traction force microscopy / atomic force microscopy
  - ...
- We can now model with **quantitative** cell-behavioral data



Yin *et al. Mol. Sys. Biol.* 2008



Reinhart-King *et al. Biophys. J.* 2005

CWI

Netherlands Consortium for Systems Biology

Acknowledgements

CWI

Margriet Palm
Josephine Daub

VUMC, Amsterdam

Pieter Koolwijk
Victor van Hinsbergh

Indiana University Bloomington

Abbas Shirinifard
James Glazier

New York Medical College

Sergey Brodsky
Stuart Newman
Michael Goligorsky

Kansas University Medical Center

Erica Perryn
Charles D. Little

Politecnico di Torino, Italy

Luigi Preziosi

Netherlands Consortium for Systems Biology and
Netherlands Genomics Initiative