Multi-Scale, Multi-Cell Modeling of Development, Homeostasis and Developmental Diseases

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Developing Multi–Scale, Multi–Cell Biological Simulations with CompuCell3D and SBW
The Hamner Institute
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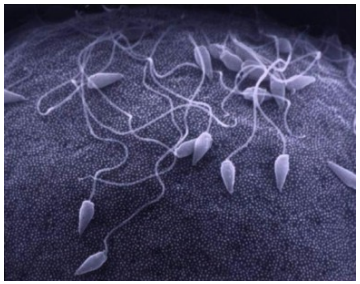
For papers on these projects, please visit http://www.biocomplexity.indiana.edu
To download software for model building, please visit http://www.compucell3d.org

Key Biological Questions

Development: How does Fertilized Egg Self-Organize into an Organism **without** a road map or plan?



http://www.stanford.edu/group/Urchin/LP/
[Lauren Palumbi]

→

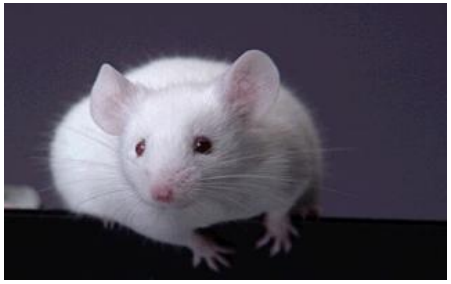
http://www.kvarkadabra.net/images/articles/Regeneracija-organov_1_original.jpg

Homeostasis: How does an Organism Maintain itself without an absolute standard of reference?

→

Key Biological Questions

Developmental Diseases: How does Failure of Homeostasis Lead to Redeployment of Developmental Mechanisms in Pathological Ways?



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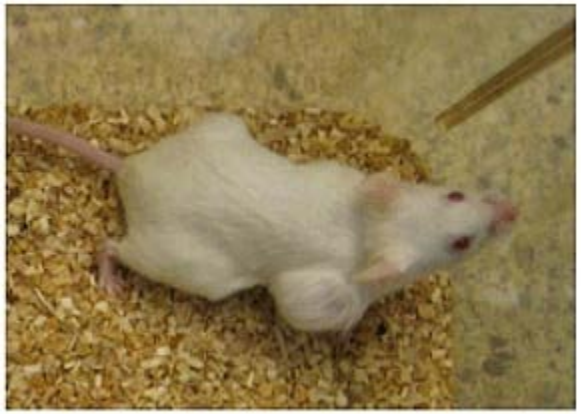


e.g., liver cirrhosis, cancer, diabetic retinopathy, polycystic kidney disease, osteoporosis,..

How do Tissues Develop, Function and Fail?

Cells: Know Their Internal State
Respond to Local Environment
Remodel their Environments
Change their Own Behaviors

Cells have no Roadmap
Cells don't know they are in an organism

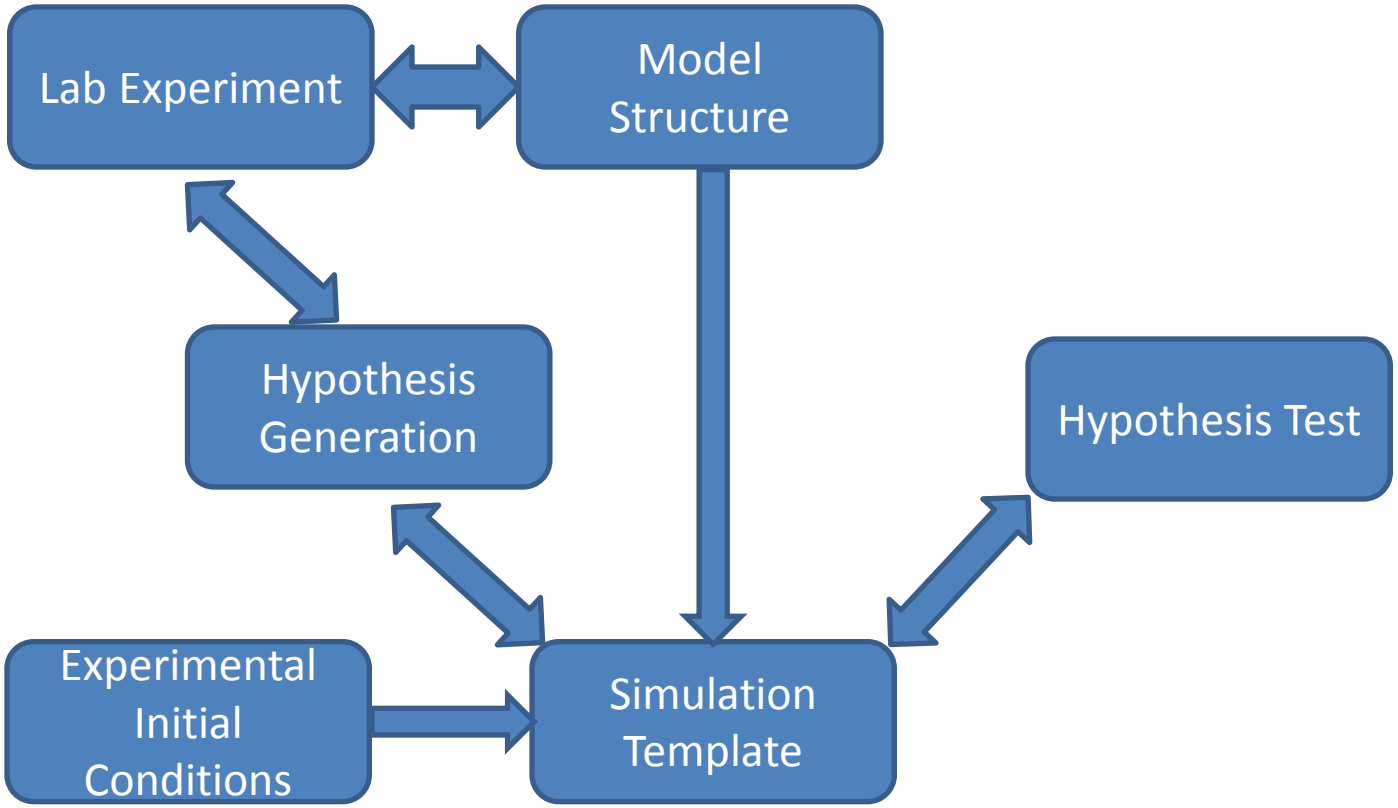
http://reslife.tamu.edu/images/maps/map3.gif

Important: Unlike an Airplane, Procedural Control (Programs) are Rare—Almost All Structures and Behaviors are Emergent (Self Organized)!

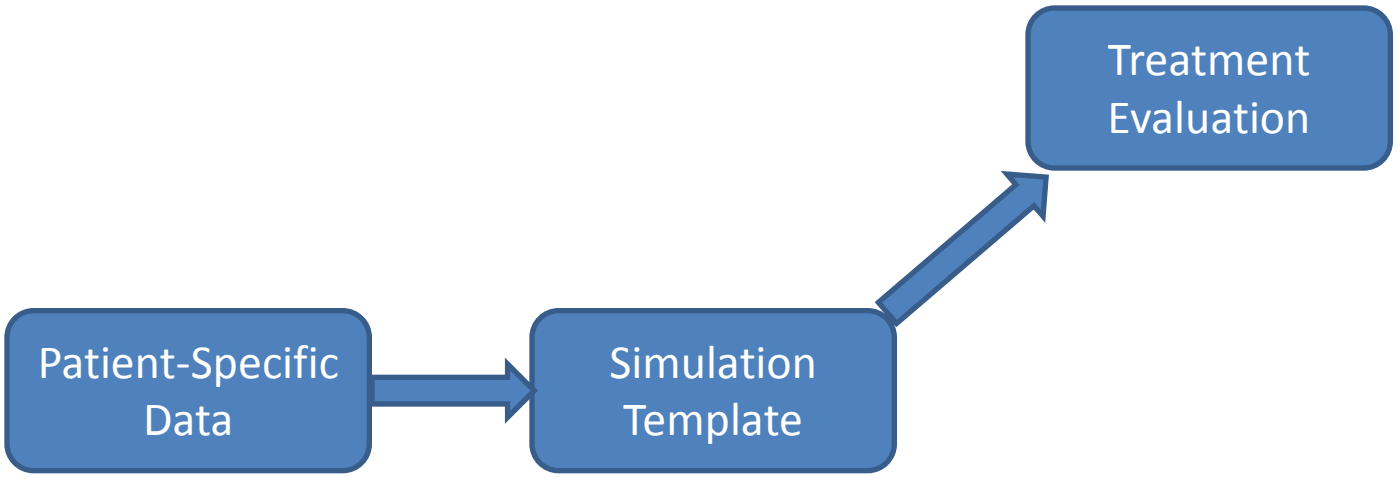
Promise of Mathematical/Mechanistic Understanding

- Fundamental understanding and control of developmental mechanisms, leading to:
  - Improved treatment regimes for cancer (ranging from more accurate tumor resection to more effective and less toxic therapies).
  - Control of stem and other human-derived cells for engineering of tissue replacements both *in vivo* and *in vitro.*
  - Induction of epimorphic regeneration *in situ*.
  - Treatments of degenerative diseases.
  - Prediction of chemical developmental toxicities.
  - …

Role of Models in Biology—Model, Experiment and Hypothesis Development

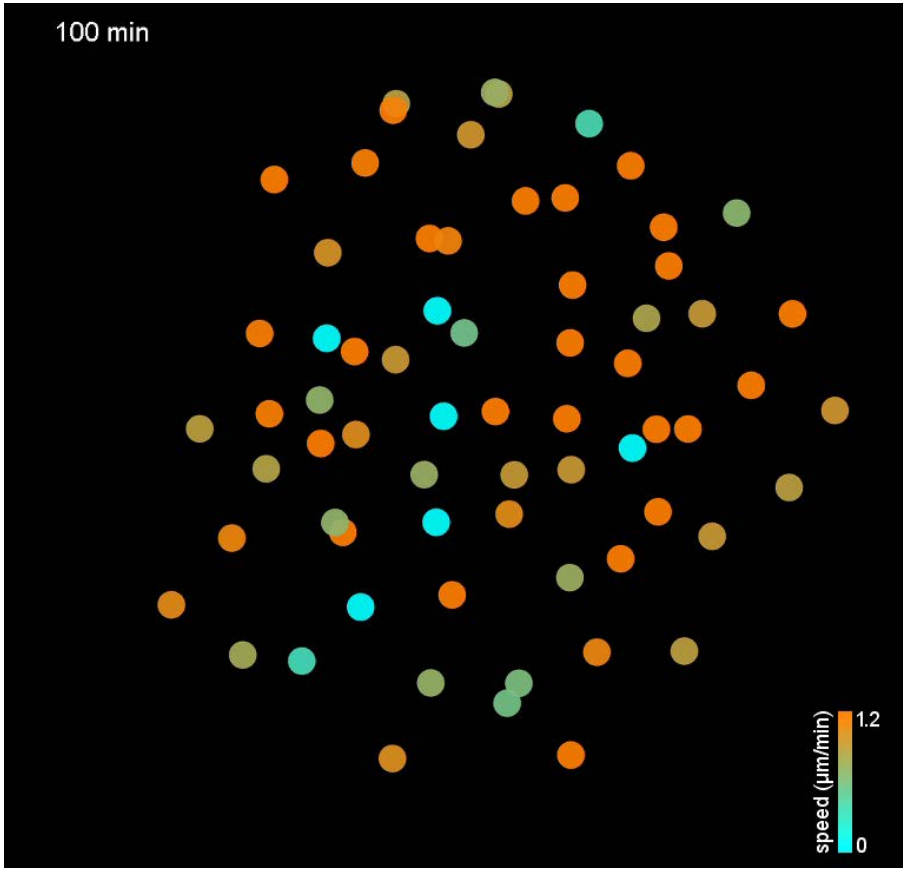


Role of Models in Biology—Model Application



Virtual Tissues Dream

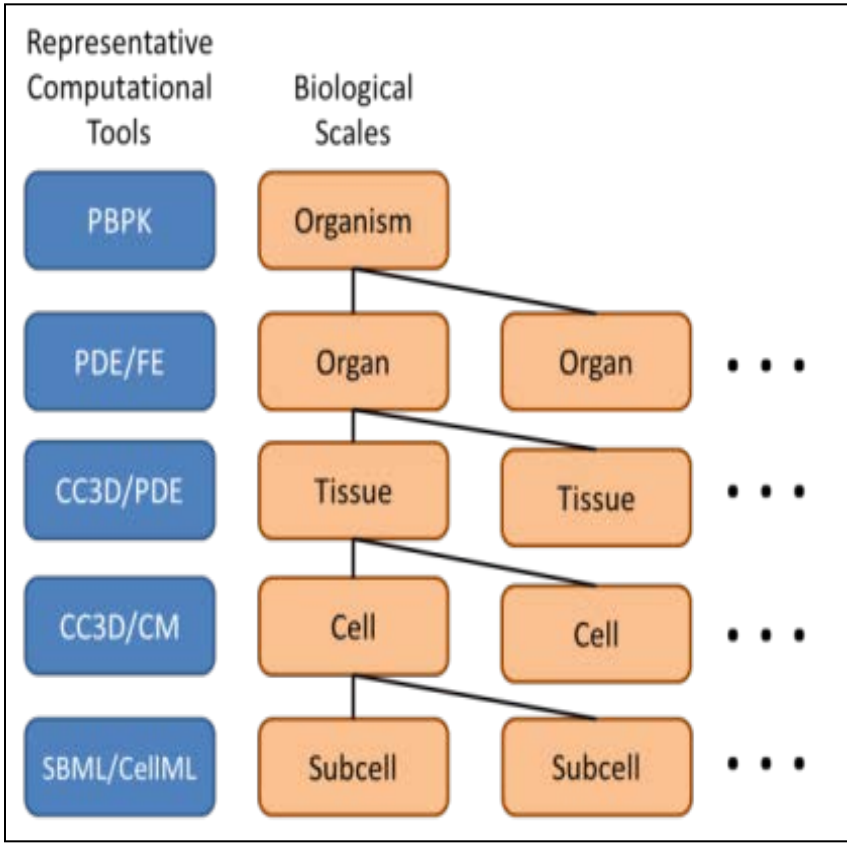
- Annotated Experimental Images ARE the Simulation.
- A Virtual Tissue Environment:
  - Reads an Annotated Image to Identify the Locations and Identity of Components.
  - Builds the Simulation by Populating the Simulation Representation of the Image with Components from the Cell Type Repository and Other Repositories.
  - Executes the Simulation using Standardized Specifications of Organ, Multi-cell, Subcell Behaviors of the Components.
  - Outputs the Simulation Results as Annotated Simulation Images for Analysis and Comparison with Experiment.
  - Functions as a Variable Power Microscope, Handling Refinement/Coarse Graining Automatically.
  - Simulates all Cells in Embryo, Tissue,…
- Ironically harder to track cells in an embryo than to position atoms in a virus!



Reconstructed zebrafish embryonic development from P. J. Keller, *et al.,* "Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy," *Science* **322**, 1065 (2008).

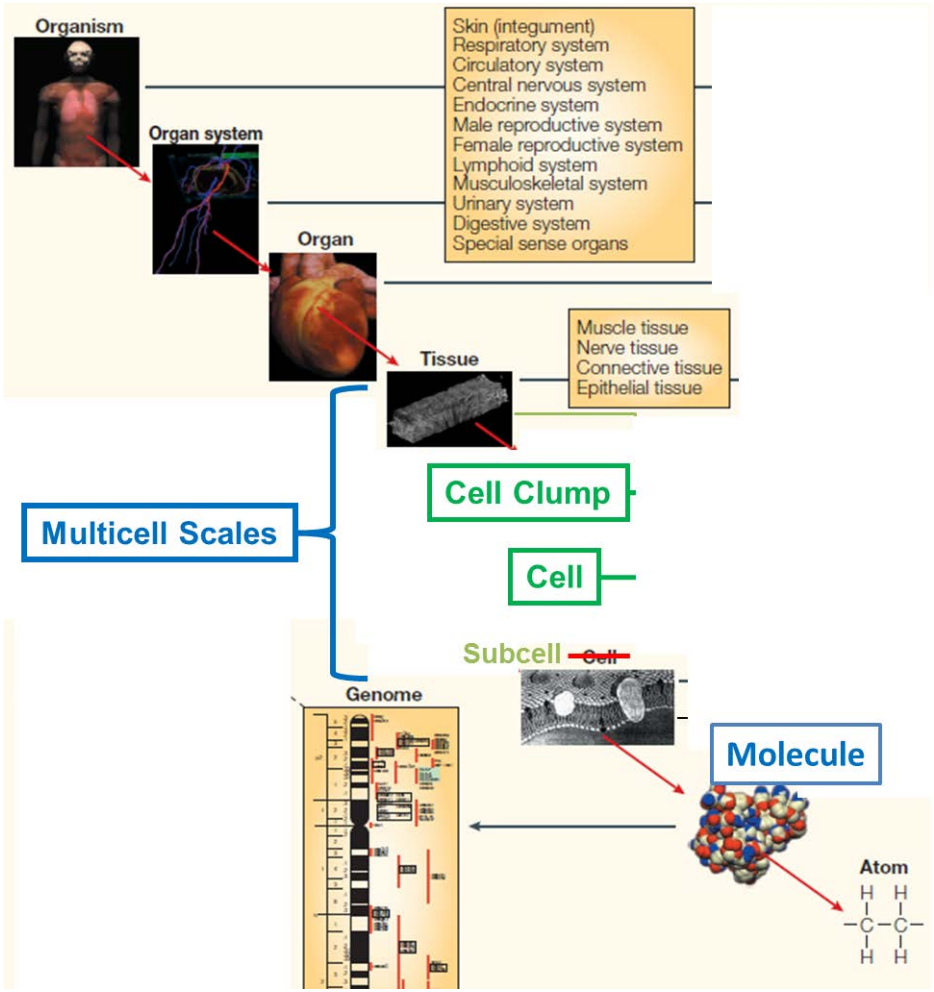
Virtual Tissues

- **Multiscale simulations of tissue function, development, disease and homeostasis integrating, subcellular, cellular, multicellular and tissue-level submodels.**
- **Integrated frameworks for organizing experiment, simulation and clinical development.**
- **Models capture the flow of molecular information across biological networks and process this information into higher-order responses.**
- **Responses depend on network topology, system state dynamics, and collective cellular behavior.**
- **Include multi-cellular behaviors that can result in emergent properties (*e.g.*, functions, phenotypes) not specified *a priori.***



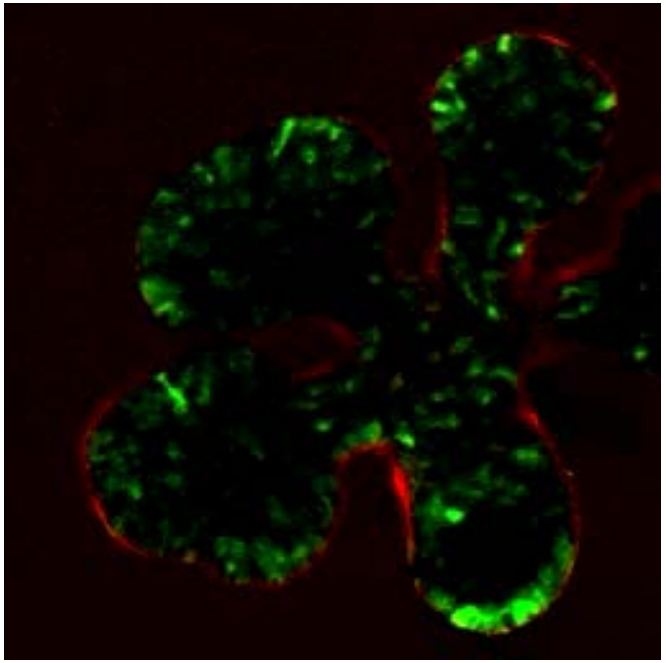
Scales Considered Determine Methodologies

- Human Brain—Many cm^3—Continuum Mechanics and PDE Methods
- Small Embryos, Adult Tissue Samples, Embryonic Organs—Several mm^3—MultiCell Methods
- One or a Few Cells—a few thousand μm^3—Macromolecular Methods
- Macromolecular Assemblies—a few thousand nm^3—Molecular Dynamics Methods
- Subcellular (Non-spatial)—Reaction Kinetics and Stochastic Methods



Multicell Models (I)—Cells

- Virtual Tissues require calculating at the coarsest level possible in each situation.
- Cells hide much of the complexity of molecular regulation.
- To understand multicellular patterning, distinguish:
  - How do moelecular interactions regulate cell phenomenology?
  - How does cell phenomenology drive multicellular patterning?

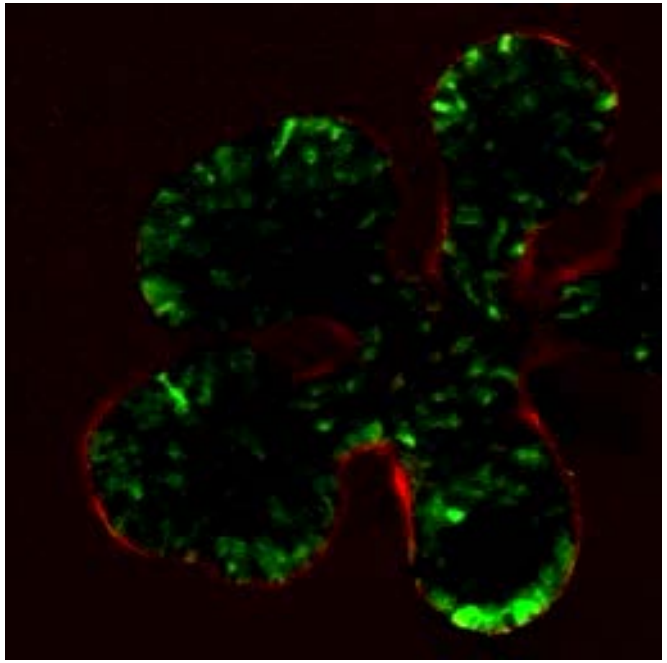


Larsen *et al.*, "Cell and fibronectin dynamics during branching morphogenesis," *Cell Sci* **119**: 3376.

Time-lapse GFP-labeled epithelial cells and labeled FN during branching morphogenesis. E12 SMGs labeled with GFP (green) and Alexa Fluor 647-FN (red), Confocal time-lapse images at 10-minute intervals for 14.5 hours shown at 10 frames/second. Epithelial cells (green) contact FN in the basement membrane (red).

Multicell Models (II)—ECM

- Much of the information in an organism is stored in the Extracellular Matrix (*ECM*)
- ECM←→Cell interaction is essential to morphogenesis and function
- Models Neglect/Oversimplify ECM because we lack:
  - Ways to characterize ECM experimentally
  - Tractable ways to describe ECM structure, mechanics and chemistry mathematically
  - Understanding of how cells move and respond to ECM
  - Understanding of how cells build and remodel ECM



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Embryogenesis at the Multicell Level

EMBRYONIC CELL BEHAVIORS	CONSEQUENCES OF DISRUPTION
cell growth & death	incorrect cell number
differentiation & function	missing cell types
cell motility & adhesion	disorganization
clocks & organizers	chaos and ataxia
genetic signals & responses	dysregulation
ECM synthesis & remodeling	loss of mechanical properties

Slide from Dr. Thomas Knudsen (EPA)

Multicell Methodologies

- Many Approaches—Different Advantages and Disadvantages
- In Rough Order of Degree of Spatial Detail
  - *Cellular Automata*
  - *Flock Models* (*SWARM*)
  - *Center Models* (Molecular Dynamics, one atom per cell)
  - ***GGH (CPM) Lattice Models*** (***CompuCell3D***, Glazier; Paulien Hogeweg, Utrecht U.; ***Tissue Simulation Toolkit***, Roeland Merks, Amsterdam; Yi Jiang, LANL)
  - **Vertex Models**
  - **Multielement Models** (Molecular Dynamics + Finite Element, many atoms per cell; Tim Newman, Arizona State U)
  - **Immersed Boundary Models** (Kasia Resniak, Moffit Cancer Center)
  - **Finite Element Models** (Drasdo, Paris)
  - …

Key:

BOLD=Cells have explicit shapes

Red—Lattice Techniques

Green—Off Lattice

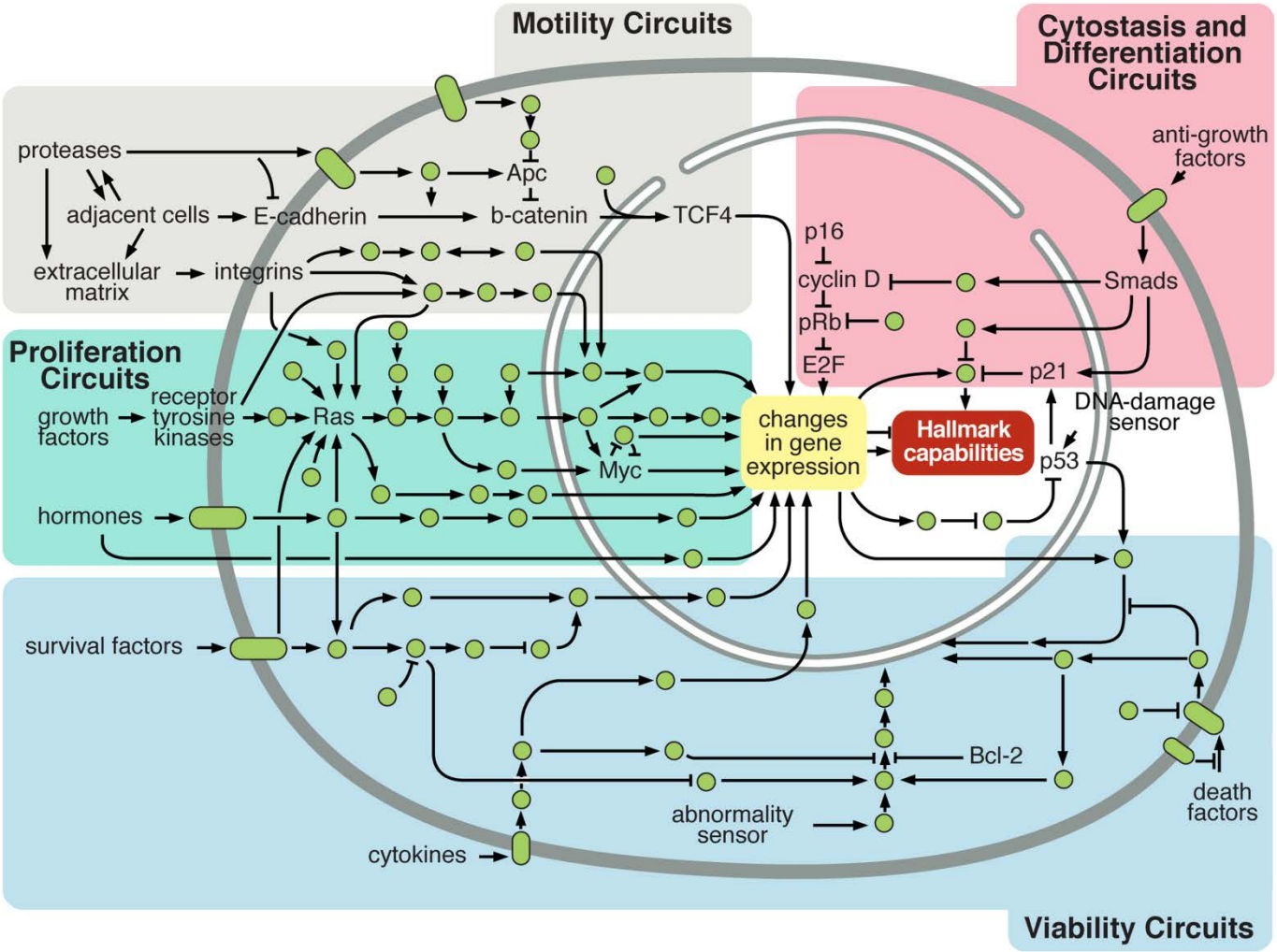
Shadow—Slow

Italics—Fast

Dashed Underline—Generic Modeling Environments Available

Underline—Specialized Open Source Modeling Environment Available

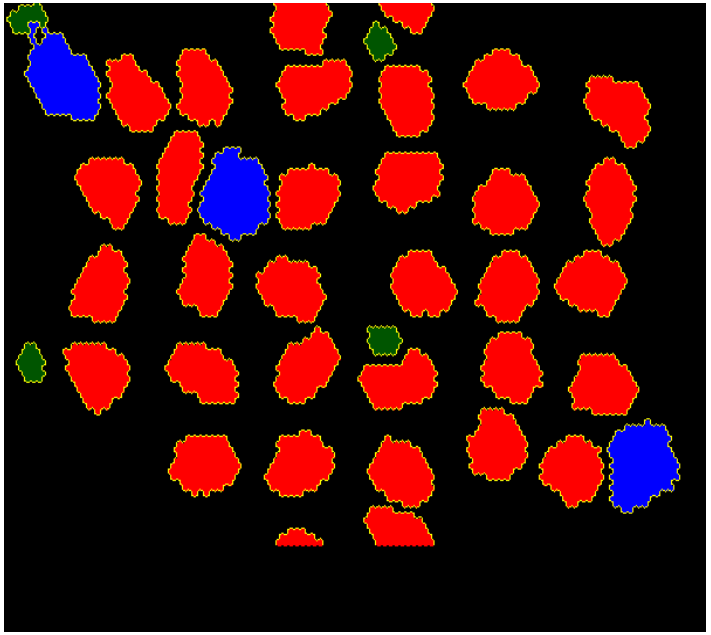
Key Intracellular Regulatory Circuits and Intercellular Signaling Pathways



Slide from Dr. Thomas Knudsen (EPA)

Simple cell-agent based model

macrophage navigating RBCs toward a microbial pathogen

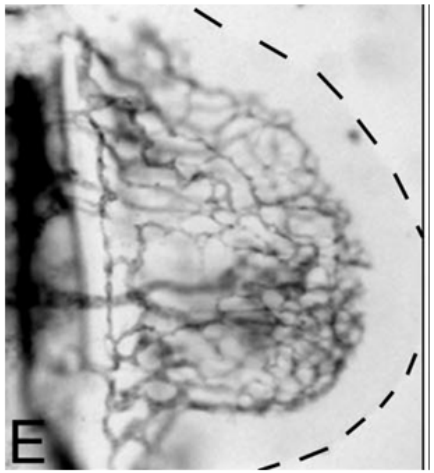


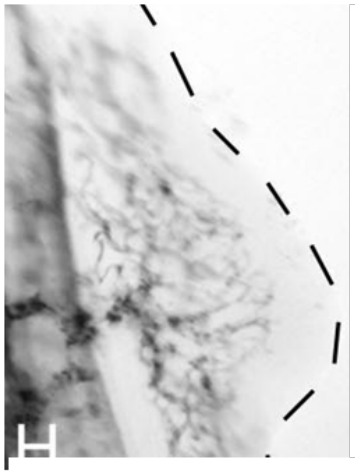
simple CompuCell3D model

Slide from Dr. Thomas Knudsen (EPA)

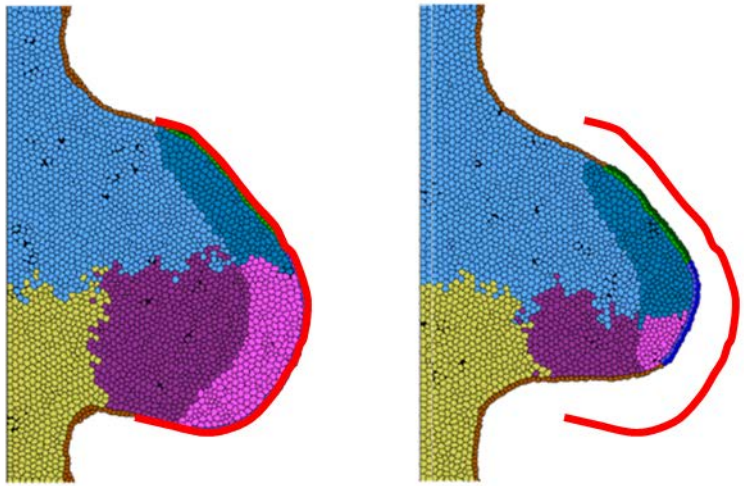
Complex cell-agent based model

Chick limb ***+CPS49***





Virtual limb



Thalidomide induces limb defects by preventing angiogenic outgrowth during early limb formation

Christina Therapontos[a,b], Lynda Erskine[b], Erin R. Gardner[c], William D. Figg[d], and Neil Vargesson[a,b,1]

Therapontos et al. PNAS 106: 8573-8578, 2009

Slide from Dr. Thomas Knudsen (EPA)

CompuCell3D Platform for Virtual Tissue Construction

- Building Virtual Tissues from scratch is difficult, time consuming and error prone.
- CompuCell3D aims to:
  - make model coding so easy, that understanding the Biology becomes the **hard** part of building multiscale, multicell biological models.
  - support modeling at scales from subcellular reaction networks, through individual cell behaviors to continuum tissue mechanics and PDEs.
  - make model specifications compact, reusable, sharable and verifiable.

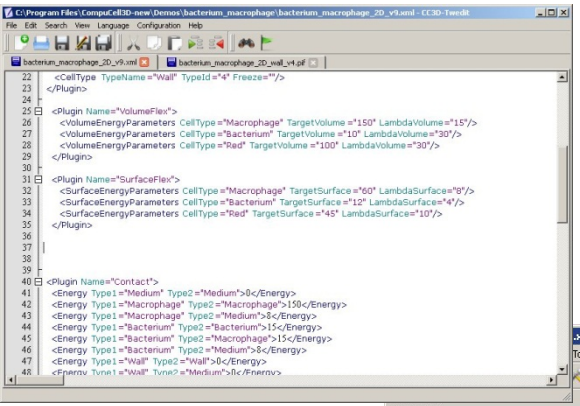
www.compucell3d.org

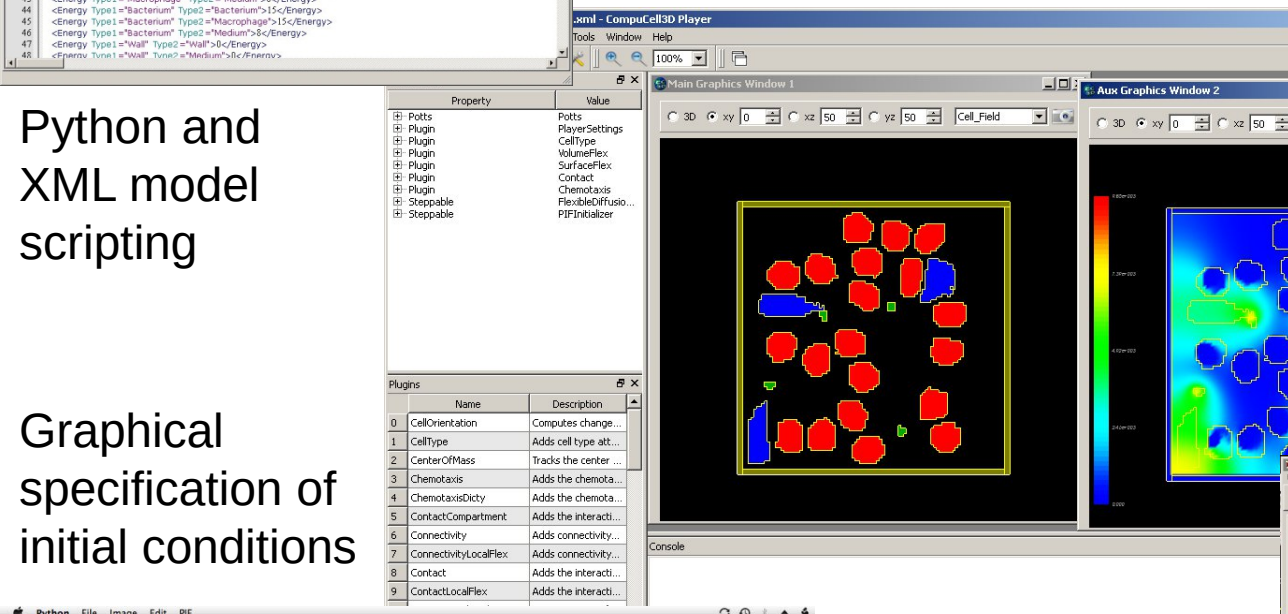
CompuCell3D - Simulation Environment for Multi-Cell, Multi-Scale Models

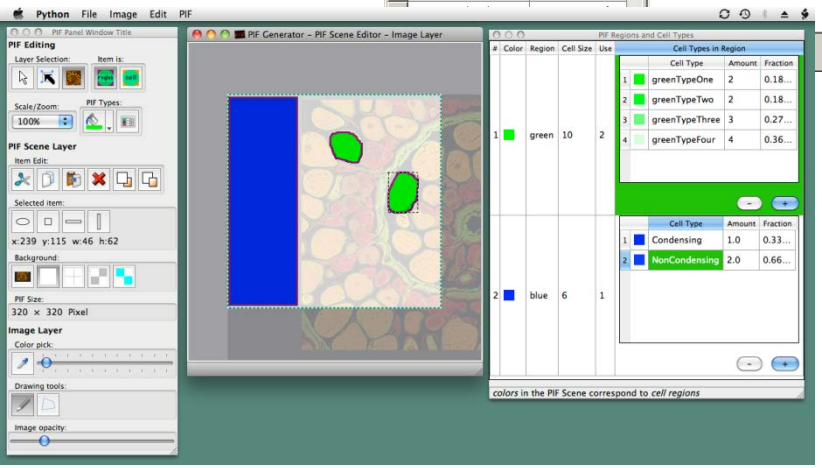
Python and XML model scripting

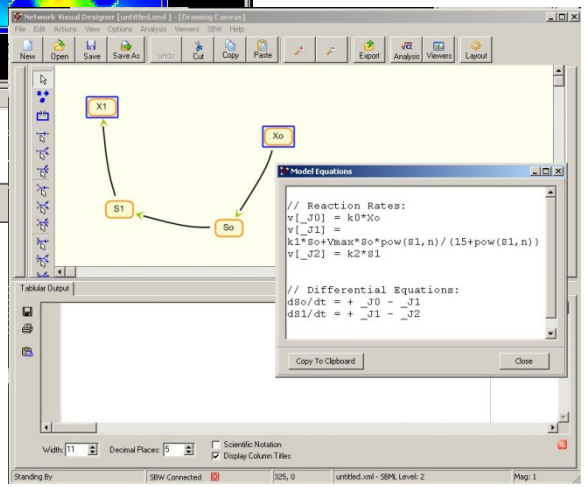
Graphical specification of initial conditions

SBML models (e.g. defined using SBW)





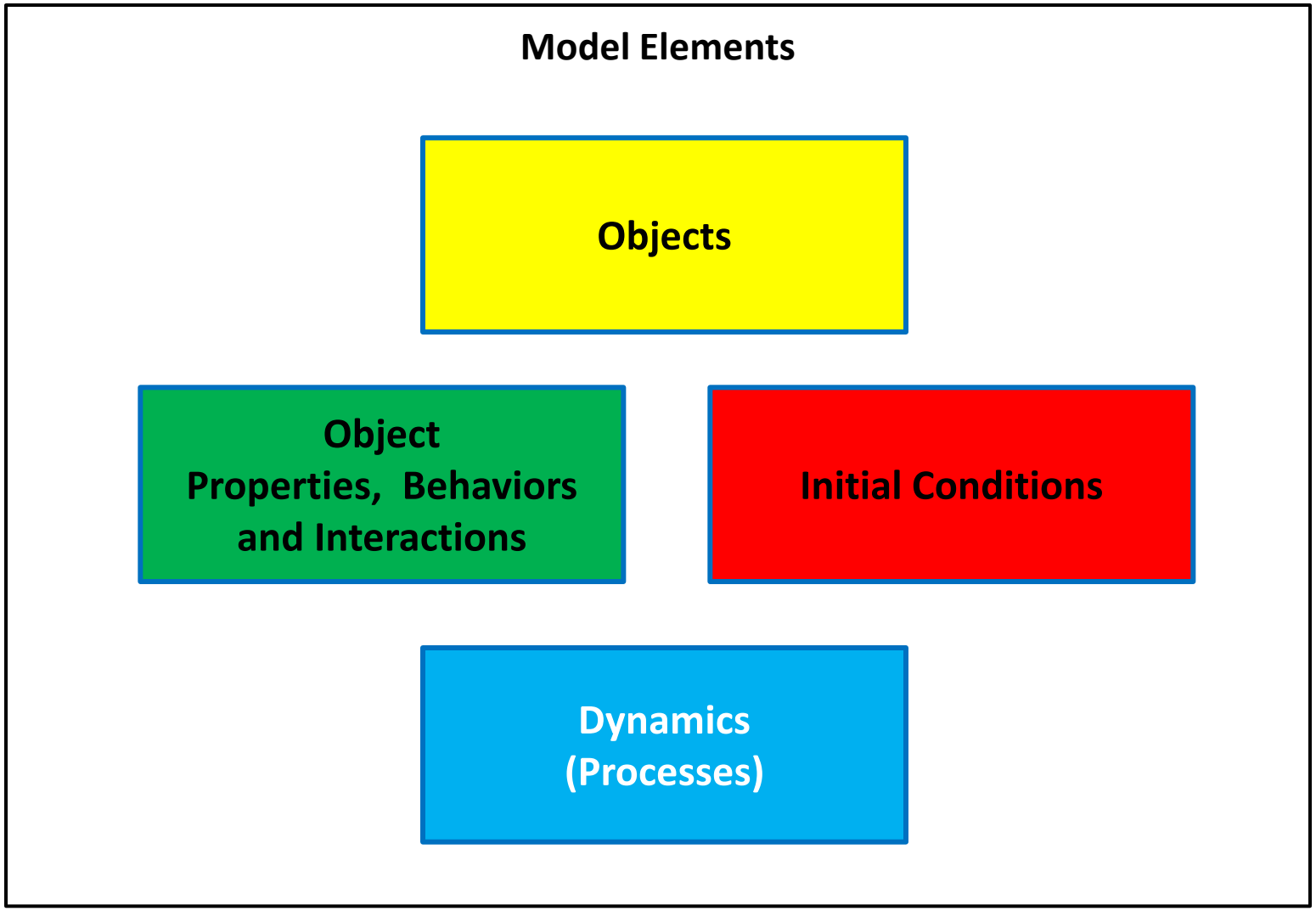




Available Mechanisms in CompuCell3D

- Control of Cell Differentiation, Signaling, Growth, … via Coupled ODEs (RK)
- Reaction-Diffusion Equations (PDEs)
- Cell Adhesion
- Membrane Areas
- Mitosis
- Apoptosis
- Secretion and Absorption of Materials
- Viscosity
- Chemotaxis
- Haptotaxis
- Rigid-Body Motion (FE)
- Links (FE)
- Inertial/Persistent Motion
- Explicit External Forces
- Gravity
- Compartmental Cell Models
- Cell Polarity
- Complex Cell Shapes and Cell-Shape Changes.
- …..

Model Components

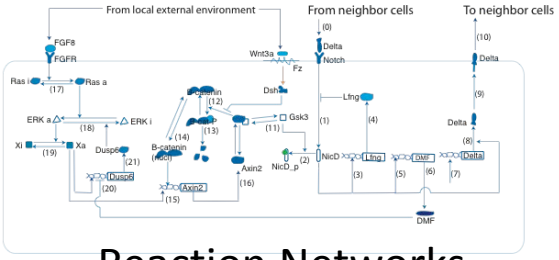


Model Components

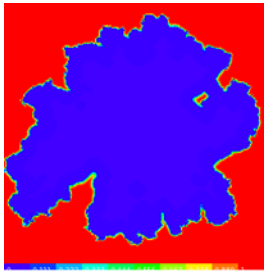
- **Objects/Representations**
- Object Properties/Interactions
- Dynamics
- ‘Tweaks’
- Initial and Boundary Conditions

CompuCell3D Objects/Representations

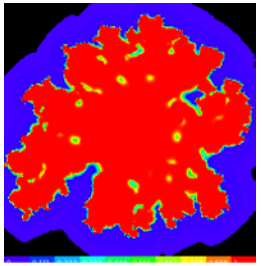
- **Cells** and **Generalized Cells** (*e.g.* mesenchymal cells, epithelial cells, ECM, medium…), represented on the primary **Cell Lattice**
- **Internal States**, **Types** and **Reaction Networks** which control their properties.
- **Fields** represented on **Auxiliary Lattices** with same geometry as the Cell Lattice.
- **Finite Element Links** for the control of Mechanical Properties



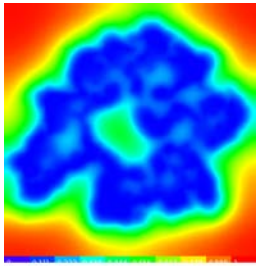
Reaction Networks



ECM

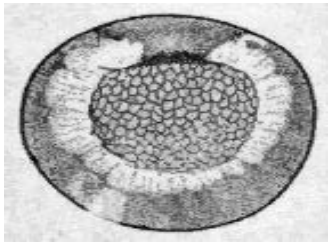


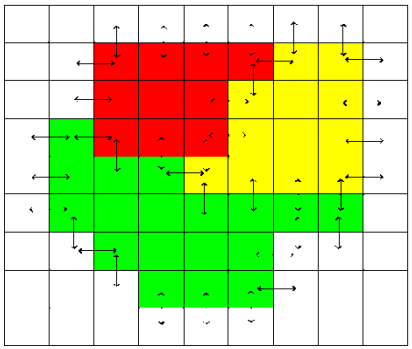
MDE



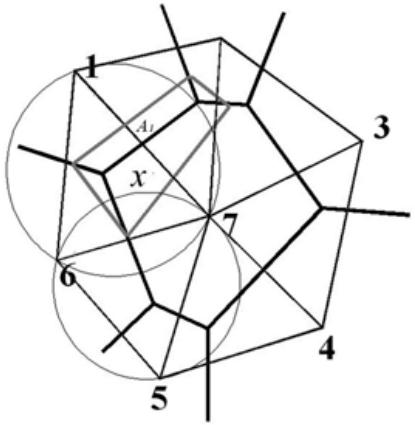
nutrient

Fields





Cell Lattice and Generalized Cells



Finite Element Links

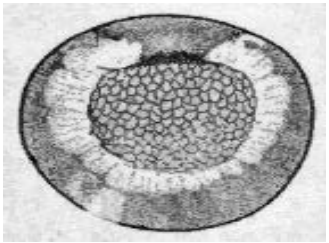
Generalized Cells

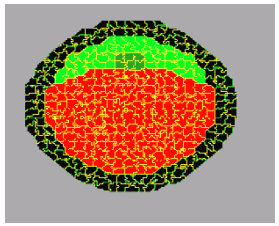
Each Cell has a unique integer Index, σ and consists of all sites on the Cell Lattice containing that Index.

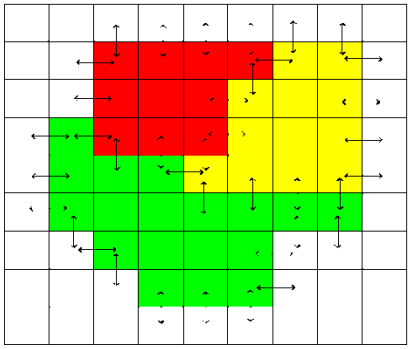
The number of Cell Lattice Sites with Index σ is the Cell's Volume, V.

The number of Lattice Sites with Index σ and, which are next to a Site with a Different Index σ' is the Cell's Surface Area, S.

Each cell also has a Type, τ.







Fields

A Field is a Lattice of (usually) positive real numbers denoting the concentration of a chemical.

Fields and the Cell Lattice usually occupy the same notional space (no excluded volume).

Fields may be Diffusing or Nondiffusing.

Fields may be confined to subregions corresponding to particular areas of the Cell Lattice (*e.g.* diffusion only outside Cells).

Diffusing Fields obey appropriate Partial Differential Diffusion Equations.

Fields may be absorbed or secreted by Cells and may Decay, or Interact with each other (Reaction-Diffusion).

Multiple Fields can represent textured materials like Extracellular Matrix.

Internal Variables and Networks

In more complex models each Cell or Field may have a complex set of auxiliary parameters and associated models, *e.g.*

Lists of Chemical Concentrations and Reaction Networks (in SBML)

Orientation Vectors and Update Rules

Boolean State Descriptors and Rules

Model Components

- Objects/Representations
- **Object Properties/Interactions**
- Dynamics
- ‘Tweaks’
- Initial and Boundary Conditions

Object Properties/Interactions

- Most biological of Cells and their interactions with each other and with Fields are Encapsulated in the Effective Energy, H.
- H is generally the sum of many separate terms.
- Each term in H encapsulates a single biological mechanism.
- Additional Cell Properties described as **Constraints**.

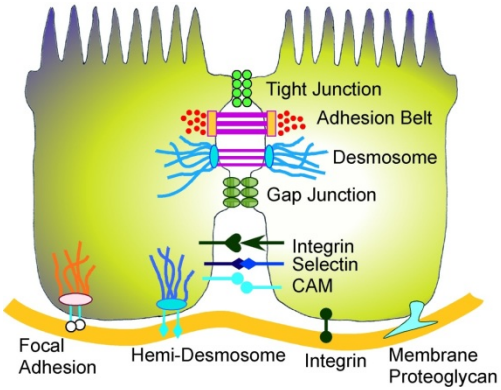
Effective Energy Terms

- The most important Effective Energy Terms describe:
- Interfacial Energy between Cells and other Cells.
- The Effective Chemical Potential which induces Chemotaxis and Haptotaxis.
- Other terms may be useful in particular situations (*e.g.* gravitational potential energy, explicit external forces).

Energy Terms: Labile Adhesion/Surface Tension

Each unit of Cell Boundary (a Link between Adjacent Lattice Sites containing different Indices) has an associated Adhesion Energy, J, which depends on the Types of the Neighboring Cells: $J\left(\tau\left(\sigma\left(\vec{i}\right)\right),\tau\left(\sigma\left(\vec{i}'\right)\right)\right)$

or the number and types of adhesion molecule on each cell: $f\left(n_j\left(\vec{i}\right),\ldots;n_k\left(\vec{i}'\right),\ldots\right)$



The Total Adhesion Energy, H_{adhesion} is:

$$H_{\text{adhesion}} = \sum_{\substack{\vec{i},\vec{i}' \\ \text{neighbors}}} J\left(\tau\left(\sigma\left(\vec{i}\right)\right),\tau\left(\sigma\left(\vec{i}'\right)\right)\right)\left\{1-\delta\left(\sigma\left(\vec{i}\right),\sigma\left(\vec{i}'\right)\right)\right\}$$

or

$$H_{\text{adhesion}} = \sum_{\substack{\vec{i},\vec{i}' \\ \text{neighbors}}} f\left(n_j\left(\vec{i}\right),\ldots;n_j\left(\vec{i}'\right),\ldots\right)\left\{1-\delta\left(\sigma\left(\vec{i}\right),\sigma\left(\vec{i}'\right)\right)\right\}$$

$$\delta\left(\sigma\left(\vec{i}\right),\sigma\left(\vec{i}'\right)\right) = \begin{cases} 1, \sigma\left(\vec{i}\right) = \sigma\left(\vec{i}'\right) \\ 0, \sigma\left(\vec{i}\right) \neq \sigma\left(\vec{i}'\right) \end{cases}$$

Energy Terms: Chemotaxis

If a Cell is attracted or repelled by a chemical, the response is represented by a Chemotaxis or Haptotaxis Effective Energy, H_{chemo}:

$$H_{\text{chemo}} = \sum_{\vec{i}} \mu\left(\tau\left(\sigma\left(\vec{i}\right)\right)\right) f\left(C\left(\vec{i}\right)\right)$$

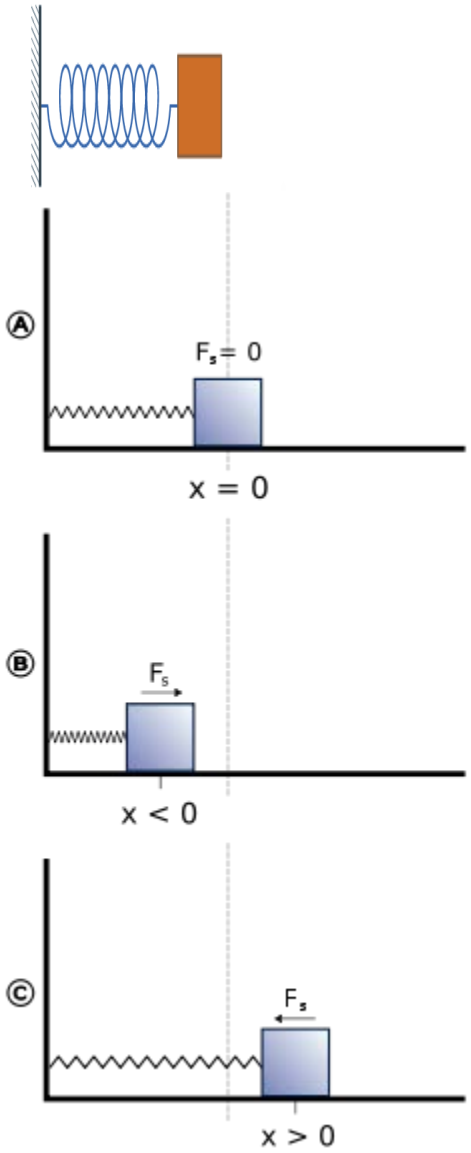
μ>0→chemorepulsion, μ<0→chemoattraction.

f is the response function of the cell to the chemoattractant.

There may be many such terms, with different responses for each cell type.

Constraints

- What is a Constraint?
- A function that pushes a system back towards some predefined state.
- *E.g.*
  - A mass on a spring
  - A ball rolling in a bowl



Constraints

- A Constraint is a very convenient method for implementing behaviors via an Effective Energy.
- In general, an elastic Constraint has the form:

$$H_{\text{constraint}} = \sum_{\text{objects}} \lambda(\text{object})\left(f(\text{object}) - f_{\text{target}}(\text{object})\right)^2$$

- λ is the Constraint Strength and f the Constraint Function. The bigger λ, the smaller the deviations of the behavior of the system from the target.
- Because of the Dynamic Behavior of Metropolis Algorithm ANY behavior can be implemented this way.

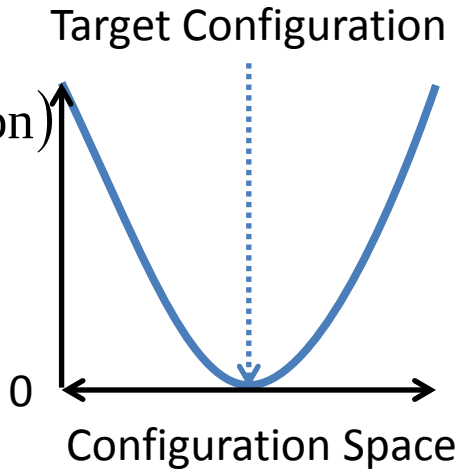
Constraints

- Saw before, the pattern configuration evolves to reduce the Effective Energy at a rate $\left|\nabla H\left(\vec{x}\right)\right|/T$

- For a constraint:

$$H_{\text{constraint}}(\text{configuration})$$

$$H_{\text{constraint}} = \sum_{\text{objects}} \lambda(\text{object})\left(f(\text{object}) - f_{\text{target}}(\text{object})\right)^2$$



- Because the energy function is smooth and has a single minimum, the pattern will evolve from any configuration to try to satisfy the constraint, at a rate proportional to $2\lambda(\text{object})\left(f(\text{object}) - f_{\text{target}}(\text{object})\right)$
- **For multiple incompatible constraints, the selected configuration will be a compromise among the constraints.**

Constraints

- Most Important Constraints:
  - Cell Volume
  - Cell Surface Area
  - Elasticity (Elastic/Plastic Solids/Junctional Adhesion)

Volume Constraints

- Most Cells (except Generalized Cells representing fluid media) have defined volumes.

$$H_{\text{volume}} = \sum_{\sigma} \lambda_{\text{volume}}(\sigma)\left(V(\sigma) - V_{\text{target}}(\sigma)\right)^2$$

$$\text{Pressure} = 2\lambda_{\text{volume}}(\sigma)\left(V(\sigma) - V_{\text{target}}(\sigma)\right)$$

- *i.e.* the cell obeys the ideal gas law.
- Provides an easy way to implement Cell Growth:

$$\frac{dV_{\text{target}}(\sigma)}{\text{dt}} = f(\text{system state, cell state})$$

- And Cell Death: $V_{\text{target}}(\sigma) = 0$

The rate of cell disappearance is proportional to $\lambda_{\text{volume}}(\sigma)$

Elastic/Plastic Solids/Junctional Adhesion

Subdivide the object into subelements, measure the center-of-mass distances between neighboring elements and constrain them to remain equal to their original values using links between subelements.

$$H_{\text{elastic}} = \sum_{\sigma} \sum_{\substack{\mu,\nu=1 \\ \text{neighbors}}}^{m(\sigma)} \lambda_{\text{elastic}}(\sigma,\mu,\nu)\left(\left\|\vec{c}m(\sigma,\mu)-\vec{c}m(\sigma,\nu)\right\| - L_{\text{target}}(\sigma,\mu,\nu)\right)^2.$$

λ_{elastic} is the Young's Modulus of the Solid.

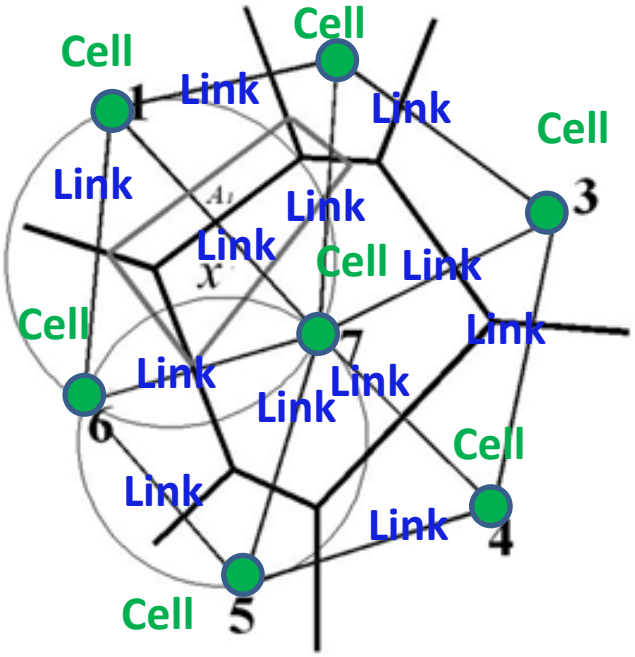
The strain on a link is:

$$\left\|\vec{c}m(\sigma,\mu)-\vec{c}m(\sigma,\nu)\right\| - L_{\text{target}}(\sigma,\mu,\nu)$$

The stress on a link is:

$$\lambda_{\text{elastic}}(\sigma,\mu,\nu)\left(\left\|\vec{c}m(\sigma,\mu)-\vec{c}m(\sigma,\nu)\right\| - L_{\text{target}}(\sigma,\mu,\nu)\right)$$

For a plastic material, define a Yield Strain (or Yield Stress at which the links break.



Model Components

- Objects/Representations
- Object Properties/Interactions
- **Dynamics**
- ‘Tweaks’
- Initial and Boundary Conditions

Model Dynamics

•To simulate the cytoskeleton-driven extension and retraction of cell membranes (including pseudopods, filopodia and lamellipodia). The GGH algorithm tries randomly to extend and retract cell boundaries one pixel at a time.

•At each attempt, it calculates the new configuration Effective Energy and accepts the new configuration according to the Metropolis algorithm: probability of configuration change:

$$P(\Delta H) = e^{-\Delta H / kT}, \Delta H > 0$$
$$P(\Delta H) = 1, \Delta H \leq 0$$

•Result is movement with velocity proportional to the gradient of the Energy, *i.e.*, linear in the applied force.

•Method breaks down if $\Delta H / kT$ too large.

•Configurations evolve to satisfy the constraints.

•When constraints conflict, evolve to balance errors.

Field Equations

- Most Fields evolve via diffusion, secretion and absorption and cells and by decay.

$$\frac{\partial C(\vec{i})}{\partial t} = D_c \nabla^2 C(\vec{i}) - \gamma_c C(\vec{i}) + S_c\left(\sigma(\vec{i})\right) - A_c\left(\sigma(\vec{i})\right)$$

Diffusion Decay Secretion Absorption

- Sometimes we couple two or more Fields via Reaction-Diffusion Equations of Form:

$$\frac{\partial C_1(\vec{i})}{\partial t} = f(C_1, C_2) + D_{c_1} \nabla^2 C_1(\vec{i}) - \gamma_{c_1} C_1(\vec{i}) + S_{c_1}\left(\sigma(\vec{i})\right) - A_{c_1}\left(\sigma(\vec{i})\right)$$

$$\frac{\partial C_2(\vec{i})}{\partial t} = g(C_1, C_2) + D_{c_2} \nabla^2 C_2(\vec{i}) - \gamma_{c_2} C_2(\vec{i}) + S_{c_2}\left(\sigma(\vec{i})\right) - A_{c_2}\left(\sigma(\vec{i})\right)$$

Model Components

- Objects/Representations
- Object Properties/Interactions
- Dynamics
- **‘Tweaks’**
- Initial and Boundary Conditions

Tweaks: Mitosis

Implement by setting a Criterion for Cell Division.

When reached, divide Cell along either random axis (random cell division) or axis with minimal moment of inertia (oriented cell division)

Assign Cell Lattice Sites in one half of Cell to a new unique Index. New Cell Inherits other properties of Parent.

Reset $V_{target}=V_{target}/2$ for both Cells.

Model Components

- Objects/Representations
- Object Properties/Interactions
- Dynamics
- ‘Tweaks’
- **Initial and Boundary Conditions**

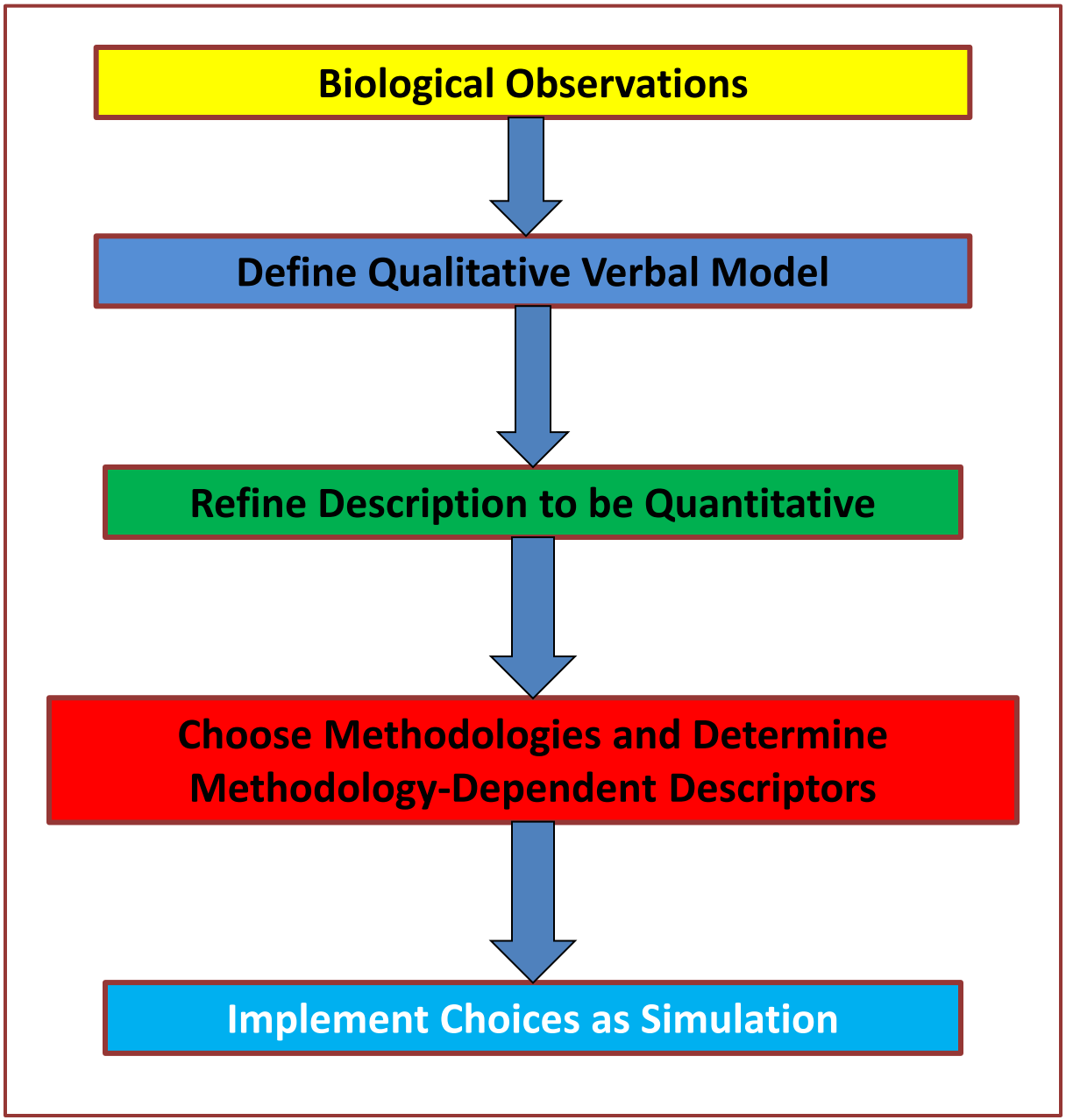
Initial and Boundary Conditions

- Need to Define Initial Configurations for All Lattices and Initial Values for all Internal Variables and Parameters.
- Need to Define Boundary Conditions of Fields and Cell Lattice (Periodic or Fixed, Absorbing or Reflecting, Excluded Volumes/No Excluded Volumes…).

Sample Current Applications

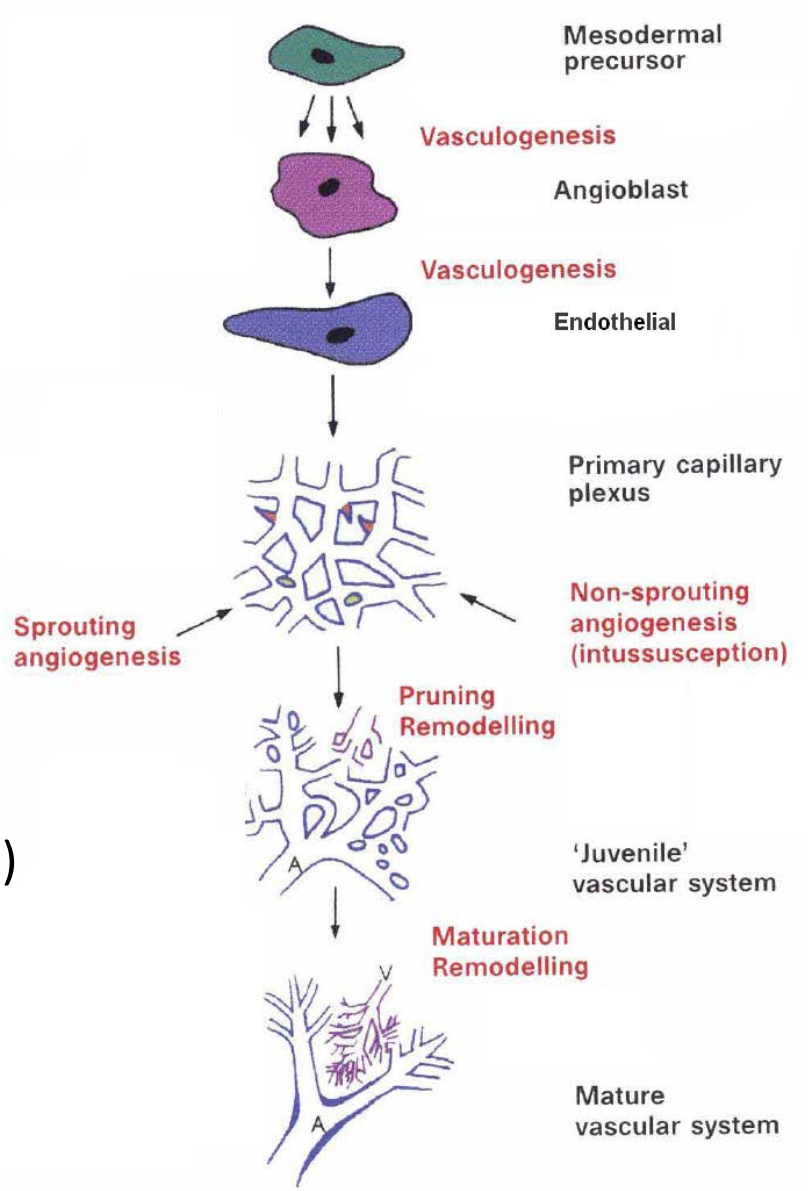
- Effects of Radiation on Tumors (Dan Lea, London)
- Vascular Tumor Growth
- Age Related Macular Degeneration (IUB, Emory)
- Computational Developmental Toxicology, Virtual Embryo, Virtual Liver (EPA)
- Drosophila Eye Development
- Gastrulation
- Segmentation

Improved Model Construction Workflow



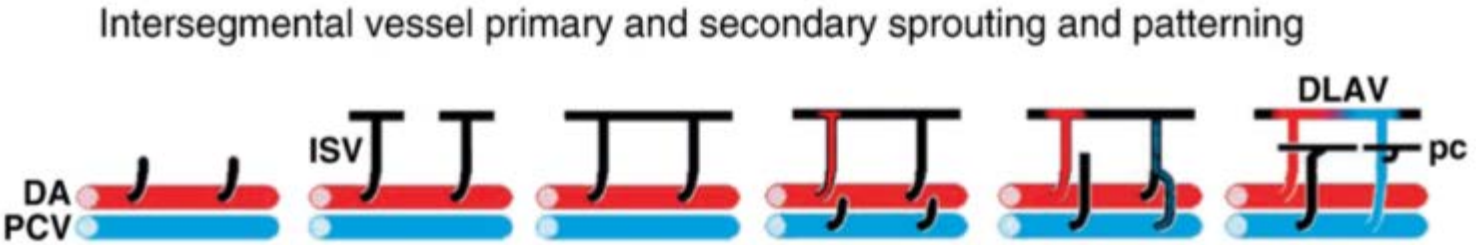
Vascular Patterning: Biology

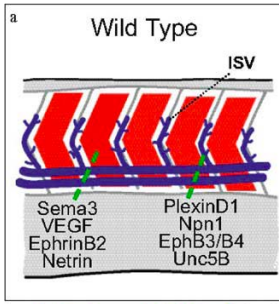
- Vasculogenesis
  - The formation of early vascular plexus from *in situ* differentiated **Endothelial Cells** (***ECs***)
- Angiogenesis
  - The formation of new blood vessels from pre-existing ones
    - **Sprouting Angiogenesis**
    - Non-sprouting Angiogenesis (Intussusceptive angiogenesis)

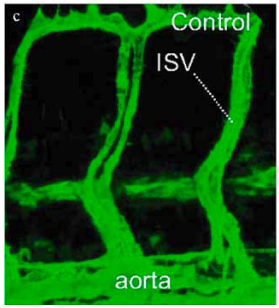


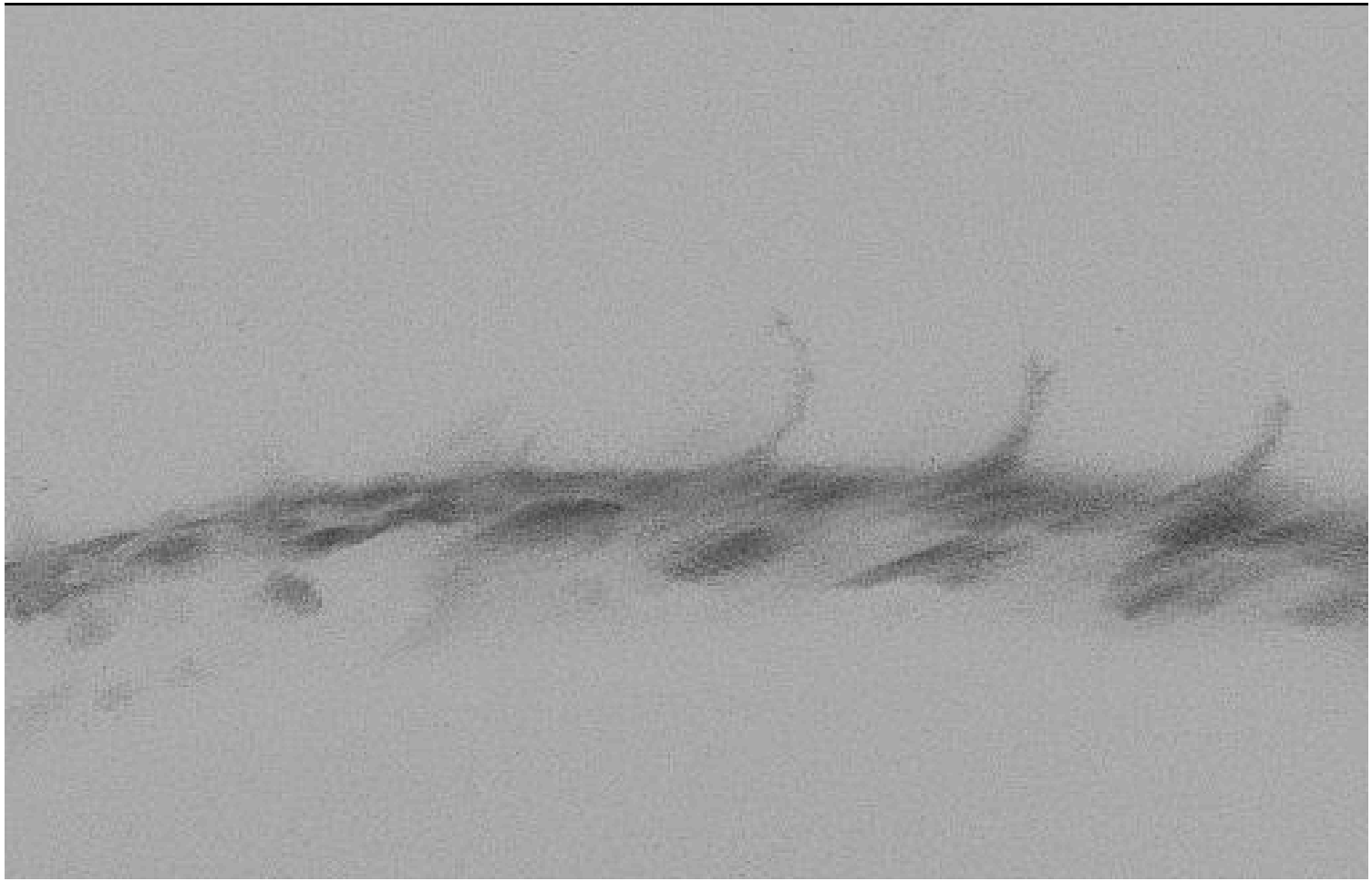
Werner Risau, *Nature* **386**, 671 - 674 (1997)

Angiogenesis in **Zebrafish Embryo (flk-1)**



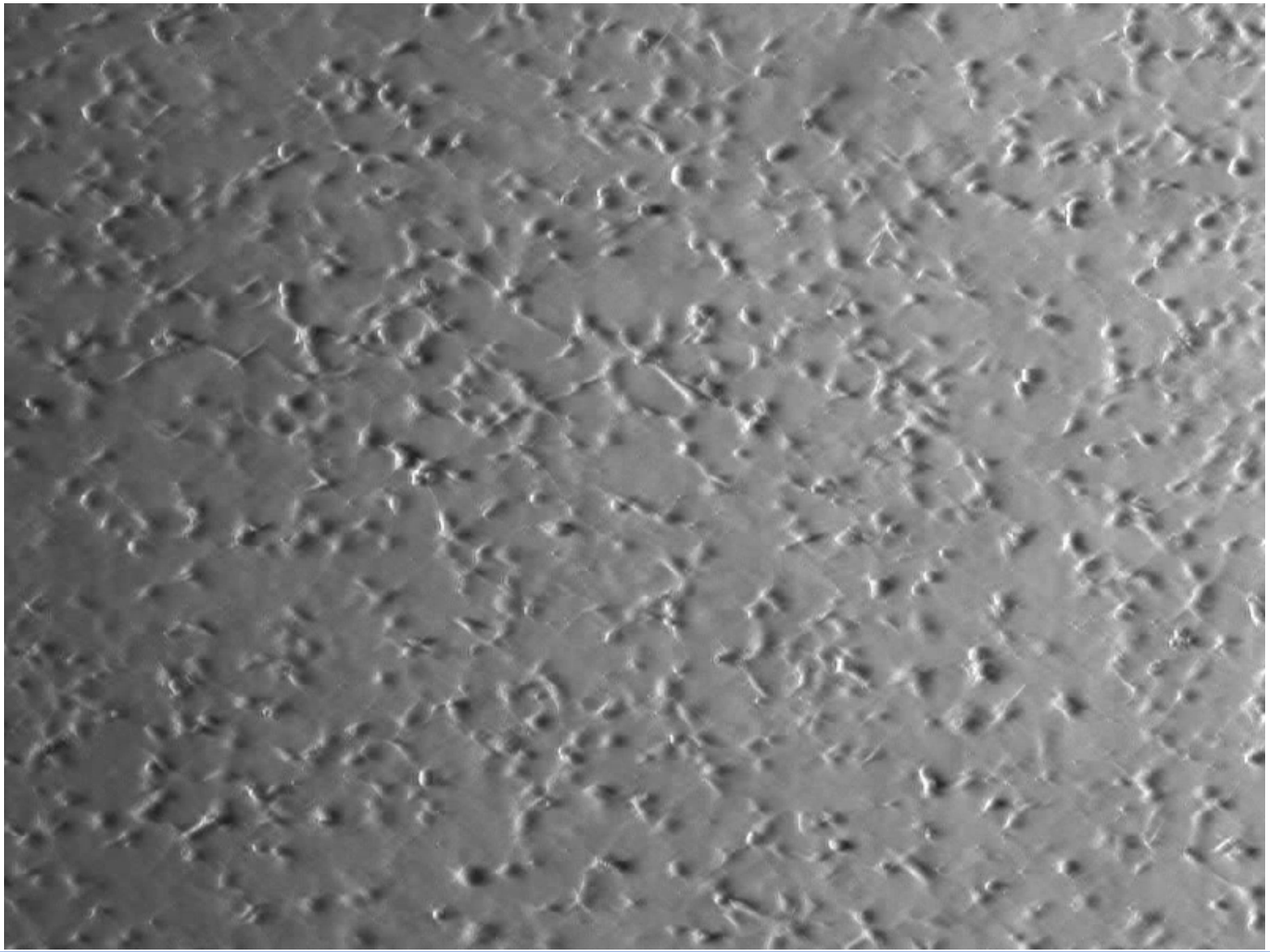






Catherine McCollum (U of Houston), Sherry Clendenon, Prof. Glazier's lab

In vitro Capillary Formation



Endothelial cells form lumenized vascular networks *in vitro* culture in 72 hours

Abbas Shirinifard, Abdelkrim Alileche, Prof. Glazier's lab

(Patent Application PCT/US2011/028492)

Vascular Patterning: Fundamental Questions

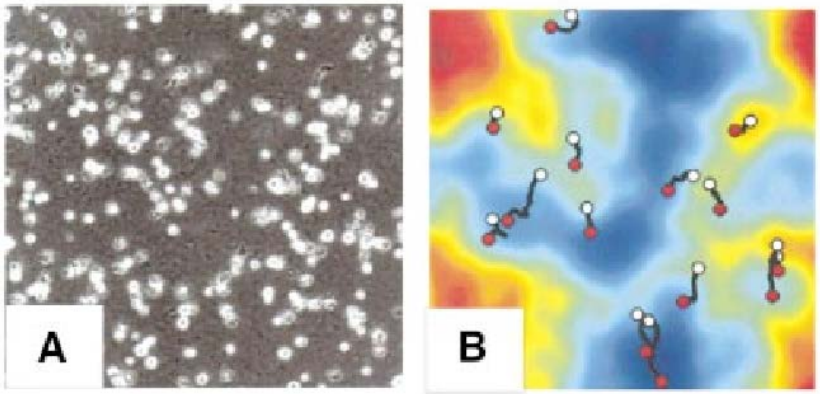
- How does blood-vessel formation function both in the presence of external patterning cues to define the precise position of the ECs, and when ECs organize into vascular patterns autonomously?
- Are there any common patterning cues?

Vascular Patterning Based on

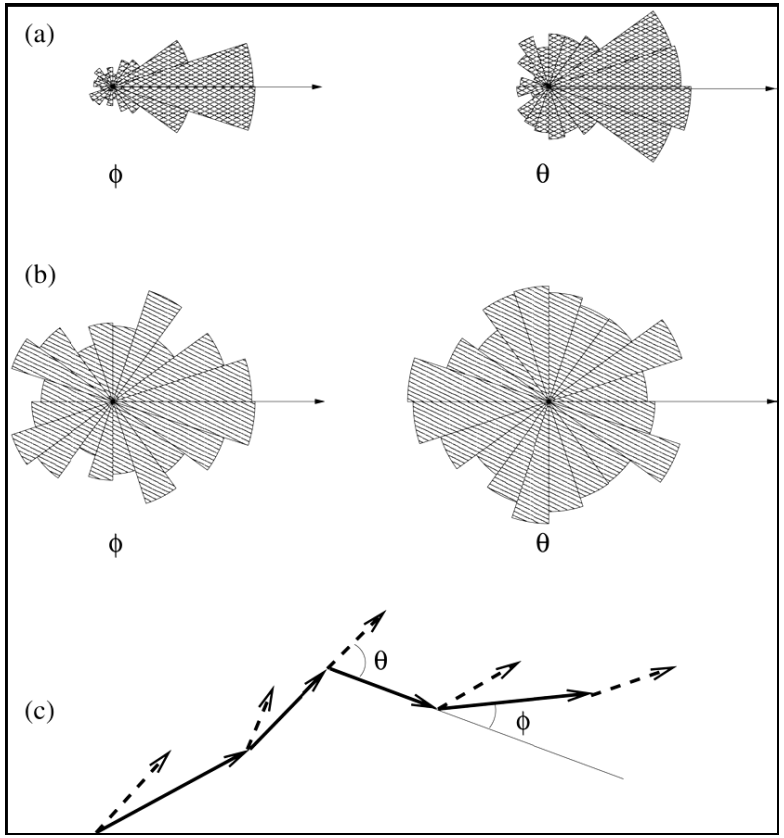
Chemotaxis Hypothesis

(Gamba *et al.* 2003; Serini *et al.*, 2003)

- ECs produce VEGF-A during first hour of vascular development
- Cells migrate to higher concentrations of cells
- Saturation of **VEGF-A gradients** inhibits directional cell migration



Red circles: starting point. White circles: arrival point.



Solid arrows represent cell displacements; dashed arrows represent chemoattractant Gradients.

Mathematical Models of Vascular Patterning

- Mechanical Models (Taxis to stress in ECM)
  - Murray, Oster, and Harris (1983)
- Chemomechanical Models
  - A. Tosin, D. Ambrosi, L. Preziosi(2006)
  - Daphne Manoussaki (2003)
- Cell-cell Mechanical model (Taxis to elongated structures)
  - Czirok A, Zamir EA, Szabo A, Little CD (2008)
- Models Based on Chemotaxis Hypothesis
  - A. Gamba, *et al*. (2003)

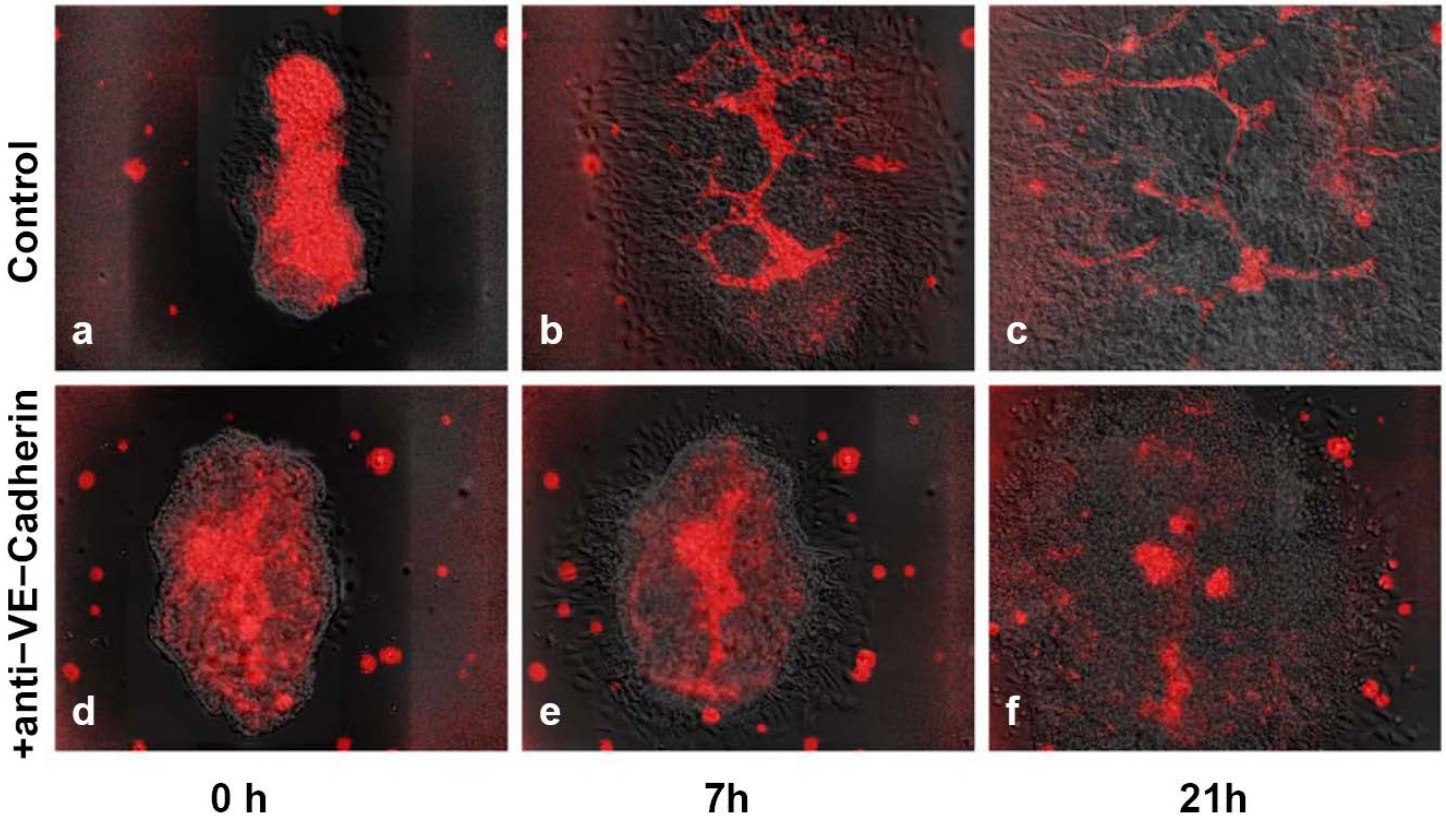
(All biological mechanisms translate to same mathematics!)

If chemotaxis to a diffusive factor guides vascular patterning:

- Can it explain aspects of both angiogenesis and vasculogenesis?
- How does vascular patterning depend on the chemoattractant properties?
- What are the properties of the chemoattractant?

Contact-Inhibited Chemotaxis

- **VE-Cadherin** (an adhesion molecule) clusters at adherens junctions between endothelial cells and **suppresses chemotaxis** at cell-cell interfaces



Anti-VE-cadherin antibody inhibits *de novo* blood-vessel growth in mouse allantois cultures. (Roeland M. H. Merks , Erica D. Perryn , Abbas Shirinifard, and James A. Glazier, *PLoS Computational Biology* 2008)

Angiogenesis Model

- Objects
  - ECs
  - VEGF Field
  - Medium
  - [Substrate]
- Behaviors
  - ECs
    - Random Motility
    - Volume
    - [Elongation]
    - Adhesivity
  - VEGF Field
    - Diffusion
    - Decay
- Interactions
  - ECs + VEGF Field
    - Secrete VEGF-A
    - Chemotax to VEGF-A
  - ECs +ECs
    - Adhere
    - [Block Chemotaxis on Adherent Surfaces]
- Dynamics
  - GGH for Cells
  - Diffusion Eqn. for Field

Vascular Development

Two Cell Types: Vascular Endothelial Cells (ECs), Medium
One Field: Vascular Endothelial Growth Factor A (VEGF-A)

$$H = \sum_{\substack{\vec{i},\vec{i}' \\ \text{neighbors}}} J\left(\tau(\sigma(\vec{i})),\tau(\sigma(\vec{i}'))\right)\left\{1-\delta\left(\sigma(\vec{i}),\sigma(\vec{i}')\right)\right\} + \sum_{\substack{\vec{i} \\ \text{restricted to Cell} \\ \text{sites next to Medium}}} \mu\left(\tau\left(\sigma\left(\vec{i}\right)\right)\right)\frac{C\left(\vec{i}\right)}{\gamma C\left(\vec{i}\right)+1}$$

$$+\sum_{\sigma}\lambda_{\text{volume}}\left(V(\sigma)-V_{\text{target}}\right)^2+\lambda_{\text{surface}}(\sigma)\left(S(\sigma)-S_{\text{target}}(\sigma)\right)^2$$

Surface tension Between Cells set to 0 (No Adhesion).

Cells are floppy.

Cells secrete and chemotax (with Contact Inhibition) to a diffusible chemical field, which decays in the external environment (autocrine signaling)

$$\frac{\partial C\left(\vec{i}\right)}{\partial t} = D_c\nabla^2 C\left(\vec{i}\right)-\gamma_c C\left(\vec{i}\right)+S_c\delta\left(\tau\left(\sigma\left(\vec{i}\right),EC\right)\right)$$

Random blob Initial Conditions or

Random Separated ECs

Vascular Development

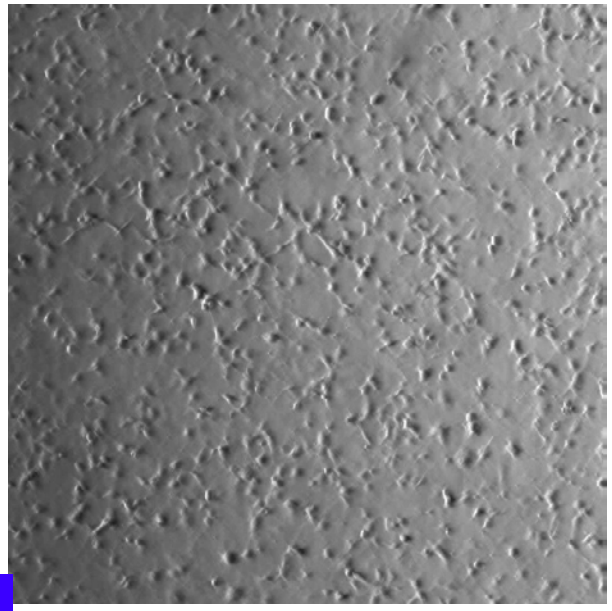
Biological System

- Umbilical Vein Endothelial Cells (HUVECs) on Matrigel

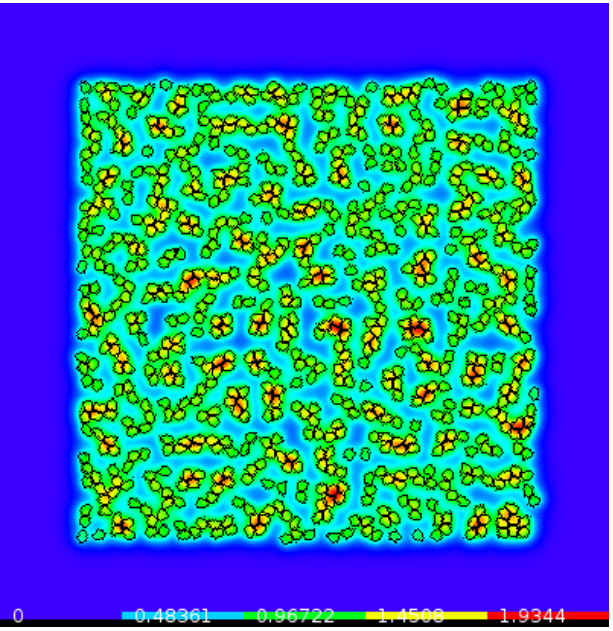
What Mechanisms give rise to these patterns?

Result: Very Short-Range Chemotaxis + Contact Inhibition can explain both angiogenesis and vasculogenesis.

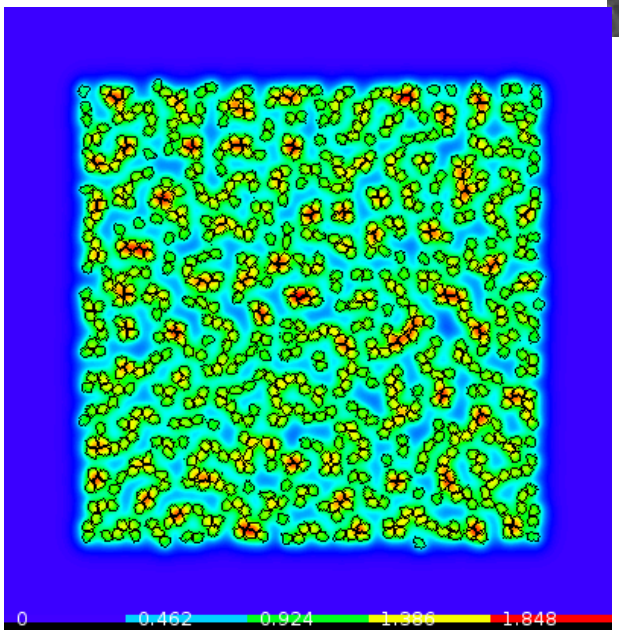
- Works in 2D and 3D.



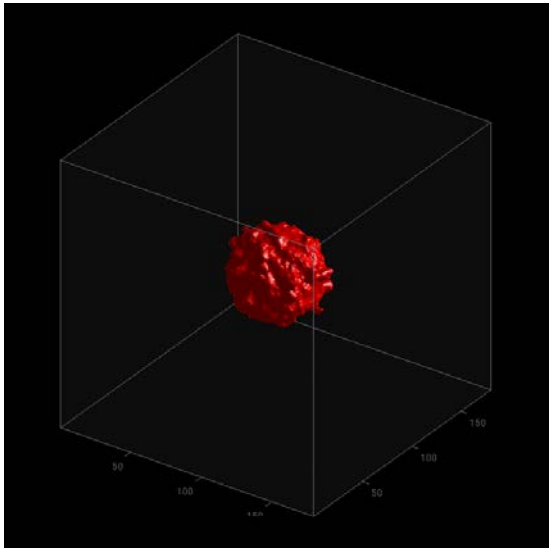
Movie 1: D. Ambrosi et al., Phys. Rev. Letters **90**, 118101



No Contact Inhibition



Contact Inhibition



Contact Inhibition in 3D

Results

- Same model reproduces both angiogenesis and vasculogenesis
- Diffusive patterning cue needs to be short-range
  - How short? One or two cell diameter!
    - VEGF-A_{165} diffuses too fast → long-range
    - VEGF-A_{189} binds to ECM and diffuses slower (ECM-bound signals also work)
- Contact Inhibition is essential
- Discrete cells are essential

Cancer as an Emergent Developmental Disease

- A disease of cell behaviors in which cells reorganize their environment and respond to that reorganization.
- As a result, study of genomics/proteomics of cancer is only relevant if the behaviors have very strong correlations with specific genes/proteins (rather rare).
- Excessive focus on mechanisms of generation of variation and insufficient attention to mechanisms of selection.

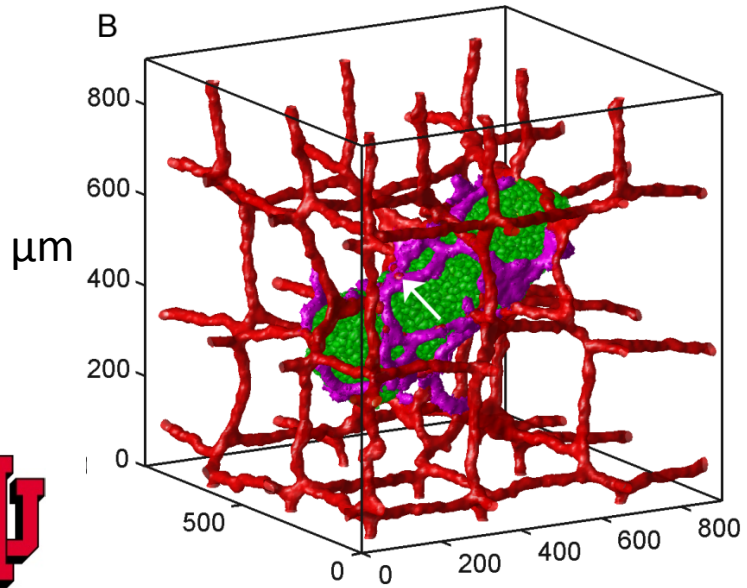
Questions Concerning Neovascular Interactions with Tumors

- **What happens to vascular patterns if ECs proliferate in response to tissue-derived angiogenic factors?**
- **How does neovasculature interact with poorly structured tissues like tumor?**
- How does neovasculature invade structured tissues like epithelium?
- What factors affect the invasion? *E.g.* cellular adhesion

3D Vascular Tumor Growth

Tumor cells	Cell behaviors
Normal	-proliferate -consume oxygen -change to hypoxic -change to necrotic
Hypoxic	-proliferate -consume oxygen field -change to normal -change to necrotic -secrete long-diffusing proangiogenic field
Necrotic	-shrink -disappear

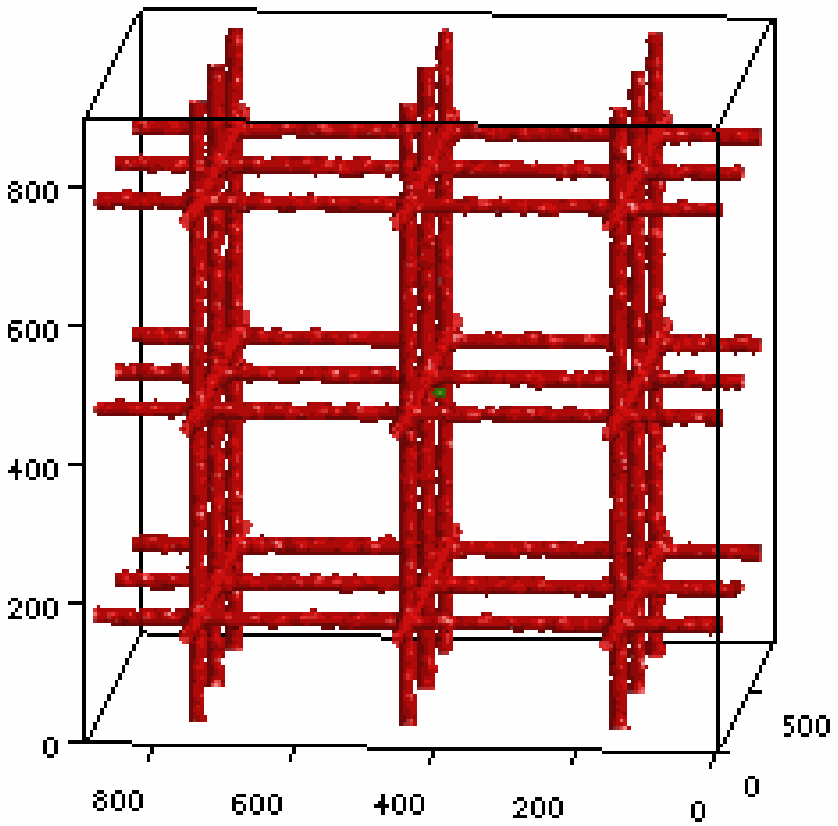
Endothelial cells	Cell behaviors
Normal	-consume oxygen field -supply oxygen field -secrete short-diffusing chemoattractant field -chemotax to short-diffusing chemoattractant -**elastically connect** to neighboring vascular and inactive neovascular cells -**lose elastic connections**
Active neovascular	-consume oxygen field -supply oxygen field -secrete short-diffusing chemoattractant field -chemotax to both short-diffusing chemoattractant and long-diffusing proangiogenic field -**proliferate**

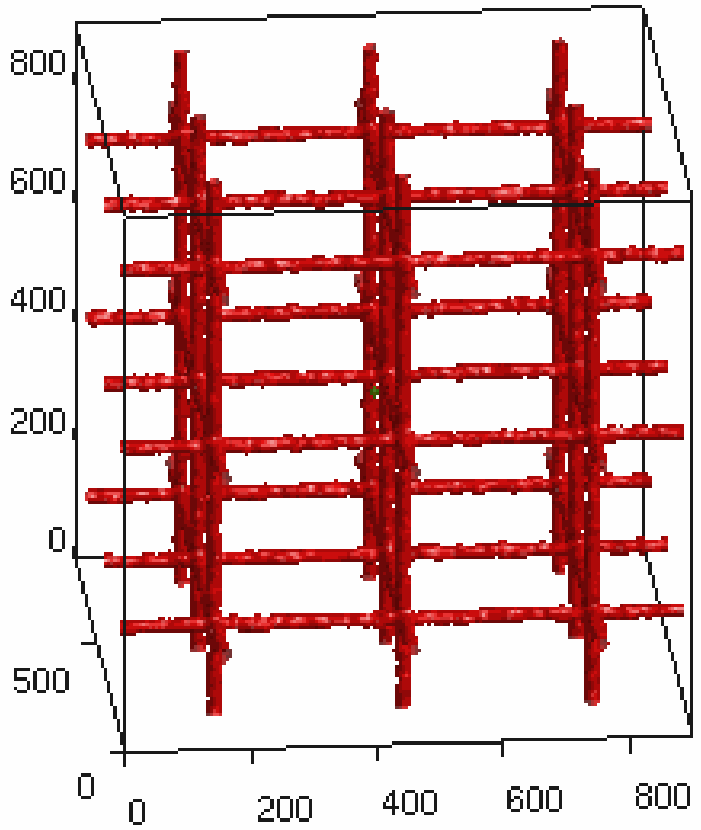


Abbas Shirinifard, J. Gens , Benjamin Zaitlen , Nikodem Poplawski , Maciej Swat , James Glazier, Sep 7, PLoSOne

Simulated Neoangiogenesis Effects on 3D Vascular Tumor Growth (75 days)

Axes are in µm





- Proliferative
- Hypoxic
- Necrotic
- Preexisting Capillaries
- Tumor-Induced Capillaries
- White—Stromal Tissue

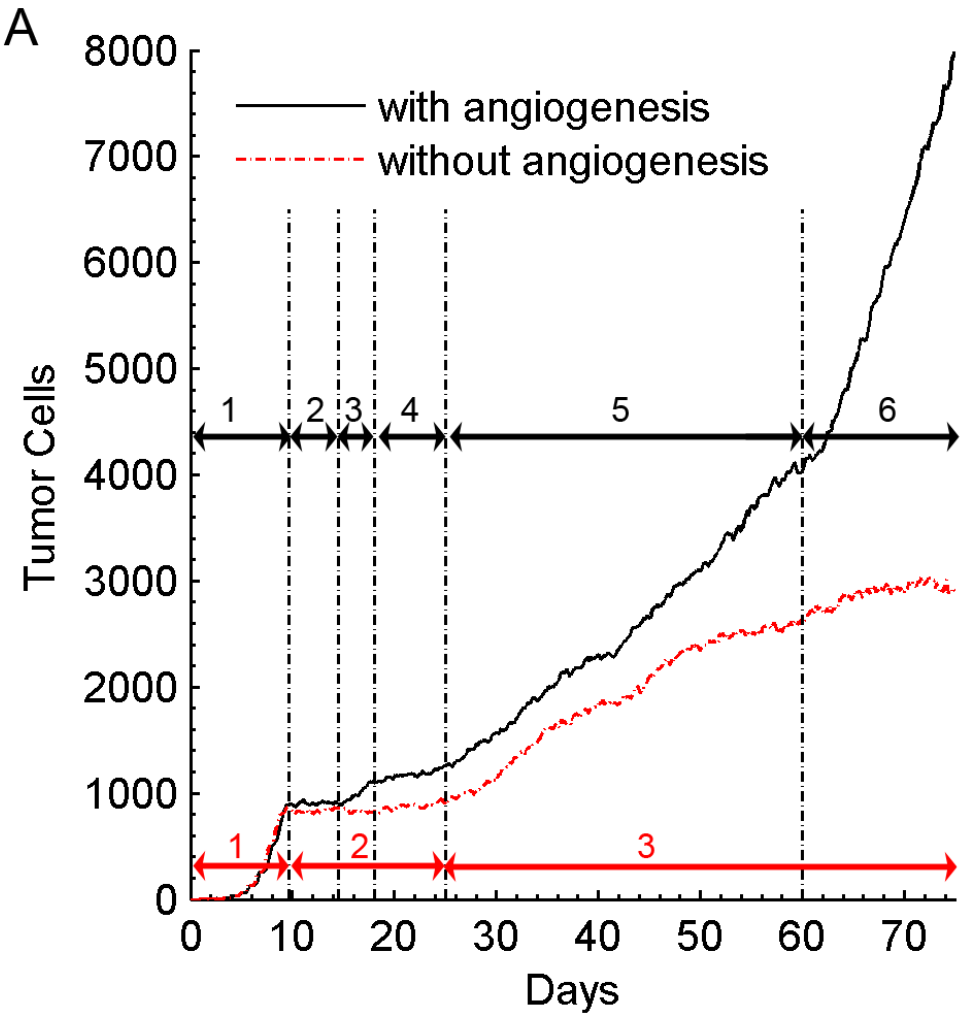
Simulated Neoangiogenesis Effects on 3D Vascular Tumor Growth

With Angiogenesis

1. exponential growth phase
2. no growth
3. linear-spherical phase
4. slow growth
5. linear-cylindrical phase
6. linear-sheet phase

Without Angiogenesis

1. exponential growth phase
2. slow growth
3. cylindrical phase



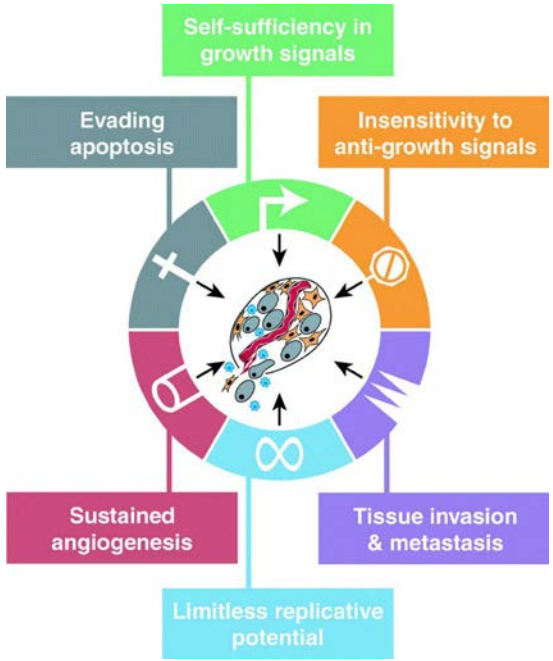
Abbas Shirinifard, J. Gens , Benjamin Zaitlen , Nikodem Poplawski , Maciej Swat , James Glazier, Sep 7, PLoSOne

3D Vascular Tumor Growth

- **Summary**
  - Tumors that induce angiogenesis grow in distinct phases
  - Avascular tumor shows more invasive morphologies (glioblastoma) due to capillary-scale nutrient inhomogeneities
  - Simulation provides an environment for studying capillary-tissue interactions

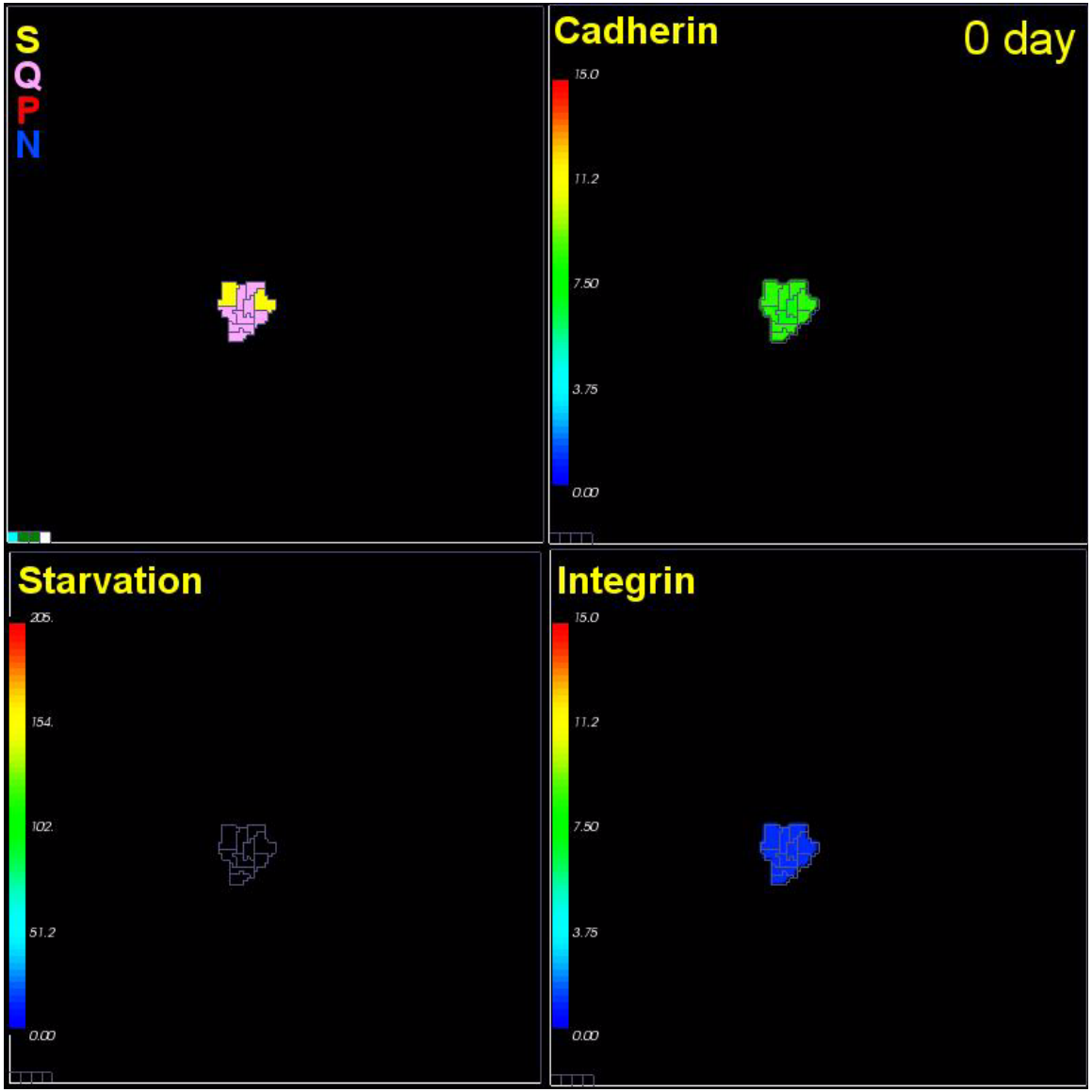
Solid Tumor Progression: Adhesion and Nutrient Heterogeneity

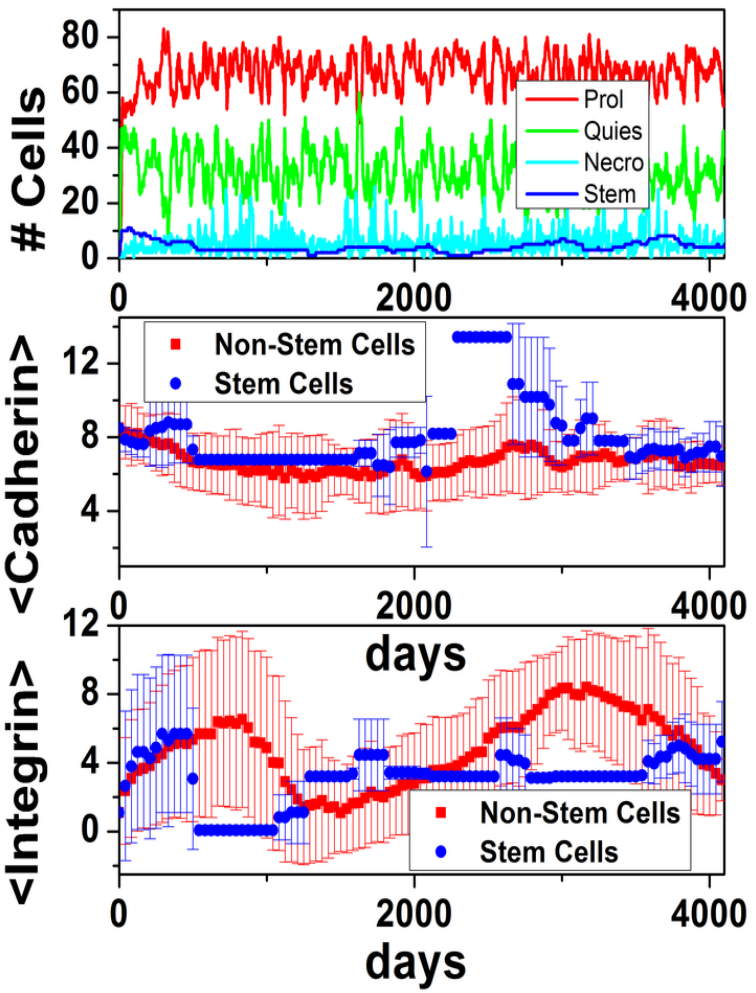
- **Paradox:**
  - **Mutation is undirected and acts on all cell behaviors simultaneously.**
  - **Why is there the appearance of directional quasi-deterministic progression?**
- **Suggested Answer:**
  - **The emergent environment of the tumor leads sequentially to selection favoring mutations of different types and in specific directions**



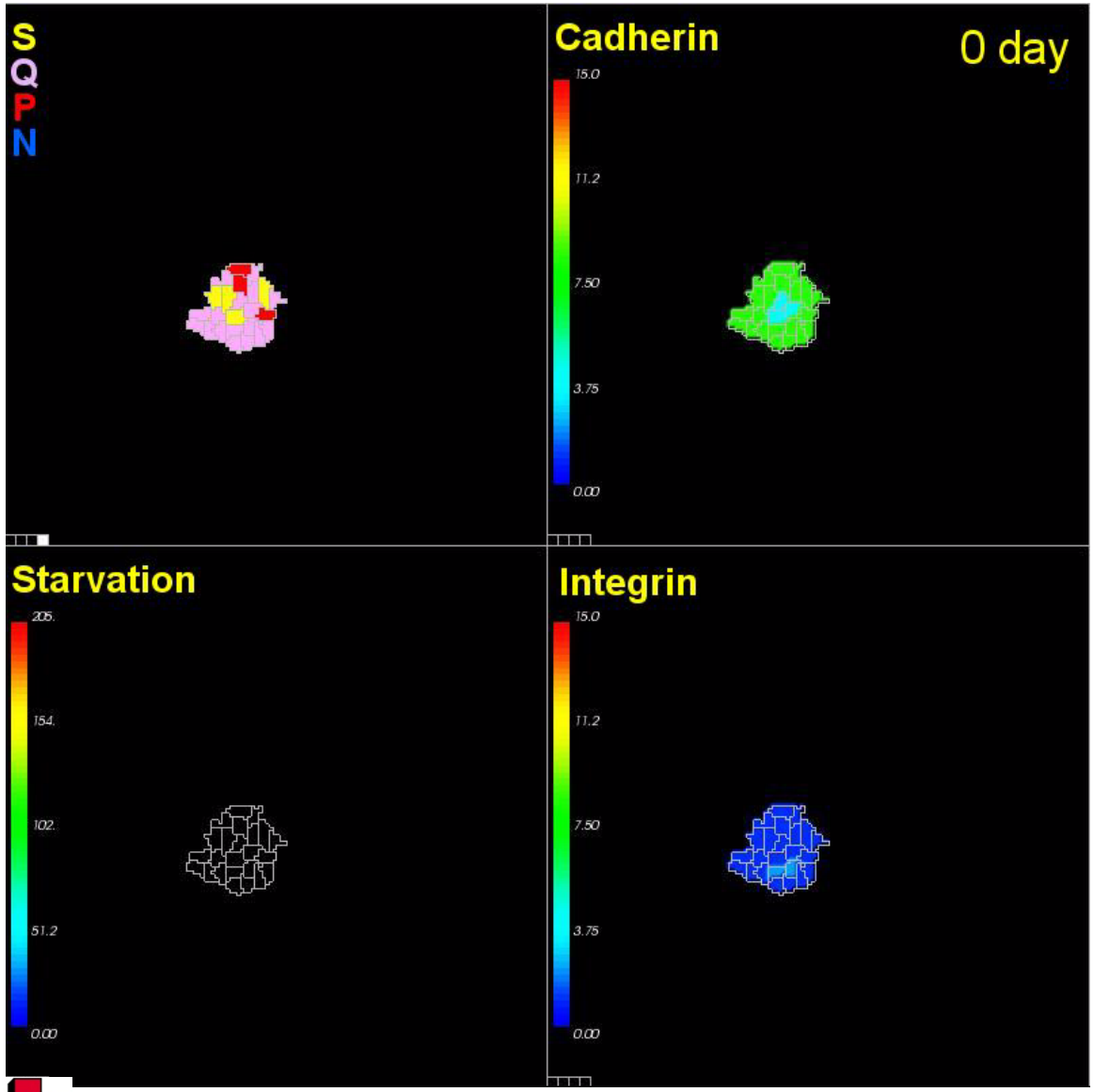
Hanahan and Weinberg, "The Hallmarks of Cancer" *Cell* **100**, 57 (2000).

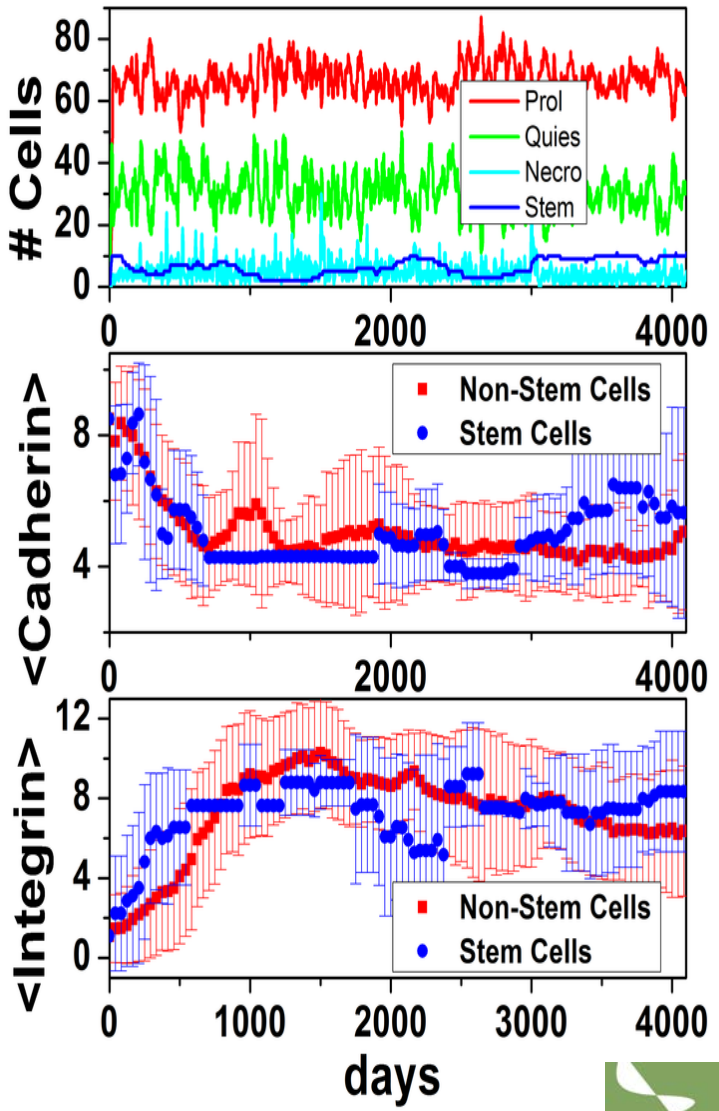
Nutrient Gradient and Strong Immune





Nutrient Gradient and Weak Immune





Nutrient Gradients and Immune Effects ⇒ Adhesion Changes ⇒ Metastasis

	Cohesiveness	Integrins	Morphology	Remission	Spread	Classification
Strong Immune	++	+	Compact	+	-	Benign
Weak Immune	--	+	Compact + Metastases	-	+	Metastatic

Summary

- Multicell models can connect heterogeneous molecular and cell-level data to predict significant tissue and organ level outcomes.
- Natural framework for studying developmental processes and failures—angiogenesis disruption, gastrulation, limb growth, liver regrowth and disfunction, polycystic kidney disease…
- Models are phenomenological.
- Models can omit key mechanisms.
- Models can only show sufficiency, not necessity.