Introduction to Virtual Tissue Modeling of Development and Developmental Diseases Using Compucell3D

Part I

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10th User Training Workshop: Developing Multi-Scale, Virtual Tissue Simulations with CompuCell3D and Tellurium
Hamner Institute, Research Triangle Park, North Carolina
Monday, August 11, 2014

IU Bloomington: [Dr. Dragos Amarie], Dr. Julio Belmonte, Dr. Sherry Clendenon, [Alin Comanescu], [Clayton Davis], Dr. Rita de Almeida, Xiao Fu, [Aaron Dy], [Garth Gast], Dr. J. Scott Gens, [Randy Heiland], [Dr. Susan Hester], [Dr. Mitja Hmeljak], [Dr. Srividhya Jayaraman], [Dr. Roeland Merks], [Dr. Nikodem Poplawski], [Ryan Roper], [Dr. Abbas Shirinifard], Dr. James Sluka, Andre Somogyi, Dr. Maciej Swat, [Dr. Gilberto Thomas], [Ruei Wu], [Benjamin Zaitlen], [Dr. Ying Zhang]. Indiana University, School of Medicine: Dr. Robert Bacallao. University of Houston: Dr. Maria Bondesson, Dr. Jan-Ake Gustafsson, Dr. Catharine McCollum. EPA: Dr. Thomas Knudsen, Dr. Imran Shah, Dr. Nicole Kleinstreuer. University of Michigan: Dr. Santiago Schnell. KUMC: Dr. Charles Little. University College London: Ana S. Dias, Irene de Almeida, Dr. Claudio Stern, University of Dundee: Dr. Mark Chaplain. Moffitt Cancer Center: Dr. Heiko Enderling. CRG Barcelona: Dr. James Sharpe. Cambridge University: Dr. Octavian Voicelescu.

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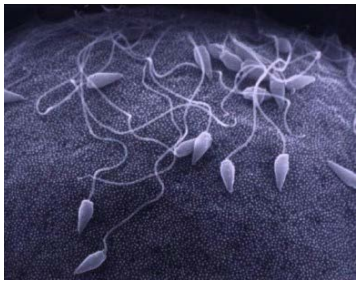
To download software for model building, please visit http://www.compucell3d.org

Key Biological Questions

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



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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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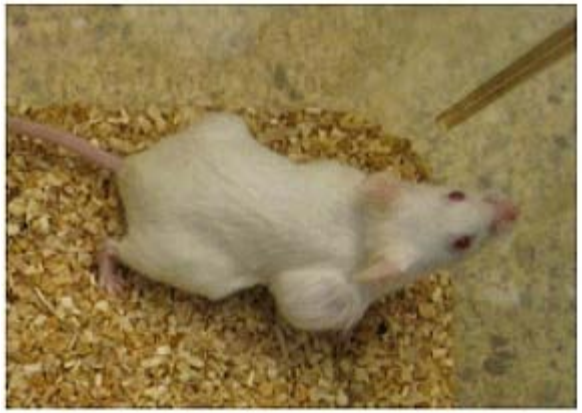


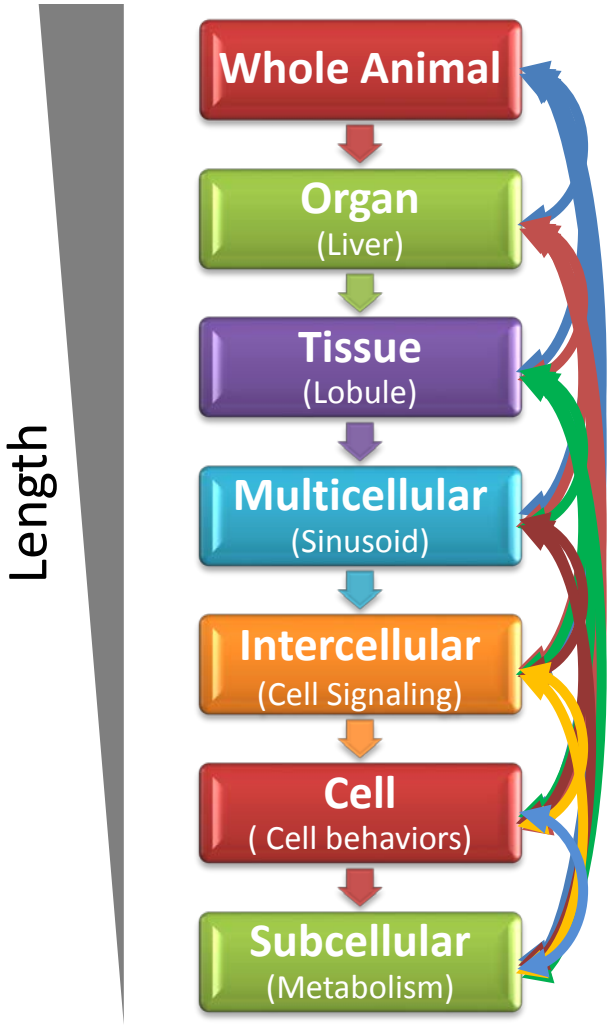
e.g., liver cirrhosis, cancer, diabetic retinopathy, polycystic kidney disease, osteoporosis,..

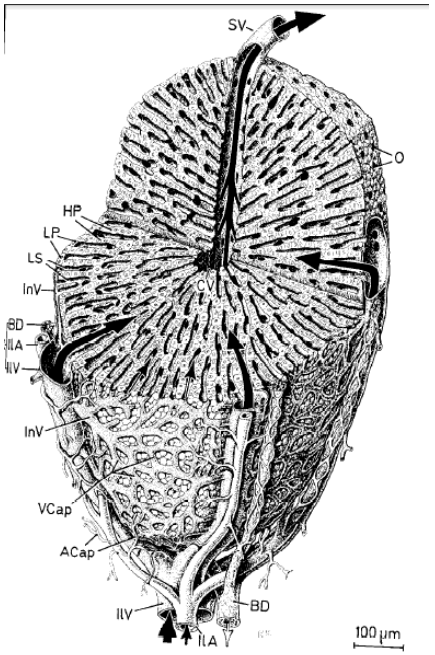
Promise of Mechanistic Understanding

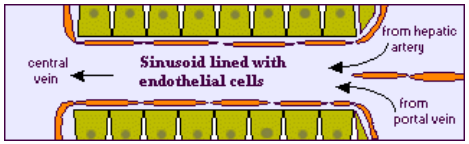
- Fundamental understanding and control of developmental mechanisms, leading to:
  - Improved treatment regimes for cancer (ranging from more accurate surgery to more effective and less toxic therapies).
  - Control of stem and other human-derived cells for engineering of tissue replacements both *in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

Biology occurs across multiple spatial and temporal scales

- Distance scales range from sub-nanometer to meters.
- Time scales range from seconds to decades.

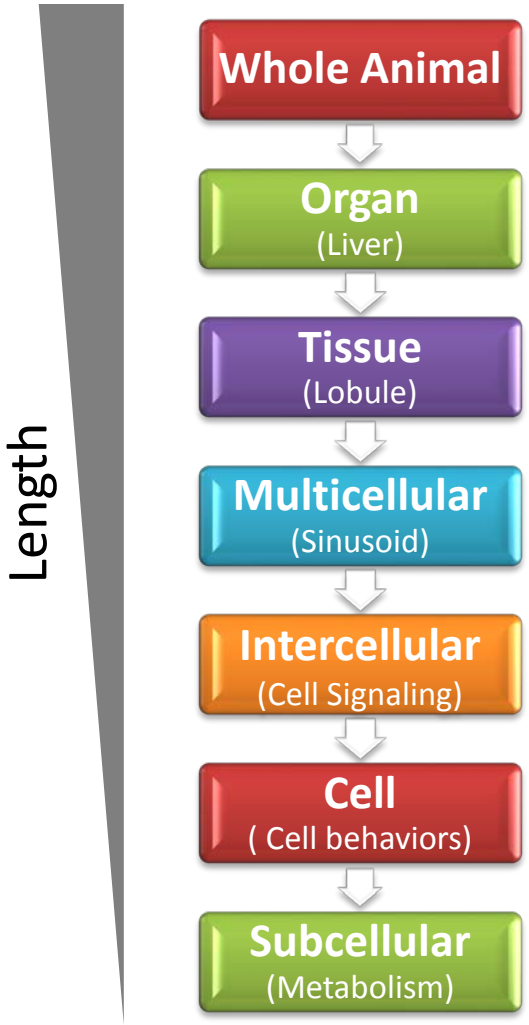






1. Human Microscopic Anatomy: An Atlas for Students of Medicine and Biology, R. V. Krstic, Springer-Verlag, 1991 (ISBN 978-3-540-53666-6).
2. http://biology.about.com/library/organs/bldigestliver.htm

Different Methods Apply to Different Scales



PBPK (Coupled ODE)

FE (Continuum Mechanics, PDE)

FE (Continuum Mechanics, PDE)

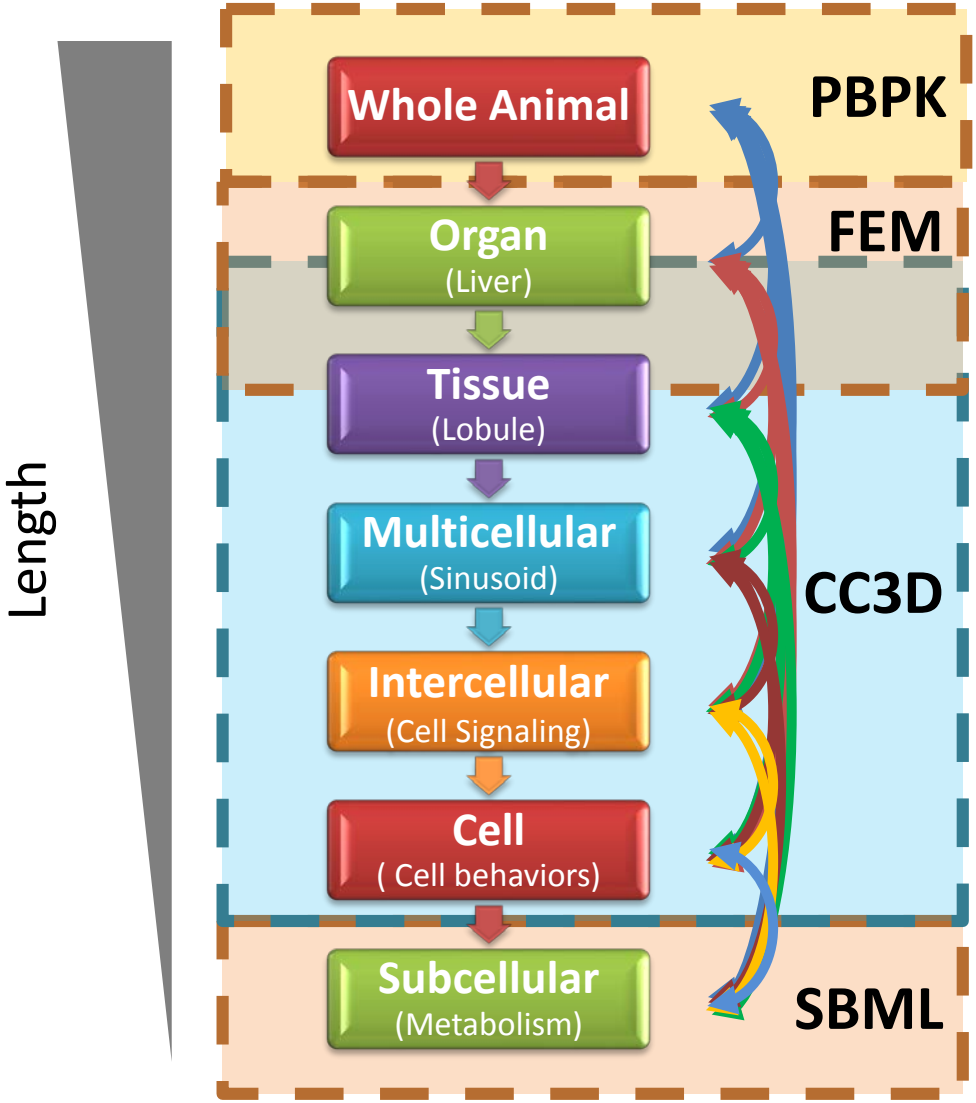
Agent-Based (GGH, Center, Vertex…)

PDE + Coupled ODE

Continuum Mechanics, PDE, Coarse-Grained Molecular Dynamics)

RK (Coupled ODE or Stochastic)

Virtual Tissues Integrate Across Scales



Where Do We Start?

Can’t model everything in detail

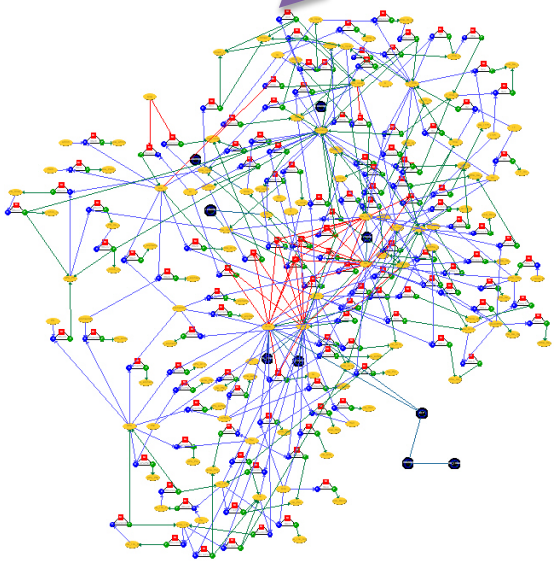
Multicell Virtual Tissue Models

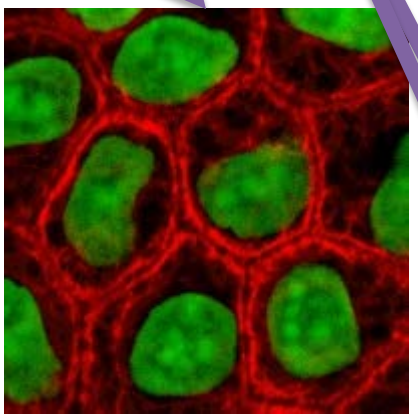
- **Separate Analysis into Three Components**

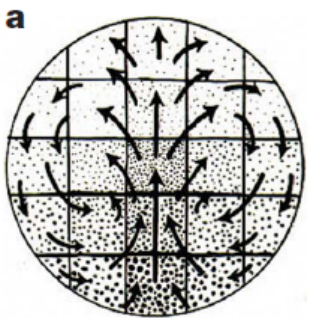
  From molecular event to molecular network behavior

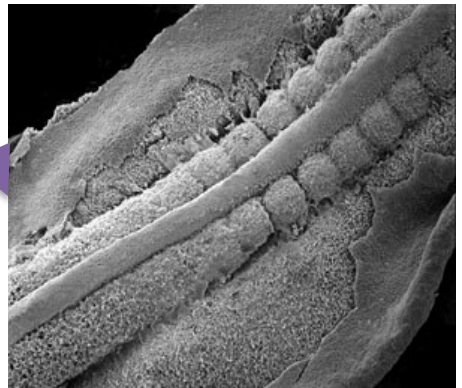
  From network behavior to cell behaviors

  From cell behavior to tissue behaviors





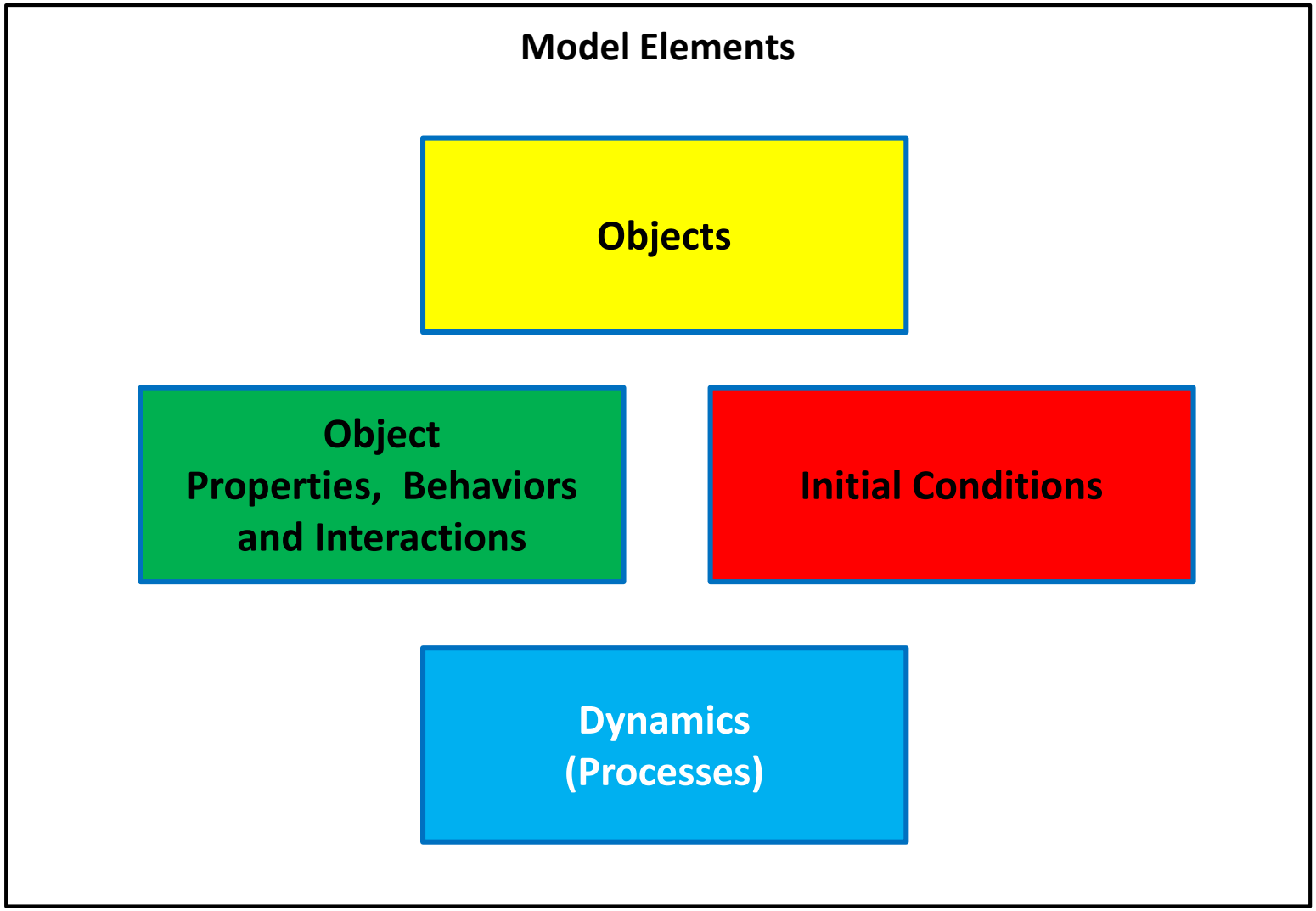




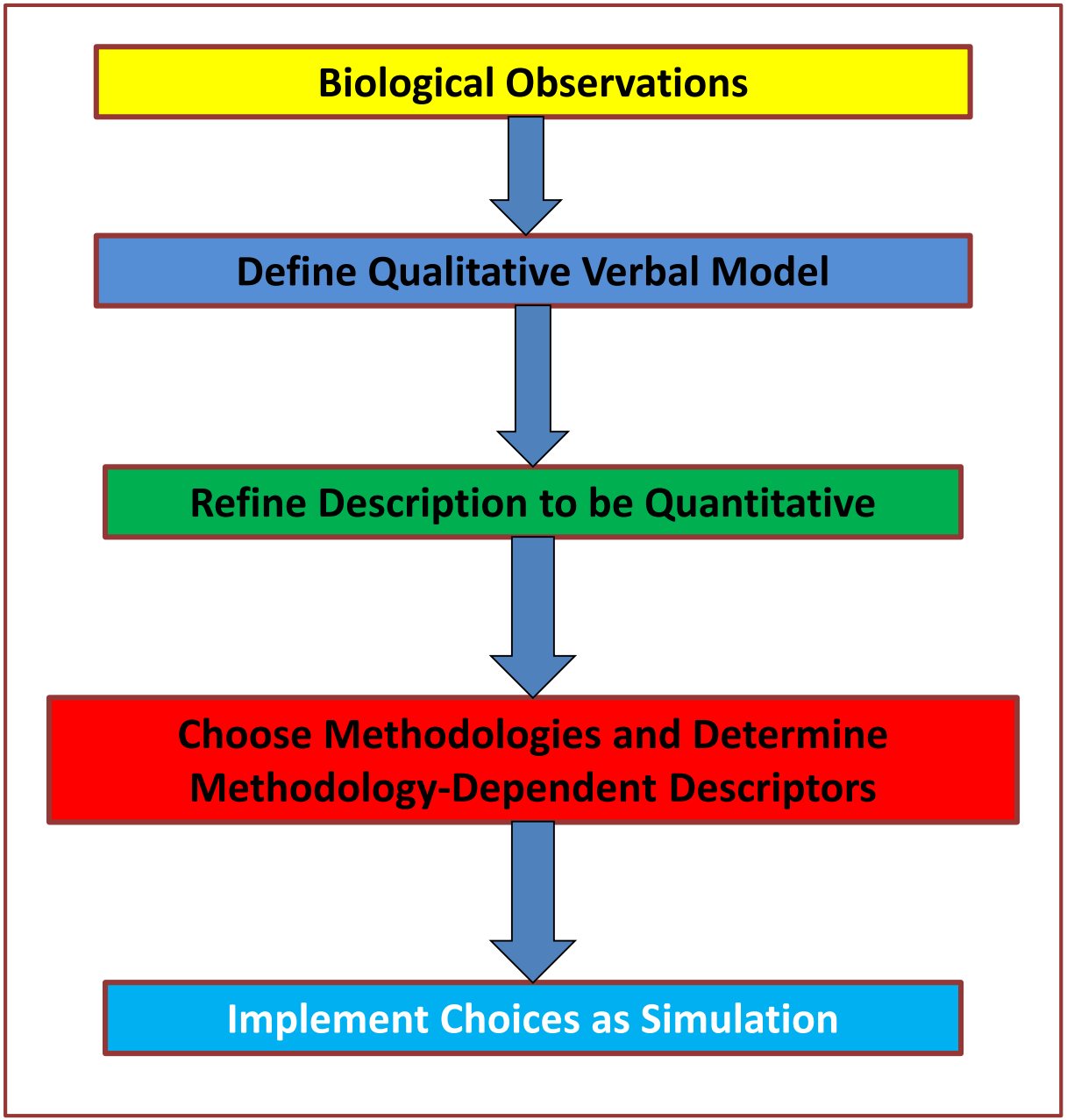
How to Start Building a VT Model?

- What are the specific questions you are trying to answer (hypotheses you are trying to test)?
- **Models can only show sufficiency, not necessity!**
- What are the scales you need to include in your model to test these hypotheses?
- What do you see as the most important components (Objects) and Mechanisms in the biology you are trying to understand?
- **A model cannot predict the importance or role of a Mechanism not included in the Model!**
- Do you have enough information to describe the Objects and Mechanisms at the scale you have chosen?
- **You cannot include a Mechanism unless you can describe it quantitatively!**
- How will you compare simulation results with experiment to test your model?
- VT models are useful for understanding emergent properties of tissues, when the behaviors of their components are relatively well understood.

Model Components



Building a Model



Sample Questions in Model Definition

- What Structural components (Objects) are important?
- Should Objects be represented individually or as continua?
- What type of Cells are important?
- What is the Chemical and Mechanical Environment around the Cells (ECM)?
- Are the Cells polar or not?
- Do Cells grow and divide?
- Do Cells differentiate or die?
- What Signaling Mechanisms are active within Cells? Between Cells?
- How strongly do Cells of one type adhere to Cells of another type?
- How strongly do Cells of a given type adhere to ECM?
- Is Cell adhesion labile (*e.g*. single molecule pair) or junctional?
- What chemicals do cells secrete and absorb?
- If Chemical Fields diffuse, how rapidly do they diffuse?
- If Chemical Fields do not diffuse, what are their mechanical properties?
- How stable are Chemical Fields (what are their decay rates)?
- What are the mechanical properties of the ECM?
- How do Cells interact mechanically (move in response to and remodel) with ECM?

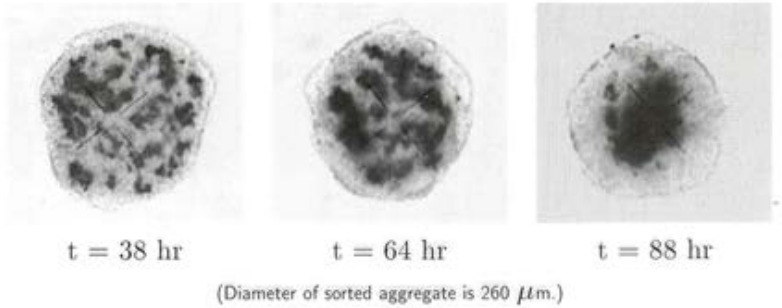
Be Aware of Feedback

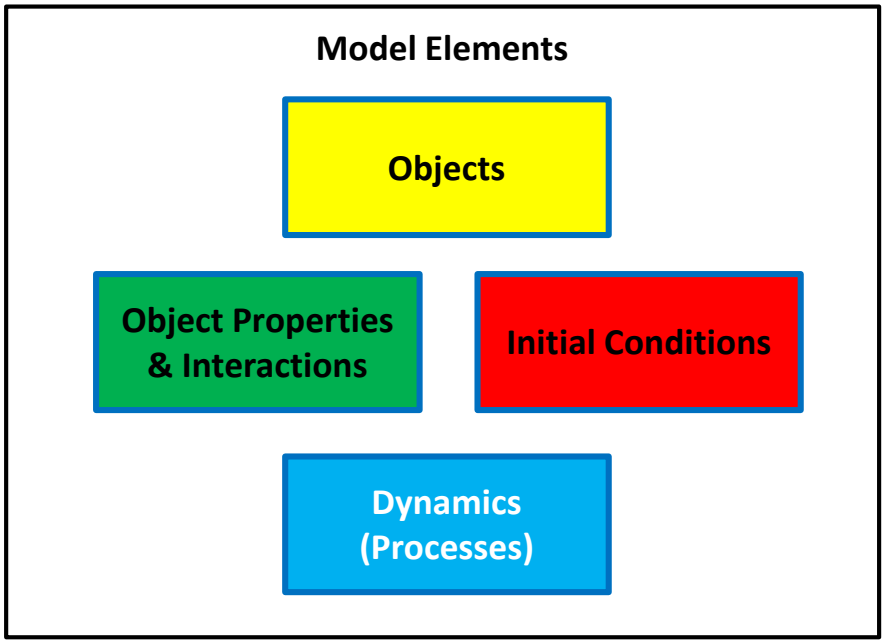
- Not Simply: Signal→Differentiation→Pattern (Known as Prepatterning)
- Cells Create Their Own Environment, by Moving and Secreting New Signals, so Signaling Feeds Back on Itself

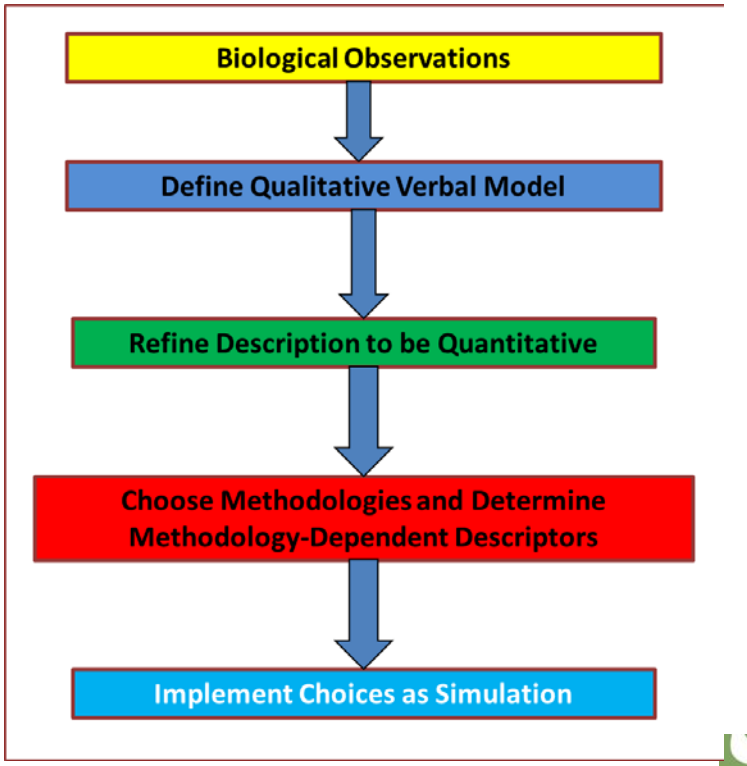
Cell Sorting—The Simplest Model

Simulate the evolution of a randomly mixed aggregate of two mesenchymal cell types due to Differential Adhesion and random cell motility.

Question—how does the outcome depend on the relative adhesion energies between the cell types and between the cells and medium?







Cell Sorting—The Simplest Model
Biological Observations

- Objects: Dark Cells, Light Cells, Medium
- Properties, Behaviors:
  - Cells do not grow, shrink, divide or die
  - No Medium added or removed
  - Cells appear isotopic or unpolarized (Mesenchymal)
  - All Dark Cells seem the same; All Light Cells seem the same
- Interactions:
  - Cells stick to each other, but can rearrange
  - Adhesion greater between 'dark' cells and other dark cells, less between light and other light cells
  - Cells seem to repel medium
- Dynamics:
  - Cells undergo random amoeboid movements
- Initial Condition:
  - A randomly mixed sphere of Cells surrounded by Medium

Cell Sorting—The Simplest Model Define Qualitative Verbal Model

- Objects: Light Cells, Dark Cells, Medium (Generalized Cell)
- Properties, Behaviors:
  - Cells have Fixed Volumes and Fixed Membrane Areas
  - Medium has Unconstrained Volume and Surface Area
  - Cells are Adhesive
  - Cells have Intrinsic Random Motility
- Interactions:
  - Cells Adhere to each other and to Medium with an Energy/Area which Depends on Cell Type (simulating different types or densities of cadherins on each Cell Type)
- Dynamics:
  - Random Cell Motility
- Initial Conditions:
  - Cells in a Blob Surrounded by Medium
  - In Blob, Cells Randomly Mixed

Cell Sorting—The Simplest Model
Refine Description to be Quantitative

Three Cell Types: More Cohesive, Less Cohesive, Medium

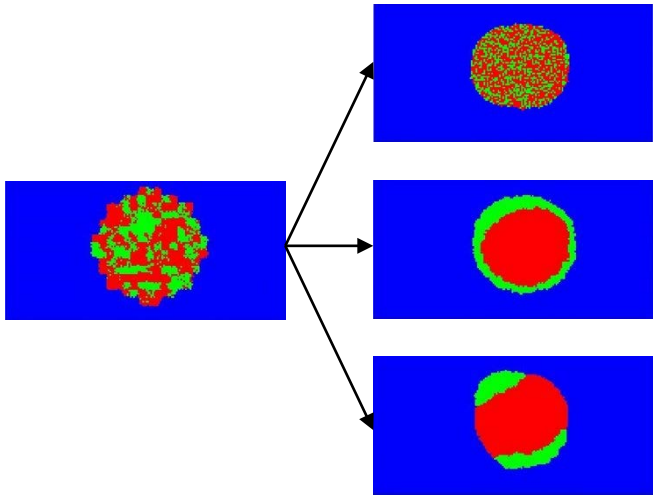
$$E = \sum_{\substack{\vec{i},\vec{i}' \\ \text{neighbors}}} J\left(\tau(\sigma(\vec{i})),\tau(\sigma(\vec{i}'))\right)\left\{1-\delta\left(\sigma(\vec{i}),\sigma(\vec{i}')\right)\right\}+\sum_{\sigma}\lambda_{\text{volume}}\left(V(\sigma)-V_{\text{target}}\right)^2$$

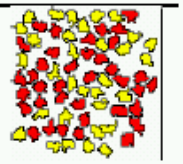
Cell Boundaries Exhibit Thermal Fluctuations (Metropolis Dynamics)

Random Blob Initial Conditions or Adjacent Domains

Cell Sorting—The Simplest Model Simulate in CC3D

Outcome Depends on *J*s

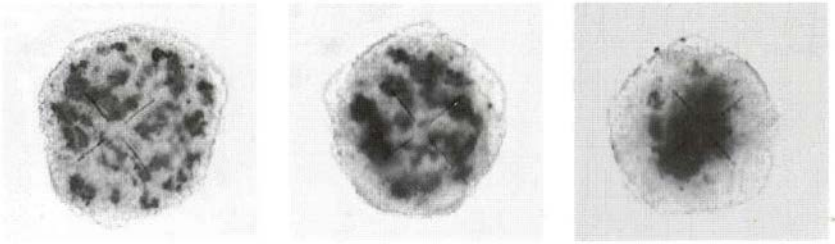


Initial configuration	
Cell Sorting $J_{white,white} = J_{grey,grey} < J_{white,grey}$	
Cell Mixing $J_{white,white} = J_{grey,grey} > J_{white,grey}$	
Engulfment $J_{white,grey} < J_{white,medium}$ $J_{grey,medium} < J_{white,medium}$	
No cell cell adhesion $J_{cell,cell} > 2J_{cell,medium}$	

Cell Sorting—The Simplest Model Compare Experiment and Simulation

Growth law in tissues

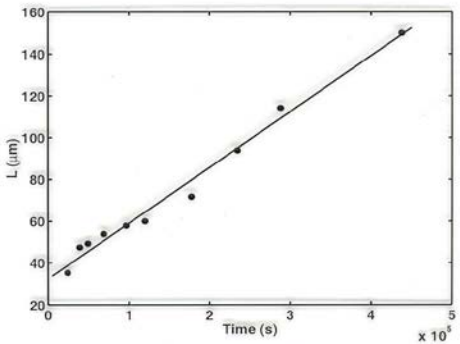
Growth of pigmented clusters in neural retinal tissue



t = 38 hr t = 64 hr t = 88 hr

(Diameter of sorted aggregate is 260 μm.)

For large volume fractions $\rightarrow$ $L \sim (\sigma/\eta)t$



Slope = 2.7 x 10^{-4} μm s^{-1}

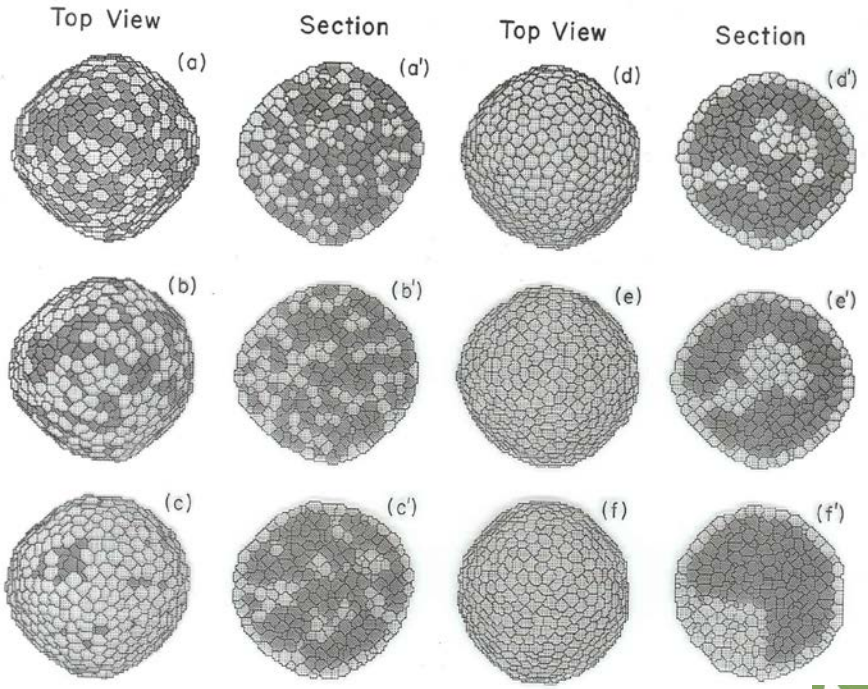


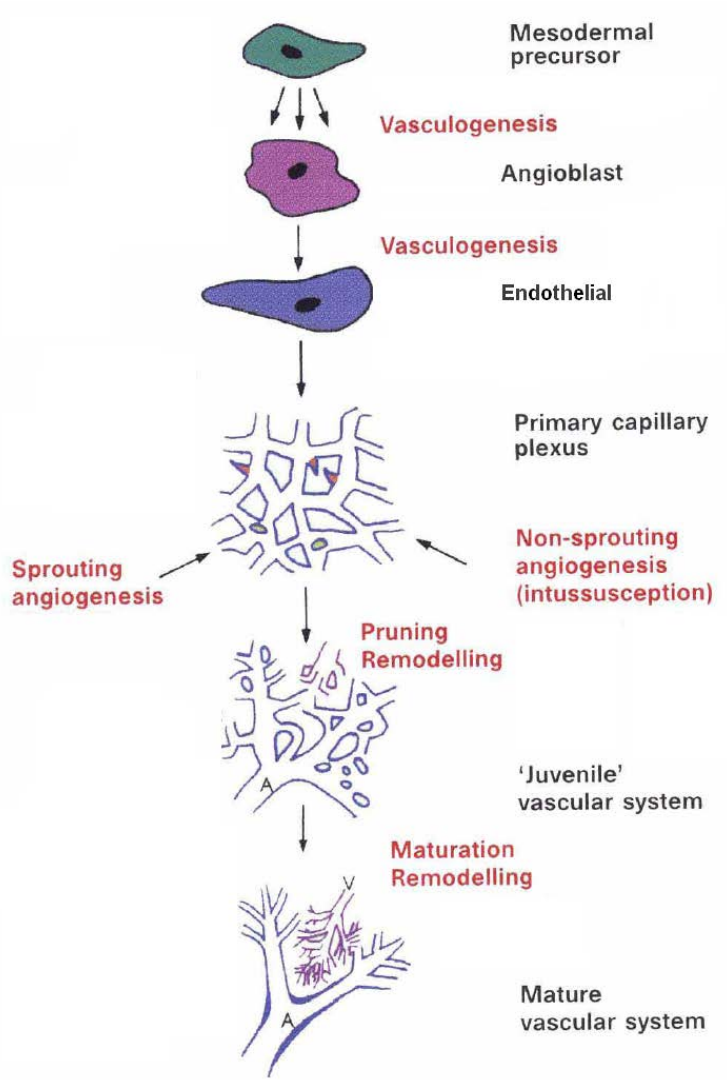
Figure 2.

Sample Current Applications

- **Angiogenesis and Vasculogenesis (EPA, CWI)**
- Vascular Tumor Growth
- Cancer Evolution
- Age Related Macular Degeneration
- Liver Toxicology (IUB, EPA)
- Polycystic Kidney Disease (IUB, IUPUI)
- Segmentation (IUB, UCL)

Simulating Vasculogenesis and Angiogenesis

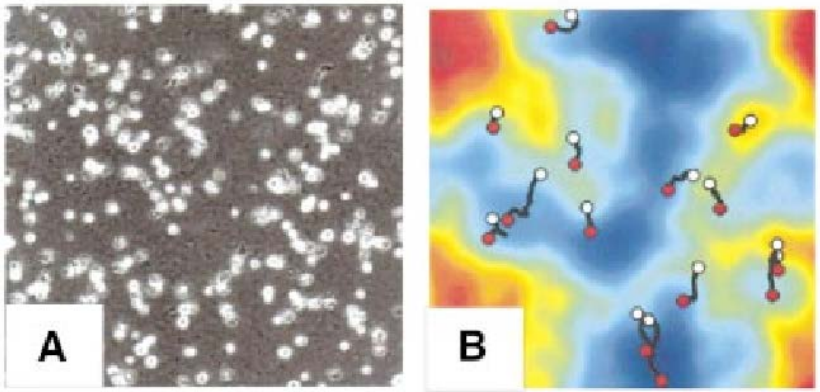
- Vasculogenesis
  - The formation of early vascular plexus from in situ differentiated **Endothelial Cells** (***ECs***)
- Angiogenesis
  - The formation of new blood vessels from pre-existing ones
    - Sprouting Angiogenesis
    - Non-sprouting Angiogenesis (**Intussusceptive angiogenesis**)
- Are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



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

Vasculogenesis via Chemotaxis

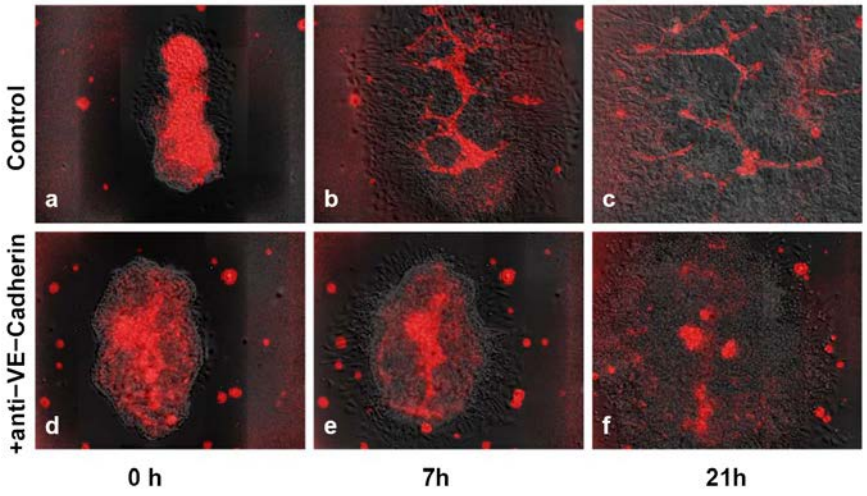
- Cells migrate to higher concentrations of cells
- Saturation of **VEGF-A gradients** inhibits directional cell migration
- ECs produce VEGF-A during first hour of vascular development



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

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

- **Contact Inhibition of Chemotaxis:**
- **VE-Cadherin** 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. (R. M. H. Merks , E.D. Perryn , A. Shirinifard, and J. A. Glazier, *PLoS Computational Biology* 2008)

Vascular Development/GGH Version

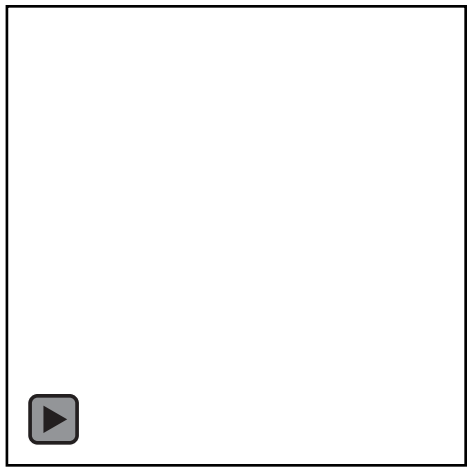
Biological System

- Umbilical Vein Endothelial Cells (HUVECs) on Matrigel

What Mechanisms give rise to these patterns?

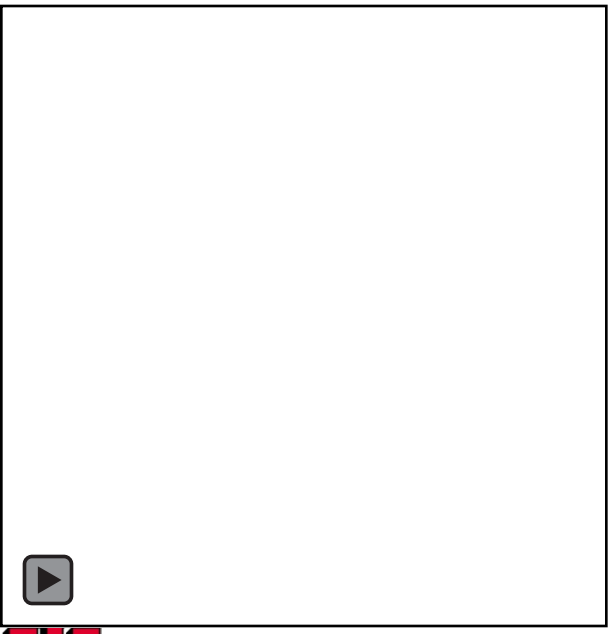
Result: Very Short-Range Chemotaxis + Contact Inhibition can explain both angiogenesis and vasculogenesis.

- Works in 2D and 3D.

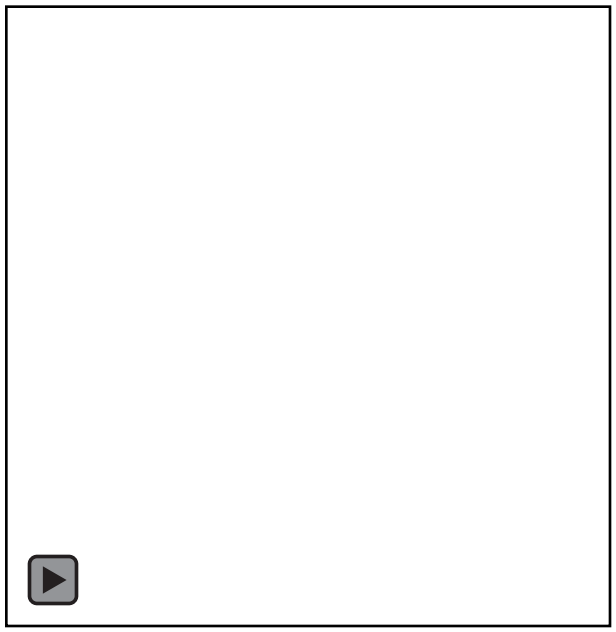


Movie 1: D. Ambrosi et al., Phys. Rev. Letters **90**, 118101

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



Contact Inhibition



Contact Inhibition

Key Physics:

Cell Diffusion

VEGF-A Diffusion

Chemical Potential Response of Cells

(Constant Pressure –Liquid Like vs Variable Pressue)

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

Sample Current Applications

- Angiogenesis and Vasculogenesis (EPA, CWI)
- **Vascular Tumor Growth**
- Cancer Evolution
- Age Related Macular Degeneration
- Liver Toxicology (IUB, EPA)
- Polycystic Kidney Disease (IUB, IUPUI)
- Segmentation (IUB, UCL)

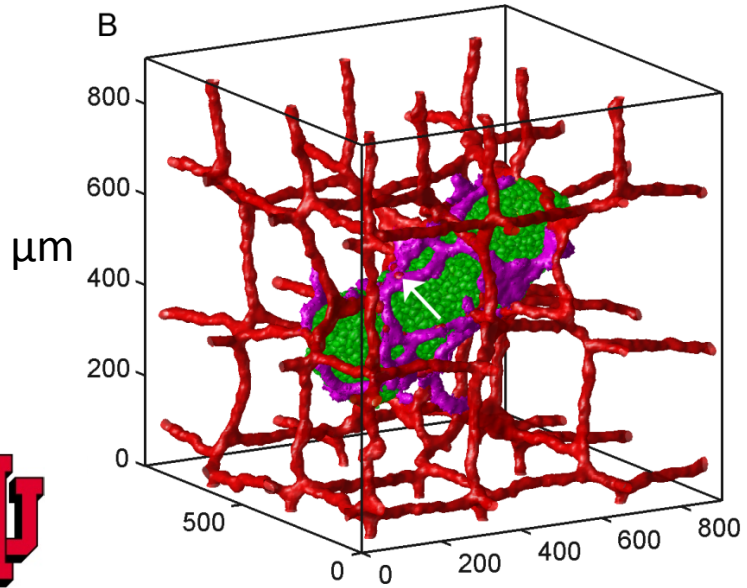
Questions Concerning Neovascular Interactions with Tumors

- **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**



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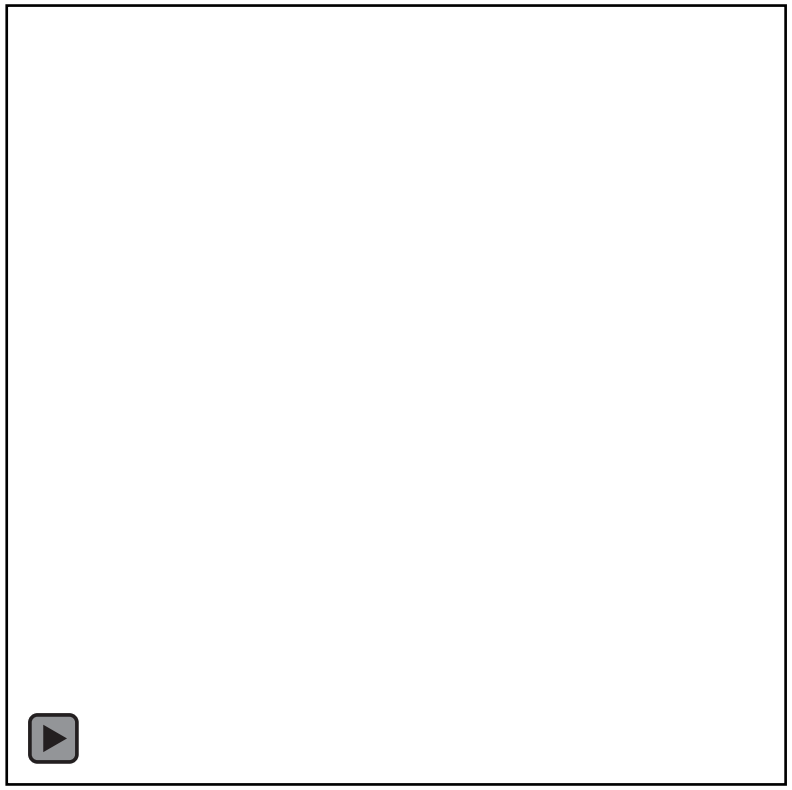
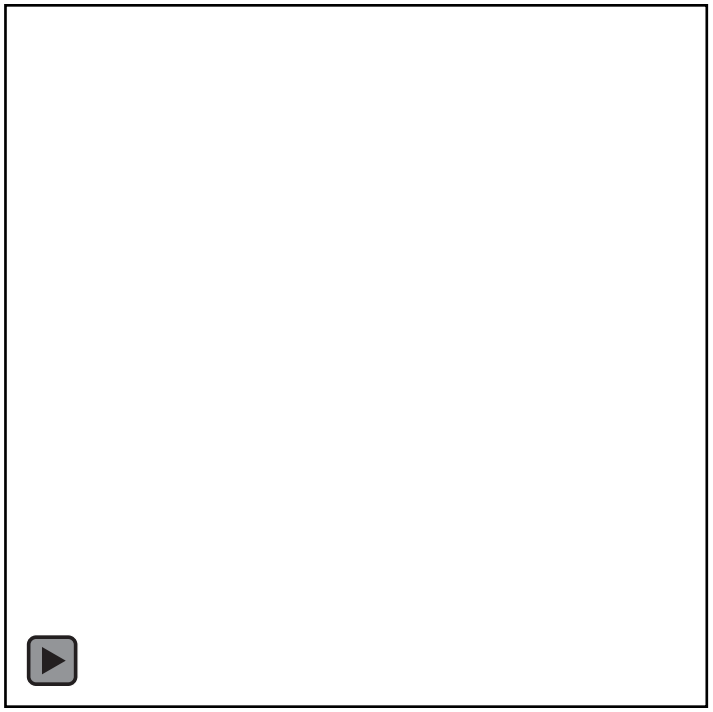
Simulated Neoangiogenesis Effects on 3D Vascular Tumor Growth (75 days)

Axes are in µm





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

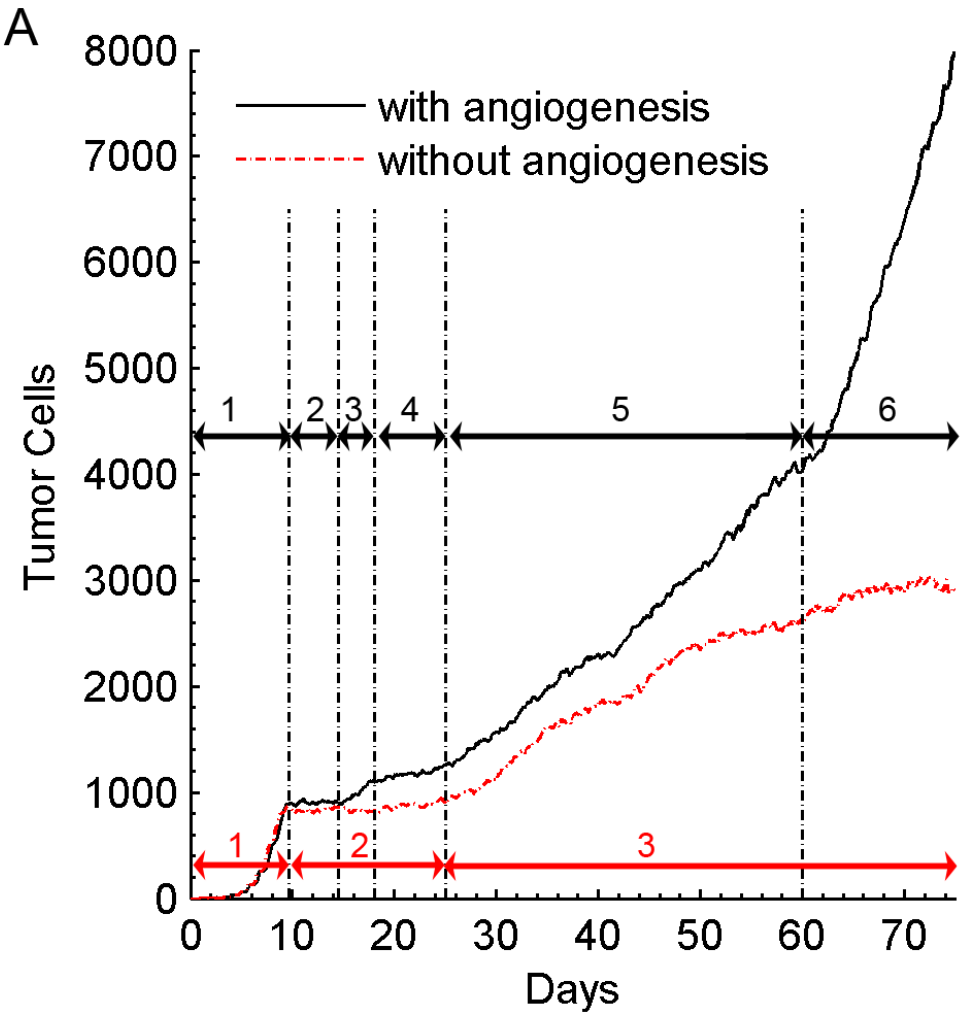
Simulated Neoongiogenesis Effects on 3D Vascular Tumor Growth

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



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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

Sample Current Applications

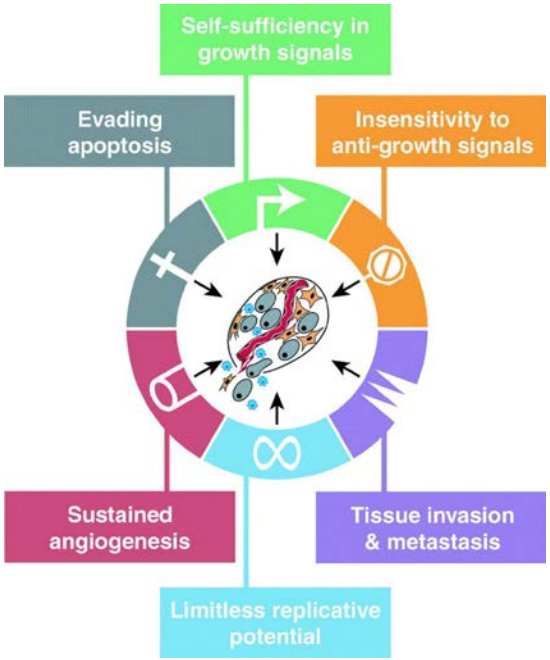
- Angiogenesis and Vasculogenesis (EPA, CWI)
- Vascular Tumor Growth
- **Cancer Evolution**
- Age Related Macular Degeneration
- Liver Toxicology (IUB, EPA)
- Polycystic Kidney Disease (IUB, IUPUI)
- Segmentation (IUB, UCL)

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.

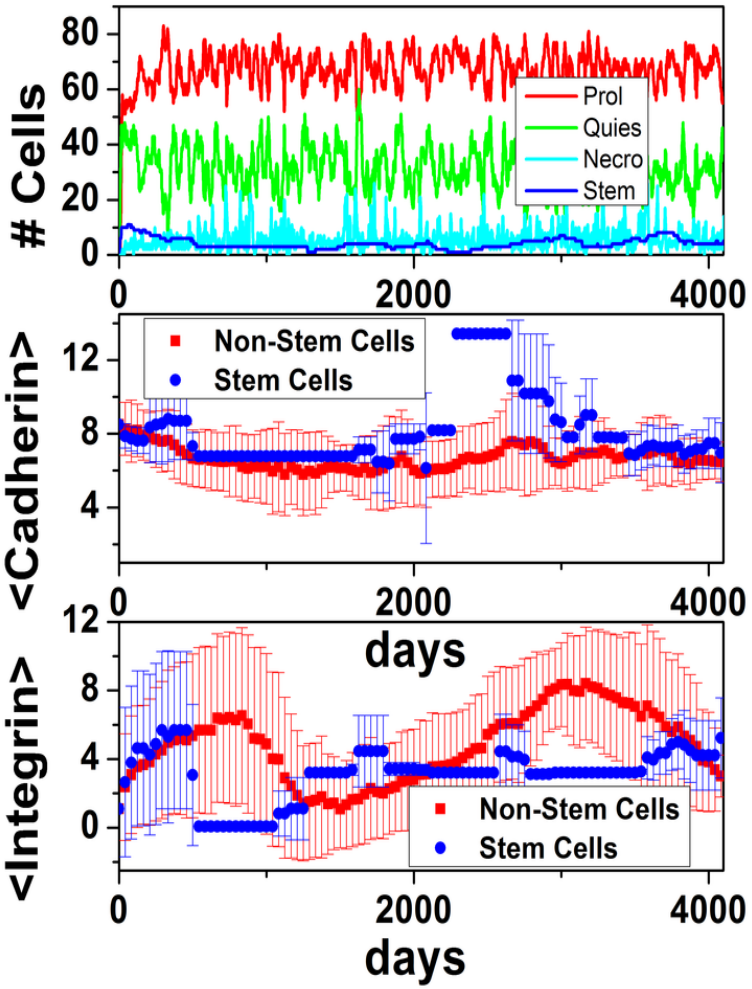
Solid Tumor Progression: Adhesion and Nutrient Heterogeneity

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

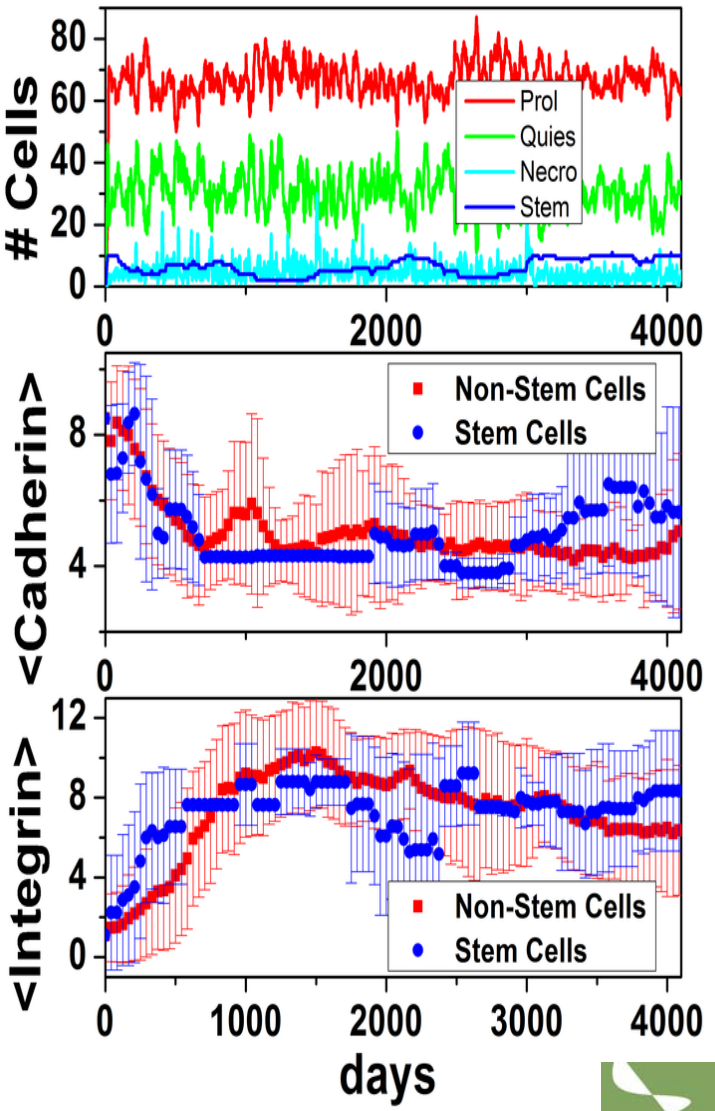


Hanahan and Weinberg, "The Hallmarks of Cancer" *Cell* **100**, 57 (2000).

Nutrient Gradient and Strong Immune



Nutrient Gradient and Weak Immune



Nutrient Gradients and Immune Effects ⇒ Adhesion Changes ⇒ Metastasis

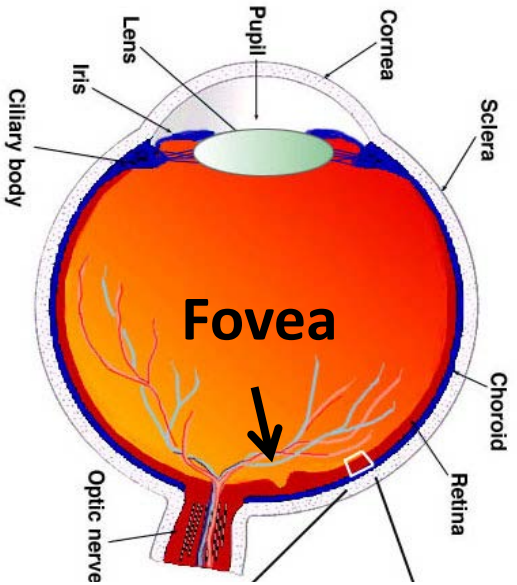
	Cohesiveness	Integrins	Morphology	Remission	Spread	Classification
Strong Immune	++	+	Compact	+	-	Benign
Weak Immune	--	+	Compact + Metastases	-	+	Metastatic

Sample Current Applications

- Angiogenesis and Vasculogenesis (EPA, CWI)
- Vascular Tumor Growth
- Cancer Evolution
- **Age Related Macular Degeneration (IUB, Emory, GSU)**
- Liver Toxicology (IUB, EPA)
- Polycystic Kidney Disease (IUB, IUPUI)
- Segmentation (IUB, UCL)

Wet Aged-Related Macular Degeneration (AMD) or Choroidal Neovascularization (CNV)

- Tiny (~2mm in diameter) abnormal growth of blood vessels into the retina → Retinal Detachment/Fibrosis→ **Blindness**
- **Severe vision loss in a year!**



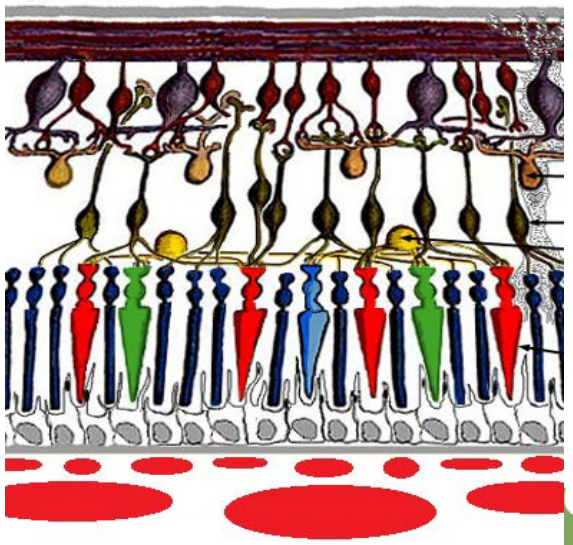
Courtesy http://webvision.med.utah.edu/

- **What causes/initiates CNV?**

Photoreceptors

Retinal Pigment Epithelium

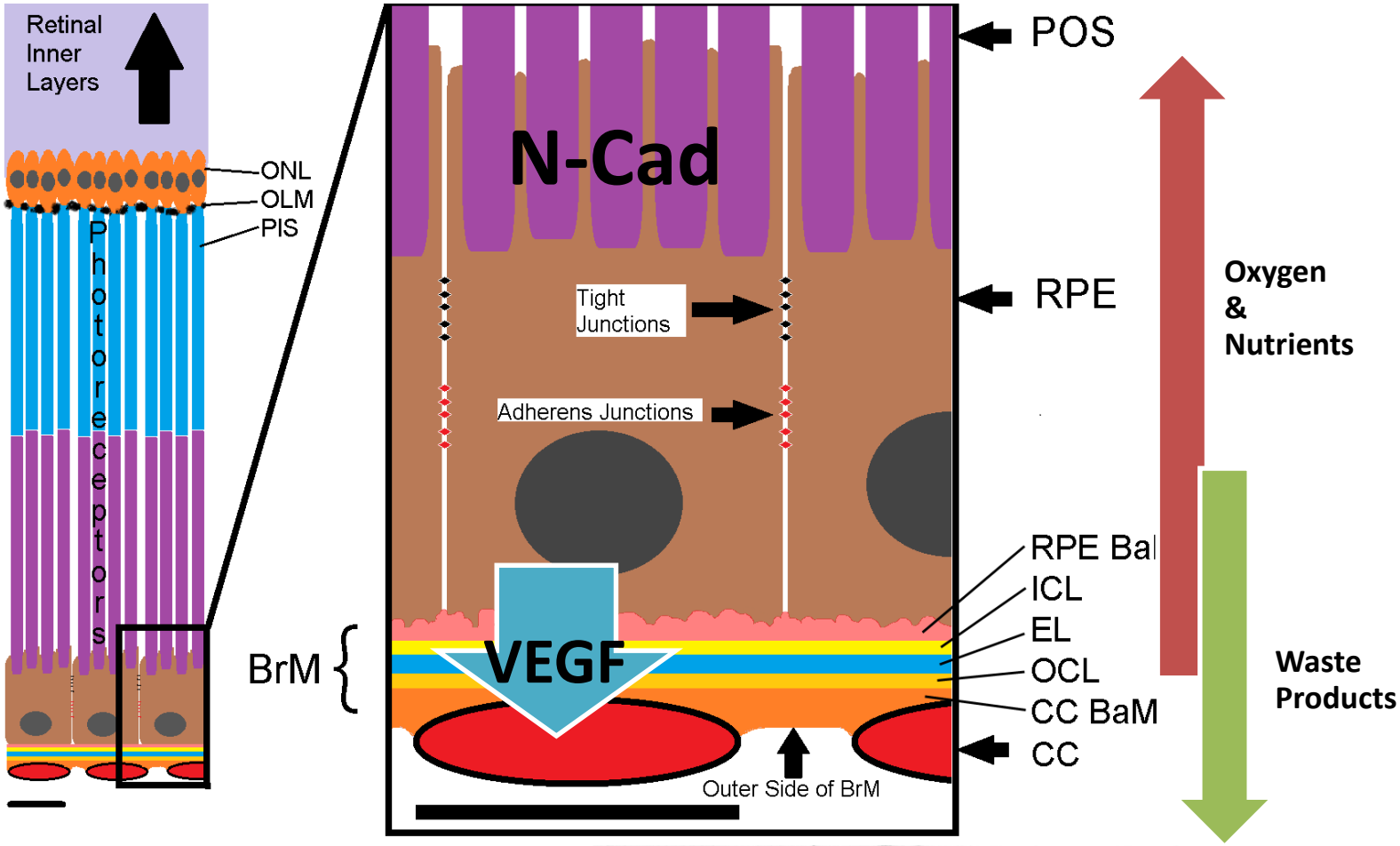
Choroidal Capillaries



AMD Significance

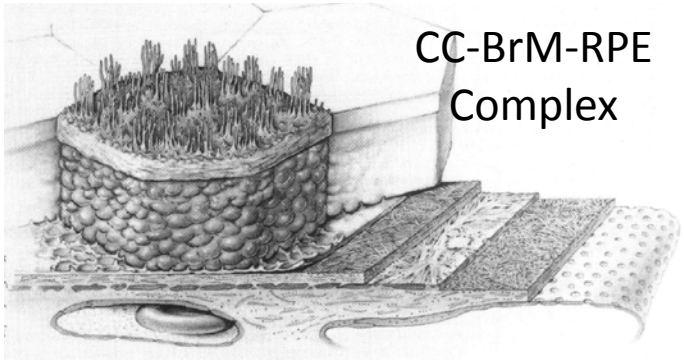
- Age-related macular degeneration (**AMD**) is a leading cause of blindness and **90%** of AMD-related blindness is due to CNV.
- **30%** of individuals 75 years or older have early signs of AMD
- **8%** of individuals 75 years or older have CNV.
- CNV also occurs in young individuals as a result of acute inflammation.

Components and Processes (Normal Retina)



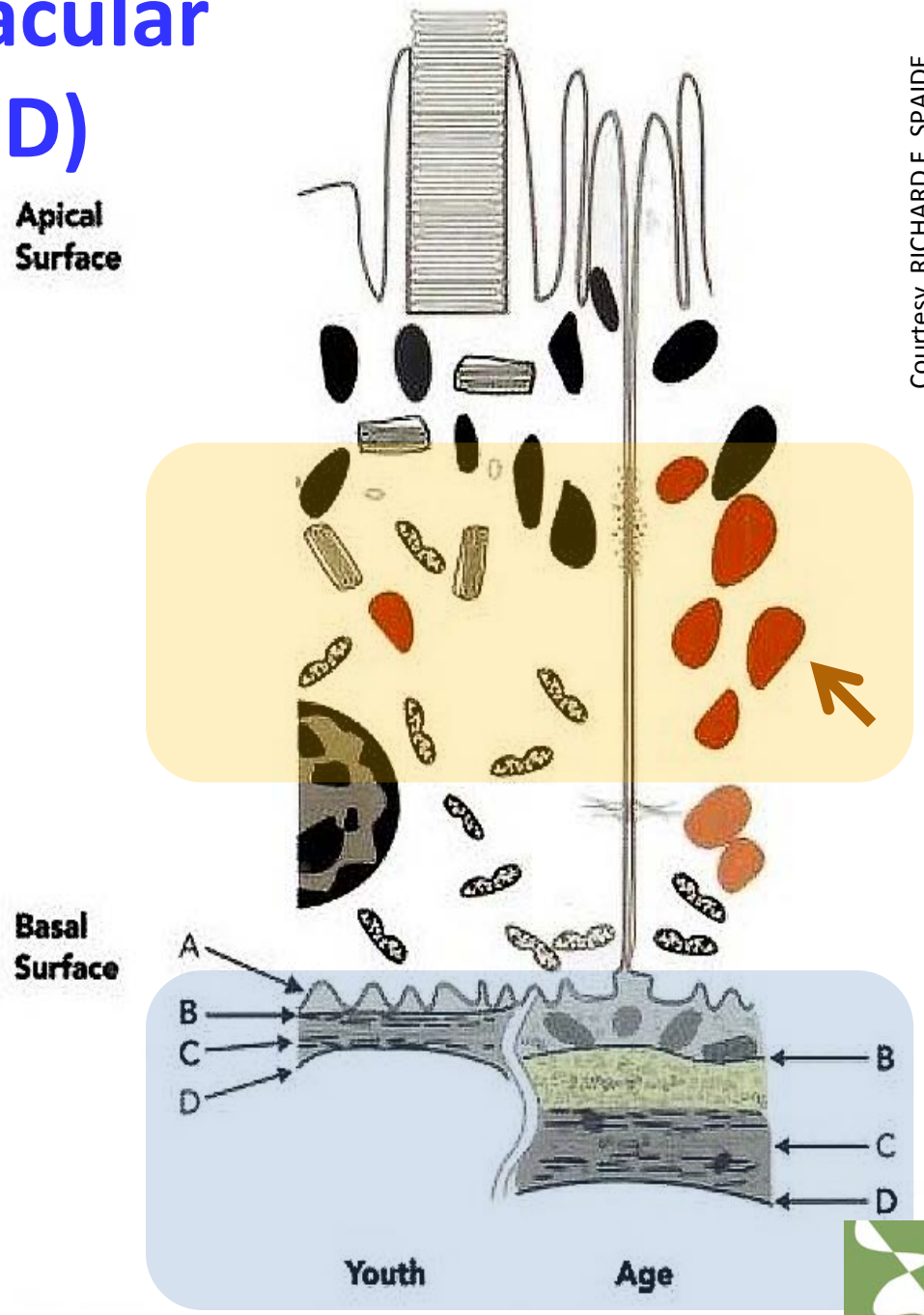
Extended List:
- Immune Cells
- Retinal Fluid Flow
- Blood Flow
- MMPs
- TIMPs

Photoreceptor Outer Segment (POS)
Photoreceptor Inner Segment (PIS)
Retinal Pigment Epithelium (RPE)
Bruch's Membrane (BrM)
Choriocapillaris (CC)



Wet Aged-Related Macular Degeneration (AMD)

- Basal Deposits → **BrM Thickening**
- Intracellular Accumulation of lipofuscin → **RPE aging** and **stress**

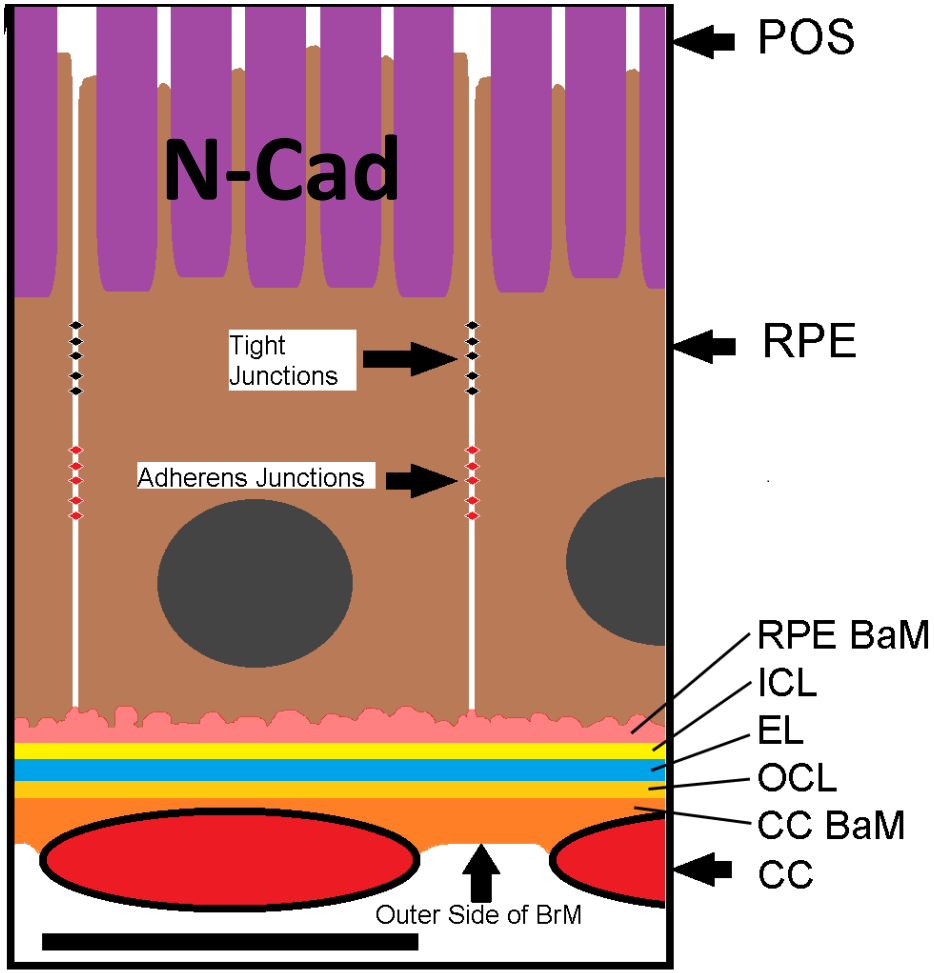


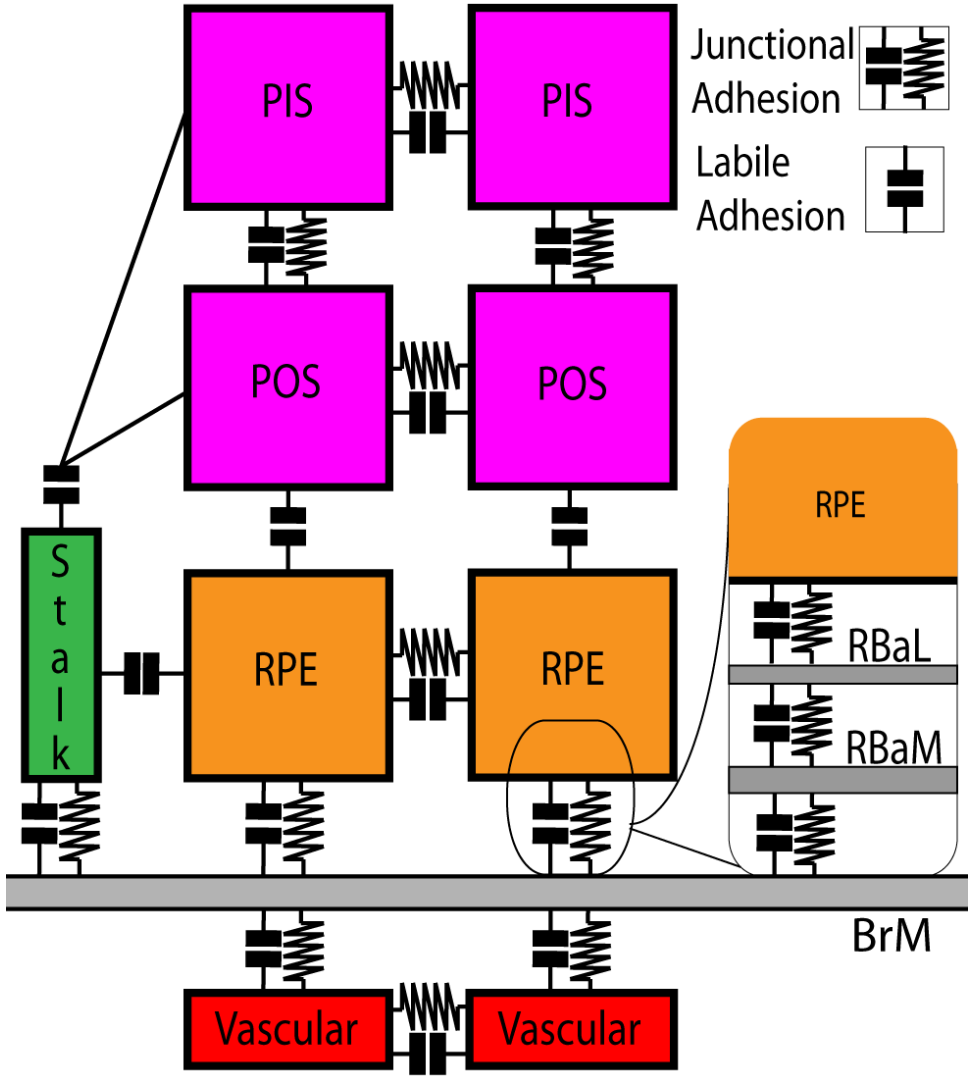
Courtesy RICHARD F. SPAIDE

Our Hypothesis

- Adhesion impairments in BrM-RPE-POS complex are **sufficient** to explain Both **CNV initiation** and **CNV patterning**

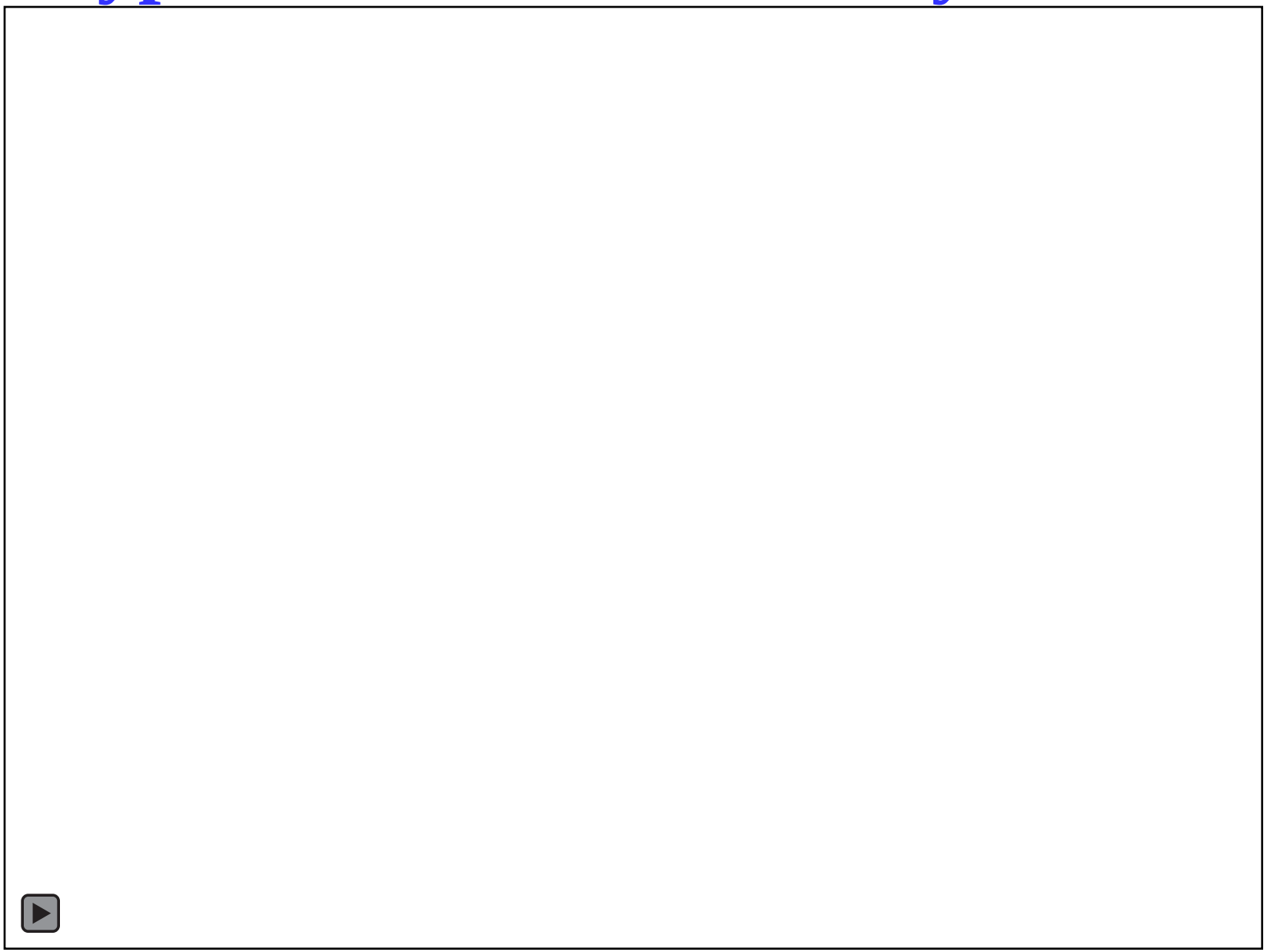
Biological Adhesion→ Modeled Adhesion



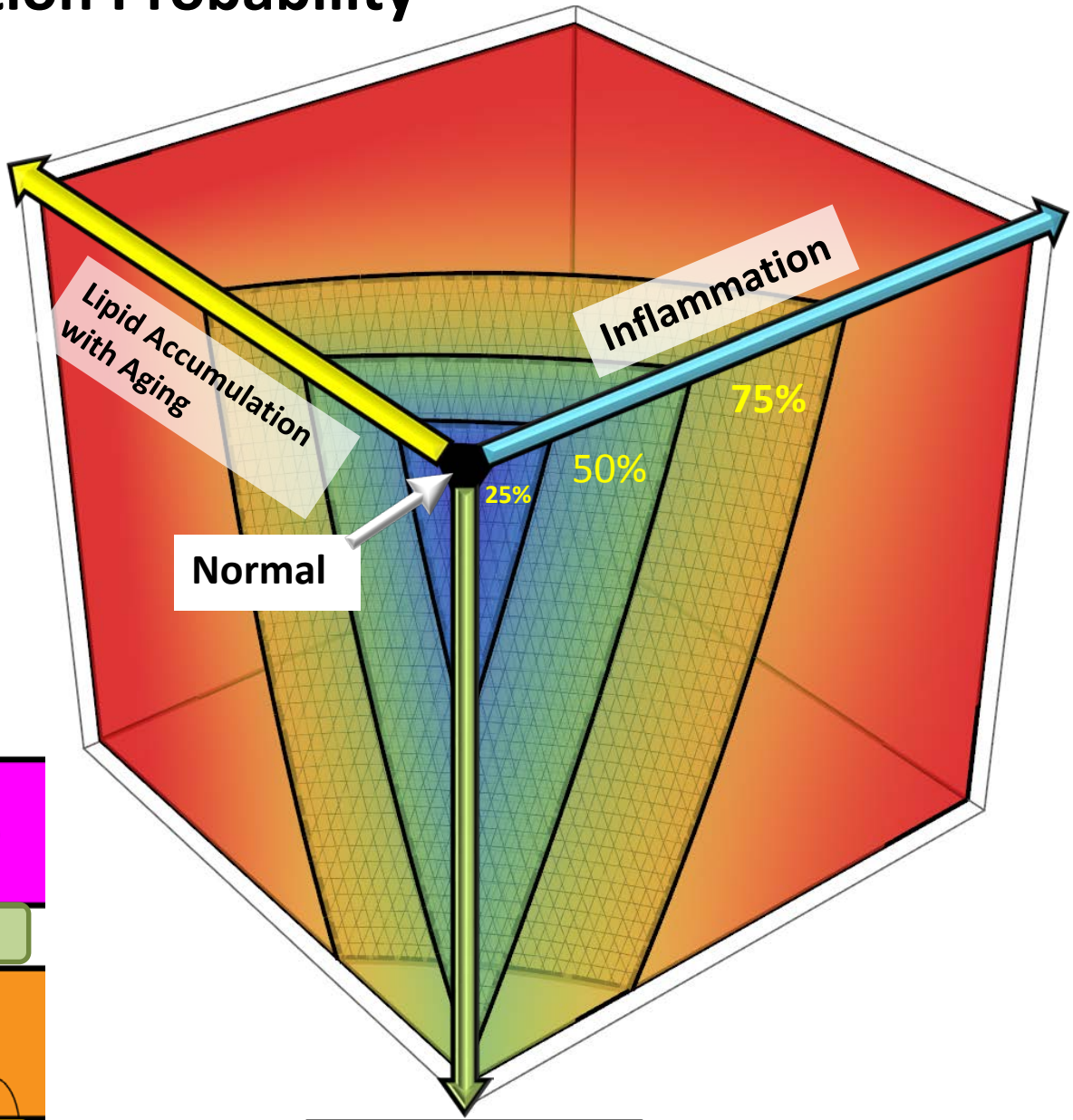


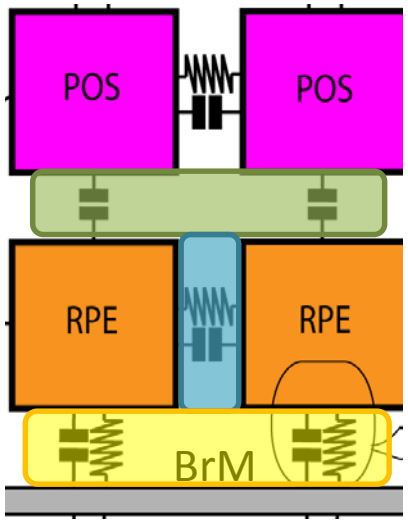
Outer Segment (POS)
Inner Segment (PIS)
Retinal Pigment Epithelium (RPE)
Bruch's Membrane (BrM)
Choriocapillaris (CC)

Typical Sub-RPE CNV Dynamics



CNV Initiation Probability





RPE Detachment (CNV Complications)

Adhesion Failures → CNV

Condition	Adhesion			Effect	Simulation
	RPE-RPE	RBaM- BrM	RPE-POS	CNV	
Normal	+	+	+	No initiation	No initiation
Lipid Accumulation	**+ / -**	**- -**	**+ / -**	**Sub-RPE**	**Sub-RPE**
Inflammation	**- -**	**+**	**-**	**Sub-retinal**	**Sub-retinal**
Retinal Detachment**	**-**	**+**	**- -**	**Sub-retinal**	**Sub-retinal**

RBaM: RPE basement membrane

** in animal models

What Prevents CNV for +60 Years?

Proper Maintenance of Differential Adhesion!

Endothelial cells mainly express **VE-cadherin**

RPE and Photoreceptors express **N-cadherin**

ECs lack adhesion mechanisms that allow macrophages cross the RPE