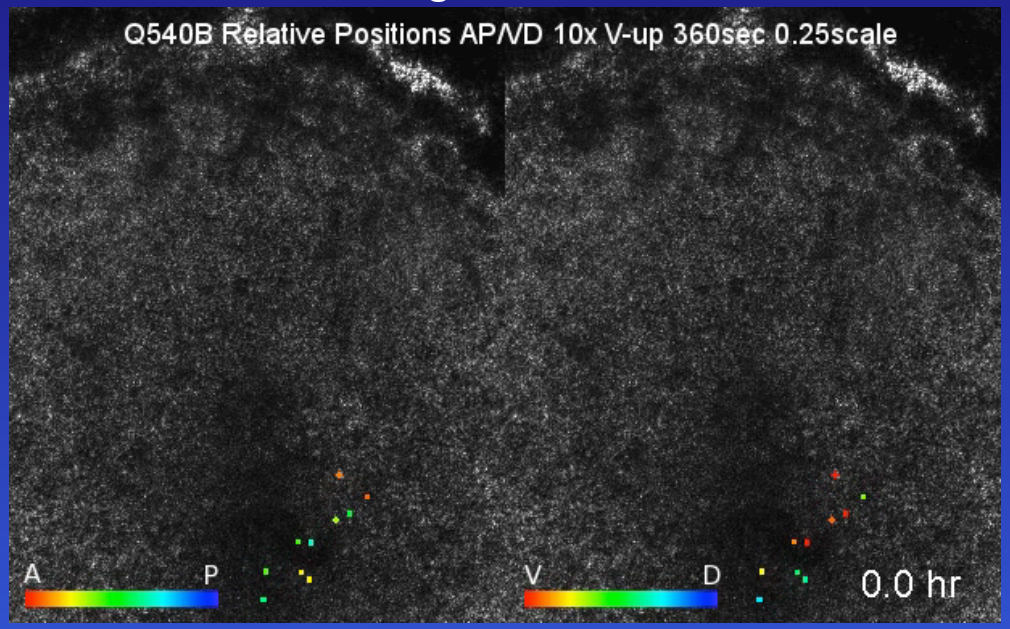
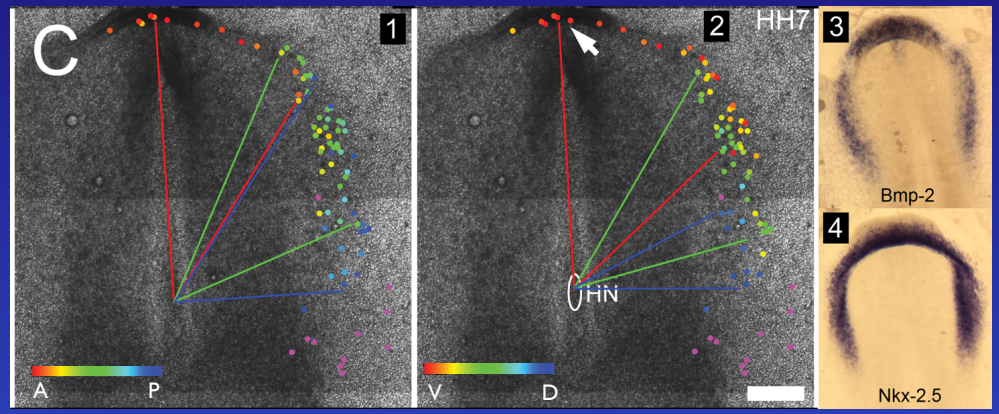
Overlay of Pseudocolored (Unilateral Tracked) Cells on Brightfield Movie

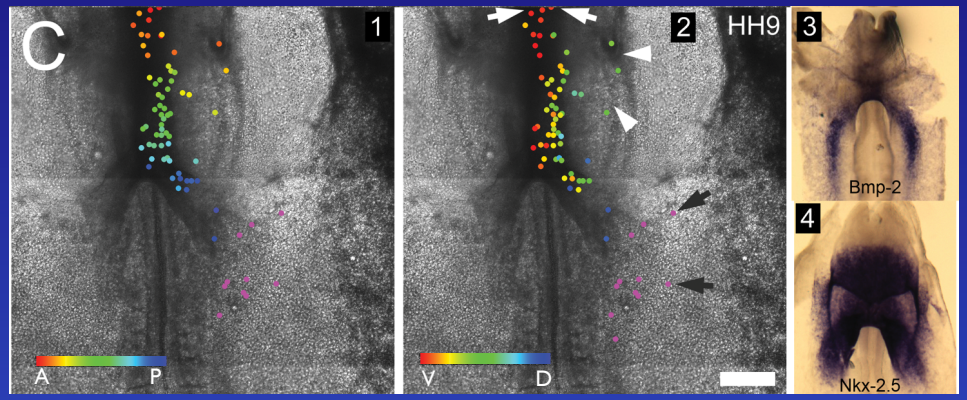


Heart Forming Regions (HFR) at HH7 (23-26h)



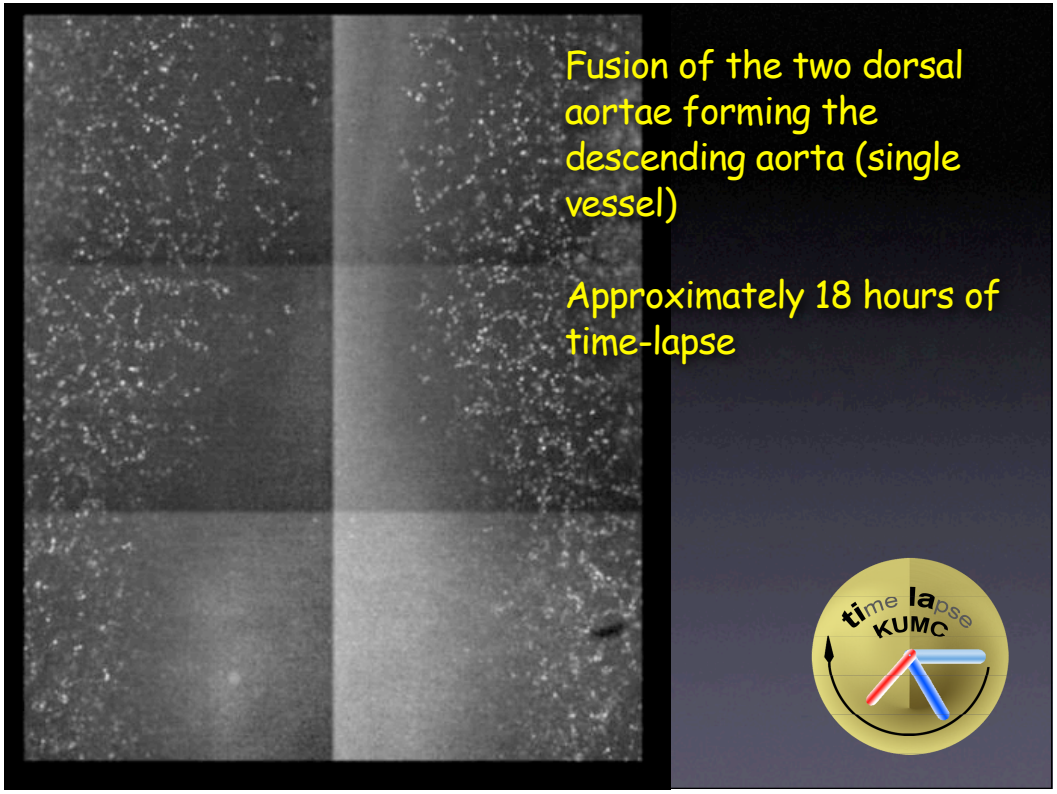
- Spatial separation of colors is observed progressing caudally.

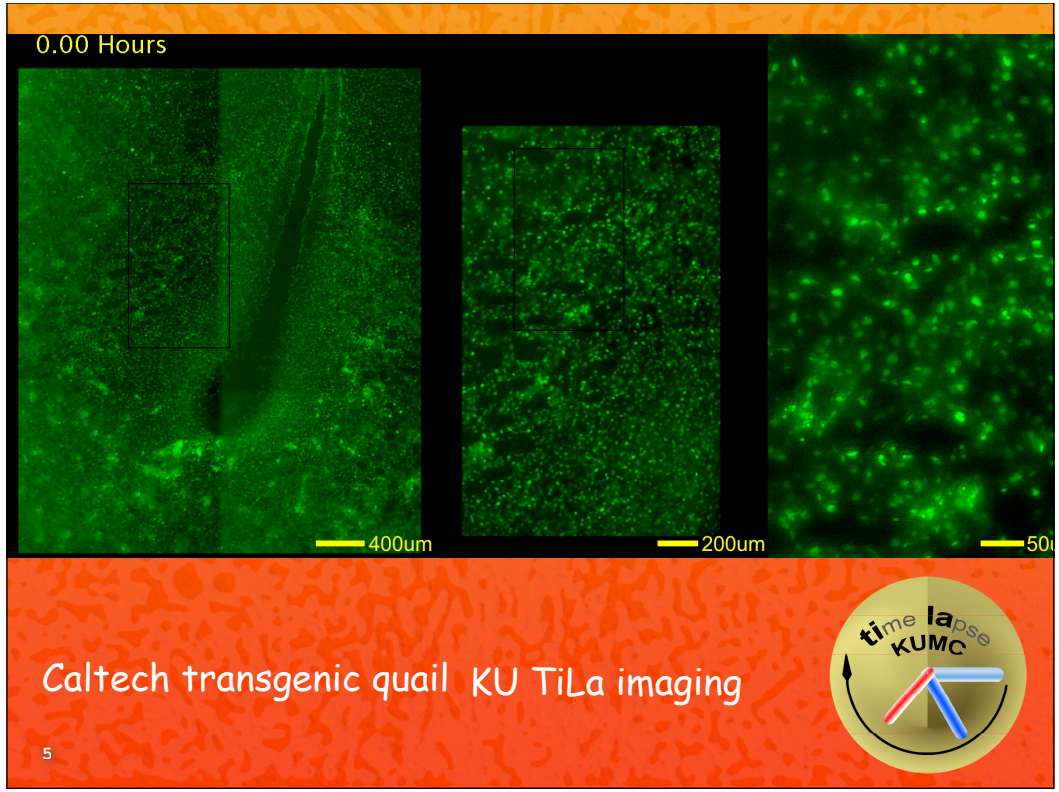
Heart Forming Regions (HFR) at HH9 (29-33h)



- Pink cells begin to contribute to inflow region of the heart
- Cells designated by arrows do not overlap with Nkx2.5(+) regions until Stage 10

Stage 9 is approx. when conduction system first develops. IFT, left side initially


Fusion of the two dorsal aortae forming the descending aorta (single vessel)
Approximately 18 hours of time-lapse
time lapse
KUMC



Caltech transgenic quail KU TiLa imaging

A hen is an egg's way of making another egg

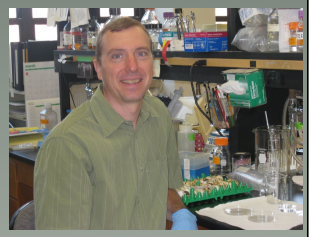
Biophysical Studies of Cellular Motility Versus Tissue Motion in Early Embryos

University of Kansas Medical Center

Computational Imaging Group

Andras Czirok

Brenda Rongish

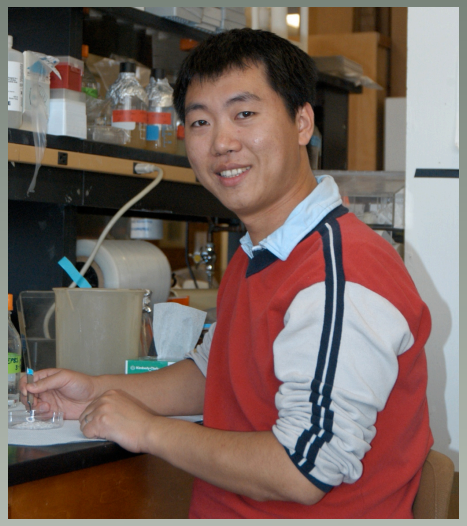


Michael Filla

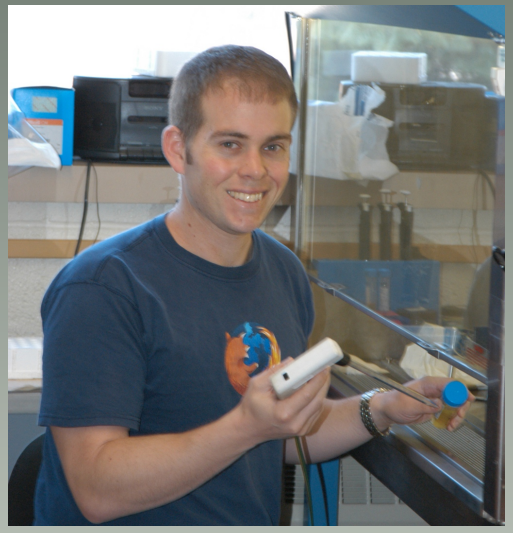
Tracey Cheuvront

Alan Petersen

Ana Aleksandrova



Cheng Cui — electrorporation based positional fate mapping; now a fellow in the Dev Biol Lab, NIHLB



Evan Zamir — PIV based motion correlation now Asst. Prof. Ga Tech

Rationale:

"Primitive animals, with few specialized cells and no tissues or organs, possess the same molecular genetic circuits that vertebrates use to make complex body parts."

(Pennisi, Science, 2008)

Therefore, if genomic complexity is not the underpinning of vertebrate organogenesis — What is? Where is the information that determines the shape of organs and tissues?

We test the hypothesis that tissues and organs largely gain shape from emergent biophysical mechnisms that are NOT encoded in DNA.

We hypotheize that collective biological motion causes large scale tissue deformations that regulate embryonic morphogenesis

We further hypothesize that relative motion, or lack thereof, between cell collectives and the extracellular matrix is an important determinant of embryonic morphogenesis

Physics and engineering provide the tools to examine these hypotheses.

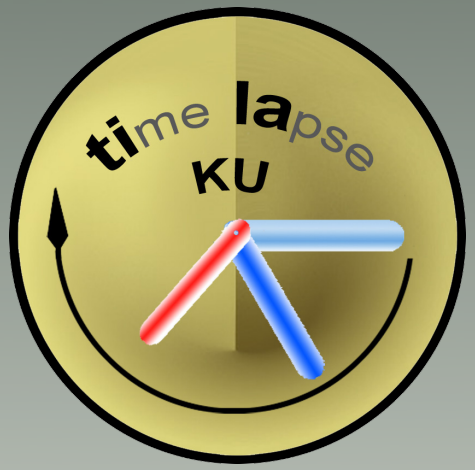
One example of an emergent pattern is the primary vascular network in warm-blooded (amniotes) embryos.

The data will show that there is no genetic regulatory circuit that governs formation of the hexagonal vascular pattern in birds and mammals.

Other data will show that tissue deformations will have a strong influence on formation of large caliber vessels.

Take Home Message:

- *Not only do cells move... but the extracellular matrix is also in constant motion during early pattern formation.*
- *Since, Cells + ECM = Tissue, it therefore follows that tissue move.*
- *Indeed, embryonic tissues are in constant motion — therefore, cellular patterns form within a moving framework.*

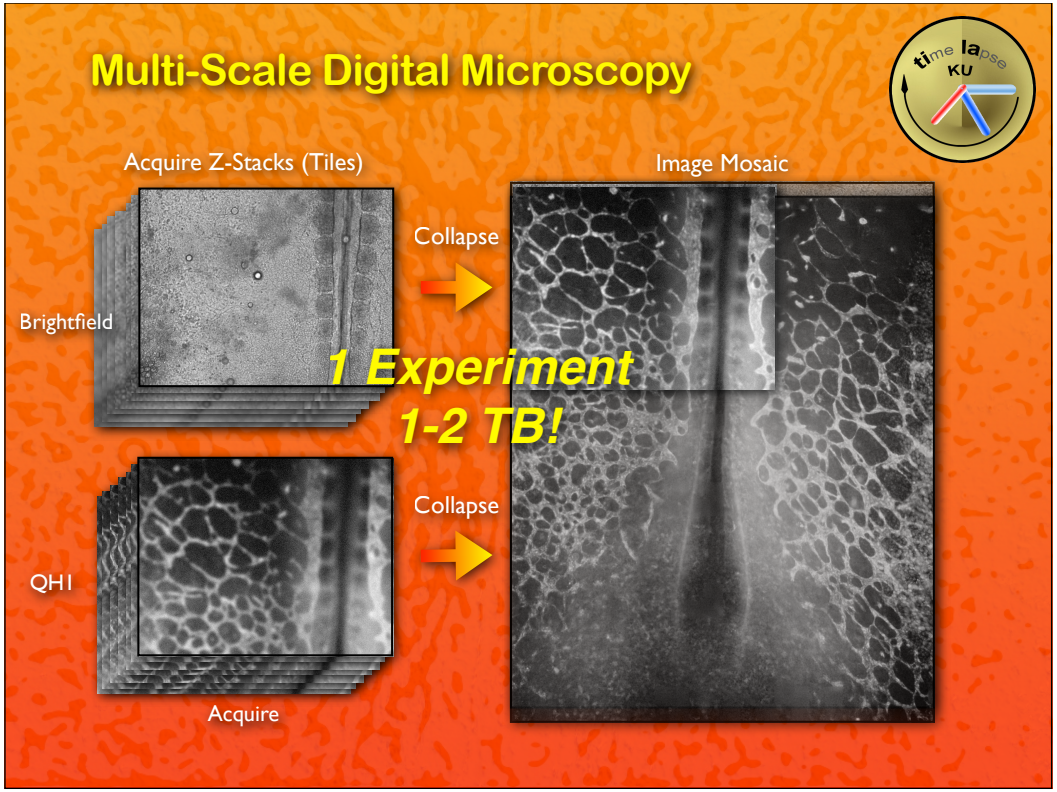


Open source computer code for scanning,
Time-Lapse Imaging.

Kansas University Medical Center

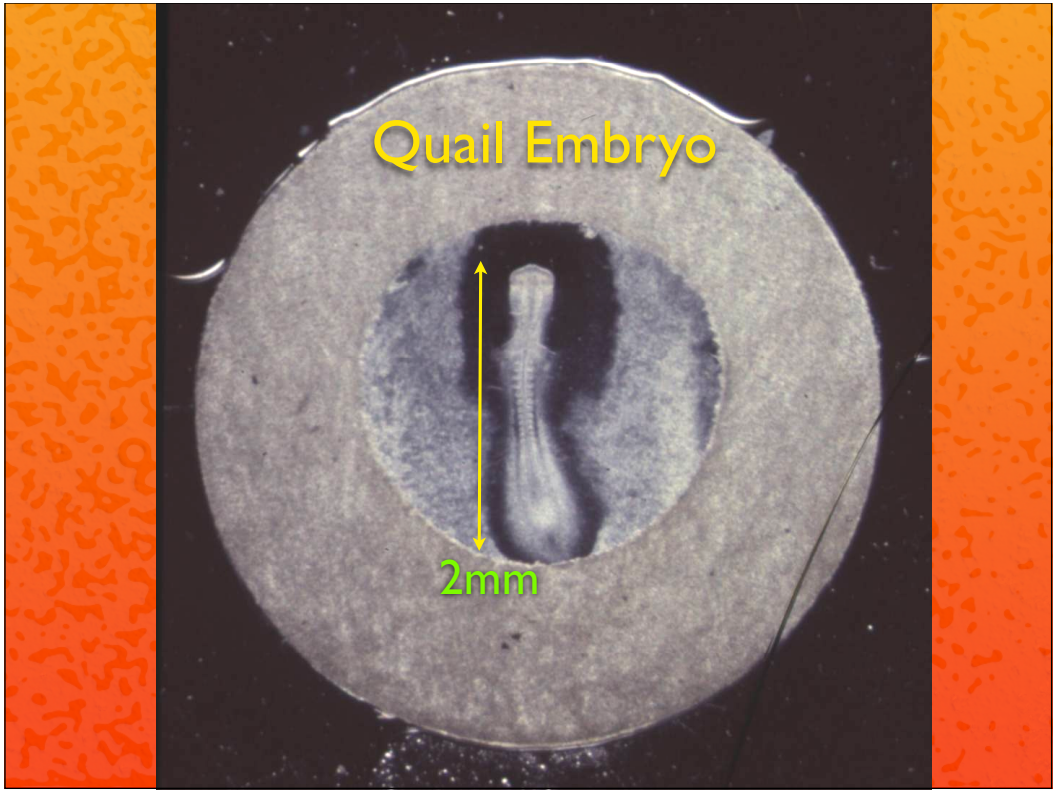
Motion Analysis of Morphogenesis

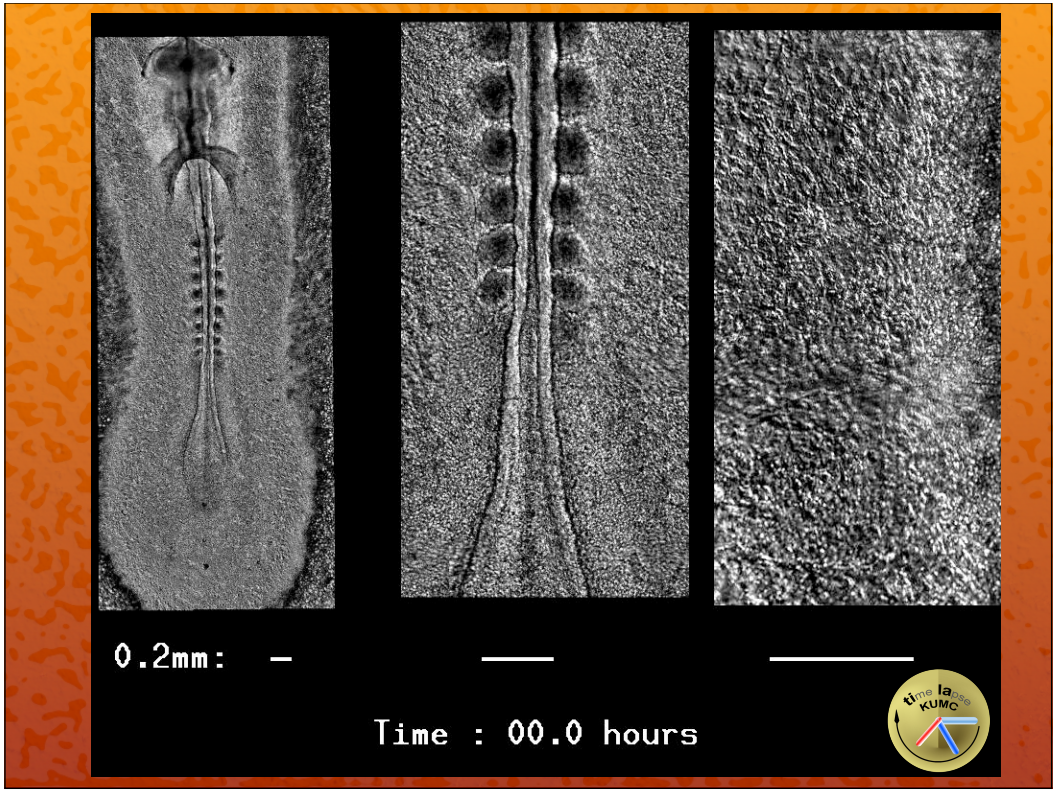
Czirok et al, J. Micro. 206:9, 2002



Our lab recently developed digital time-lapse microscopy and image processing techniques to capture both large-scale, macroscopic (tissue-level) and cell-level or microscopic processes in the avian embryo. This is truly a multi-scale method as the variation in length-scales, from micron to mm, spans three orders of magnitude.

1. Using custom-written software to control the microscope and CCD camera, we rapidly acquire overlapping image stacks at different x-y locations spanning the entire embryo or some region of interest.
2. The image stacks are then collapsed into a single best-focused plane or tile, and
3. The tiles are then arranged and merged together, thus creating a single mosaick-ed image of the embryo for a specific illumination mode at a single time point.
4. This process is then repeated for different illumination modes (enabling the capture of various microinjected fluorescent labels or fluorescent microspheres), and can be done on several embryos (up to six currently) in a single experiment.
5. A single experiment can generate up to 20 GB of data in the form of images - data storage and automated analysis is key!


Quail Embryo
2mm



Remember to thank everyone for coming!

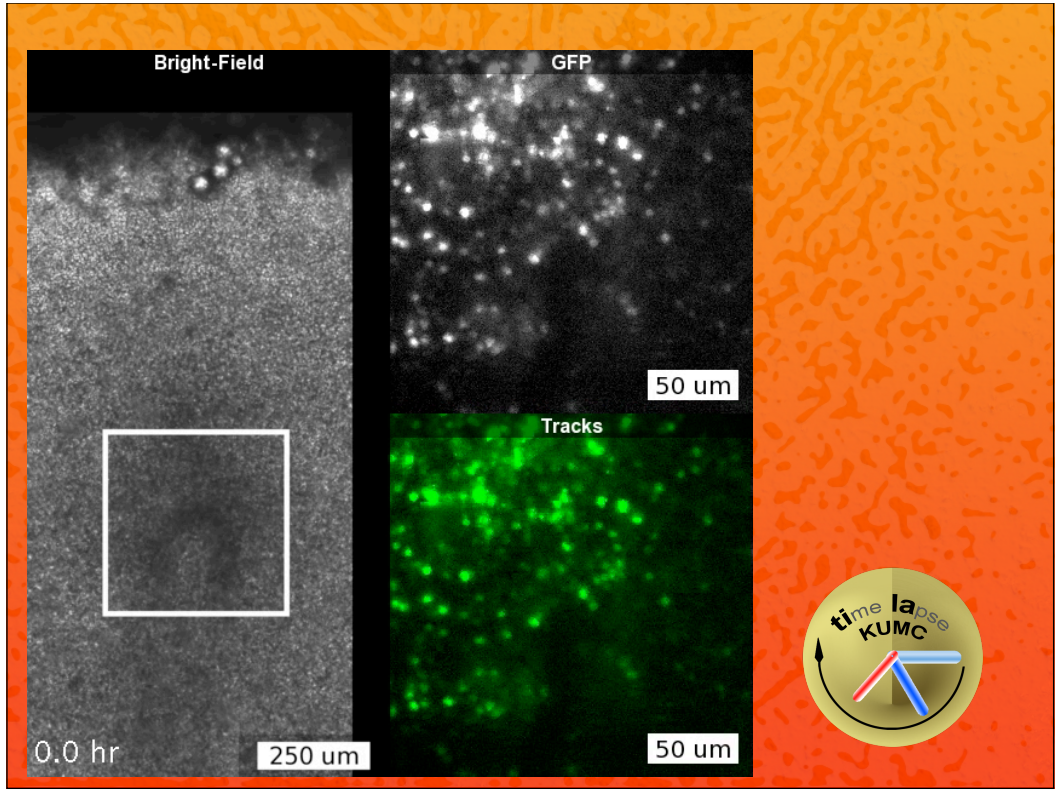
Disclaimer: As you can tell from the title, this talk has somewhat of a computational or engineering emphasis, but I promise, there are no equations and only a couple of mathematical expressions.

Three ways to introduce fluorochomes:

1) Microinjection of fluorescently tagged antibodies

2) Electroporation of DNA plasmids encoding florescent protein

3) Transgenic quail bearing lineage specific fluoescent protein reporters

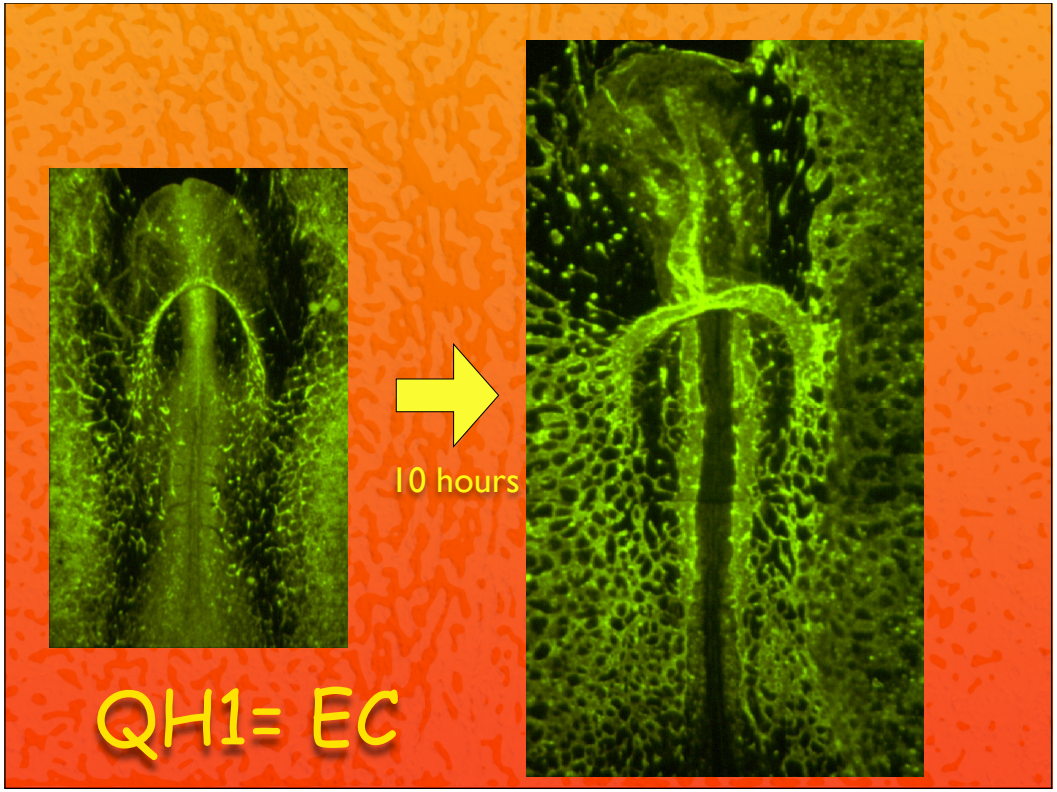

Bright-Field
GFP
50 um
Tracks
0.0 hr
250 um
50 um
time lapse
KUMC

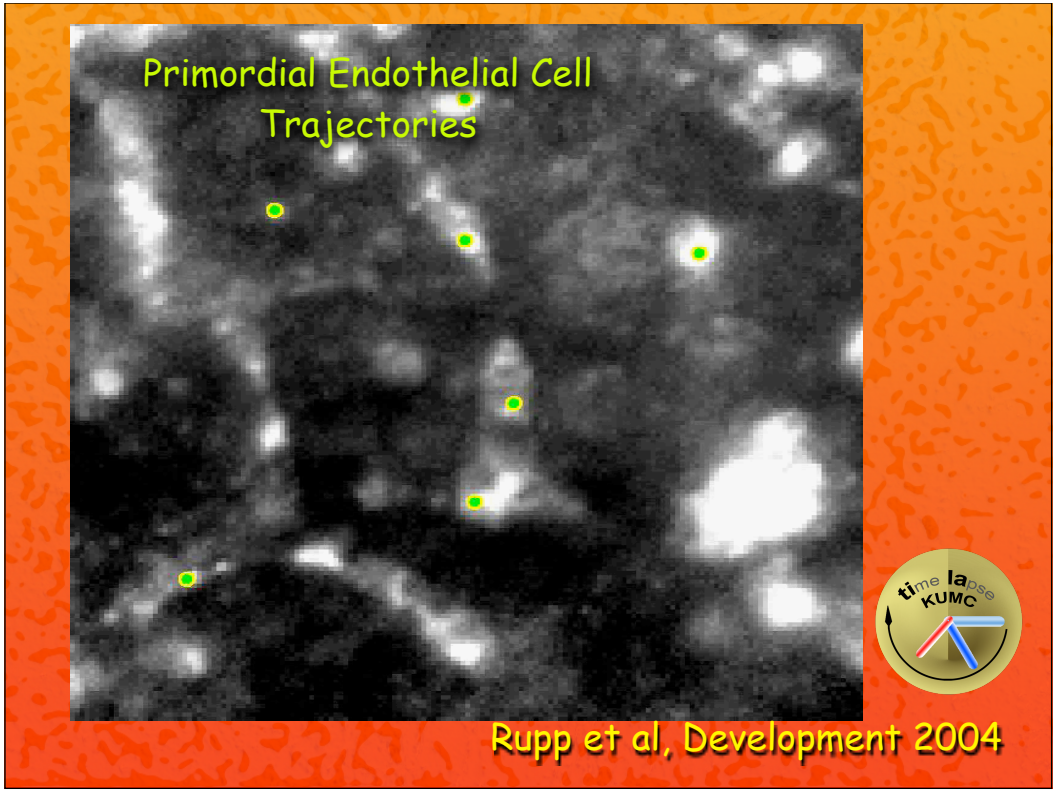
Consider the case of primary vascular pattern formation.

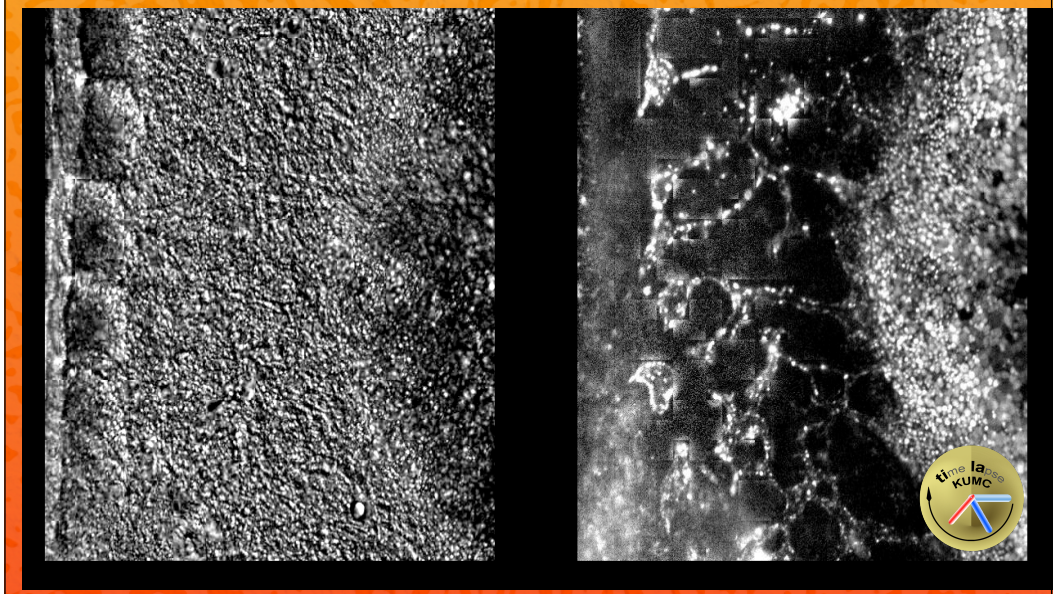
If vascular pattern formation occurs in a constantly moving milieu (in warm-blooded embryos) — certain experimental questions then arise:

1) To what extent are vascular cells "really" moving compared to composite tissue drift, and where are they going?

2) To what extent is vascular pattern formation an emergent process? That is, dependent on biophysical cues in the microenvironment and not directly regulated by genes that specify descrete pattern elements.

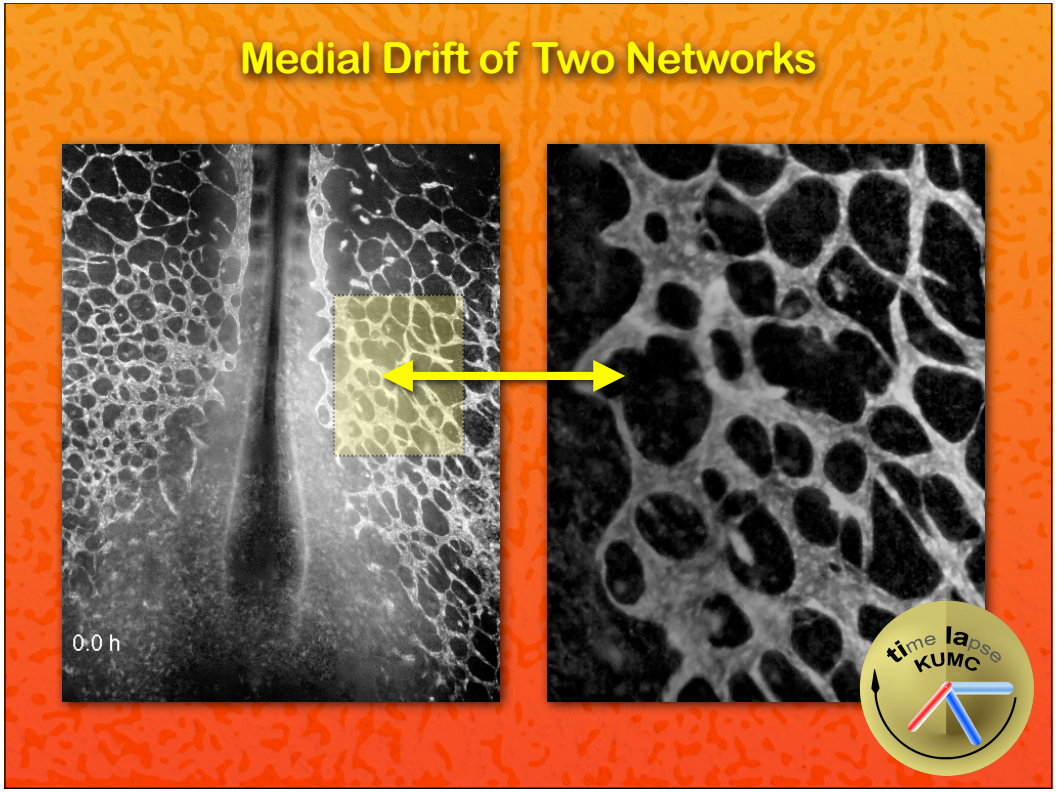

10 hours
QH1= EC


Primordial Endothelial Cell
Trajectories
time lapse
KUMC
Rupp et al, Development 2004



High velocity endothelial cells moving on existing vascular elements

Rupp et al, Development 2004

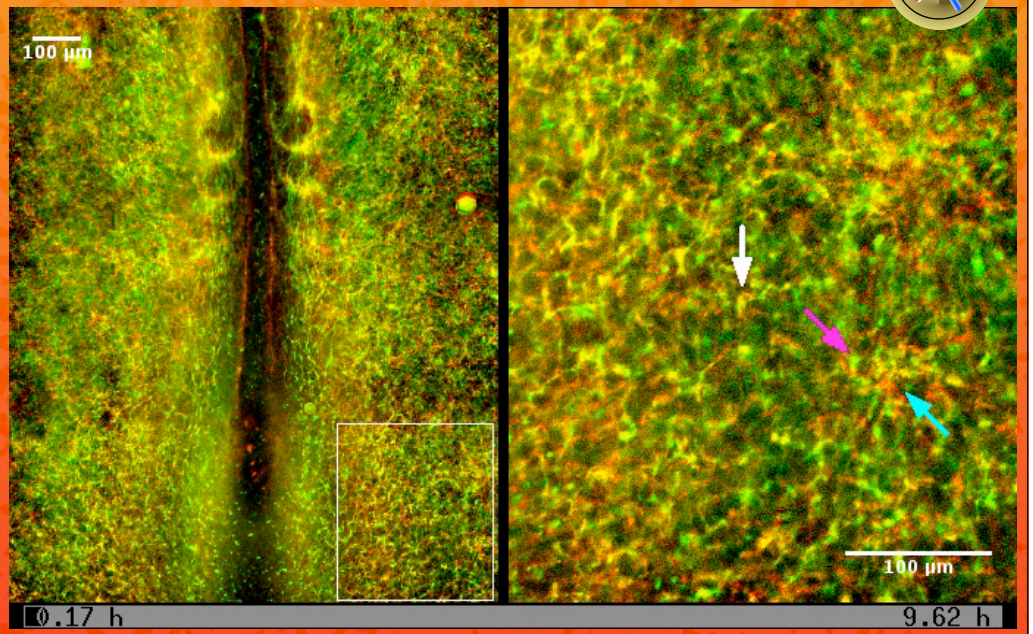


Here is a completed movie of an embryo labeled with QH1 for a period of approximately ten hours.

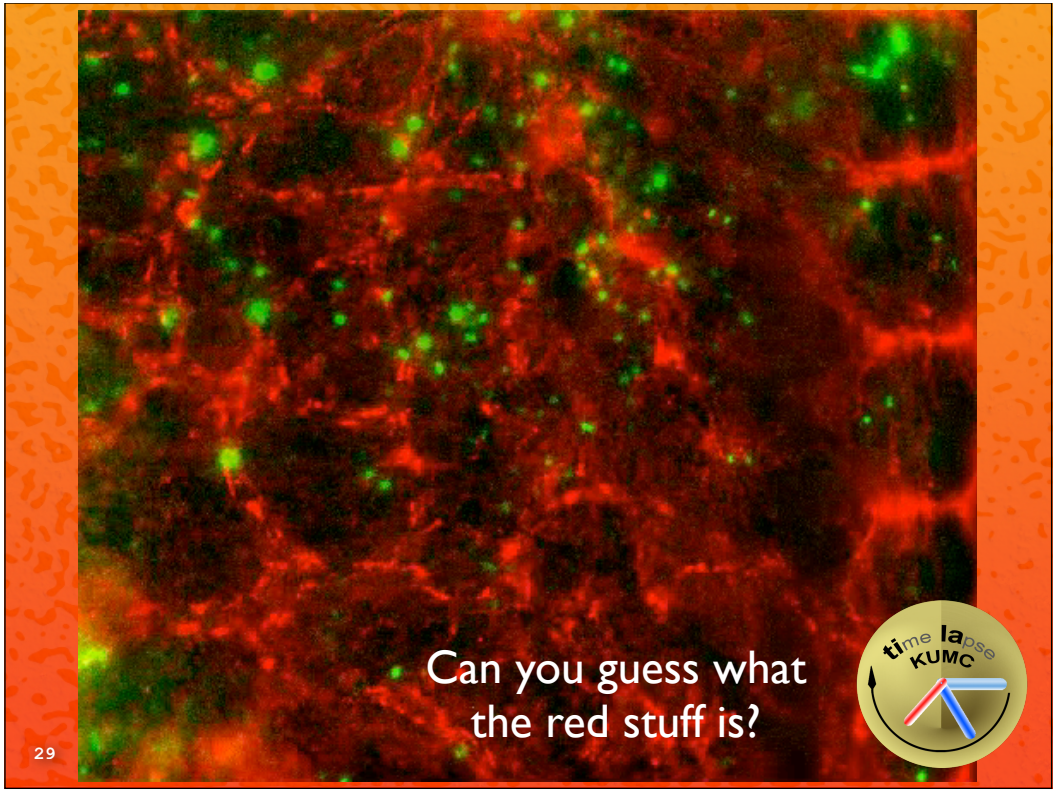
(Point out where somites are and Hensen's node)

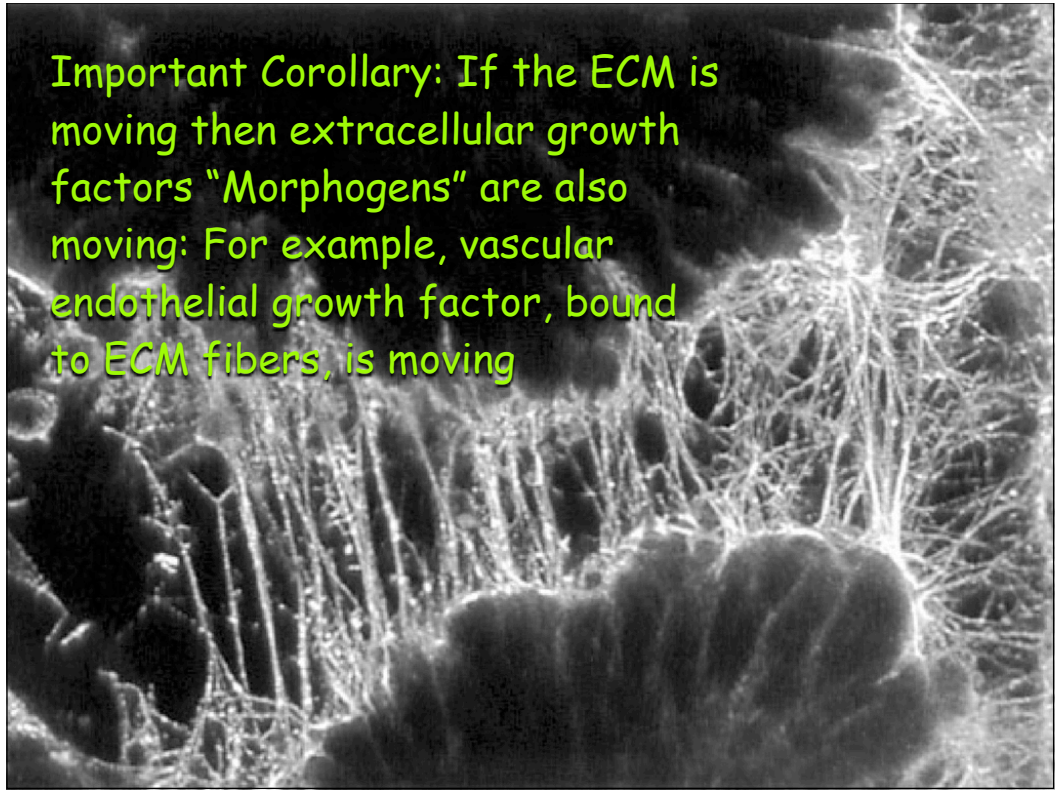
(Click to zoom in). Now you can see at the right a portion of the movie close-up showing the more local or cell-level remodeling of the vascular network.

The ECM moves!



Czirok et al, Curr Top Dev Biol. 2006


Can you guess what
the red stuff is?
time lapse
KUMC


Important Corollary: If the ECM is moving then extracellular growth factors "Morphogens" are also moving: For example, vascular endothelial growth factor, bound to ECM fibers, is moving

OK, but what about vascular pattern formation in mammals?

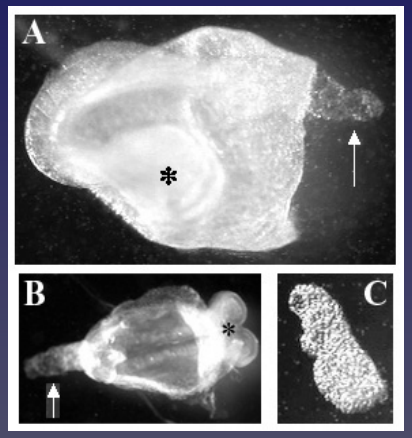
Futher, how can I be sure that genetic circuits are not directly responsible for a hexagonal pattern?

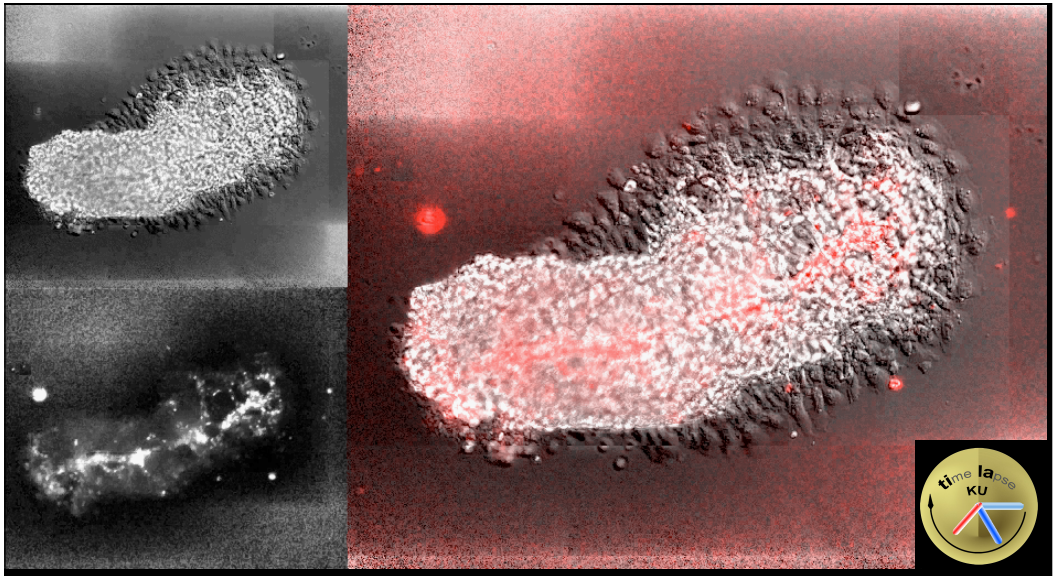
Model System -- Explanted Allantois MOUSE

Future umbilical cord and vessels

Entirely mesoderm

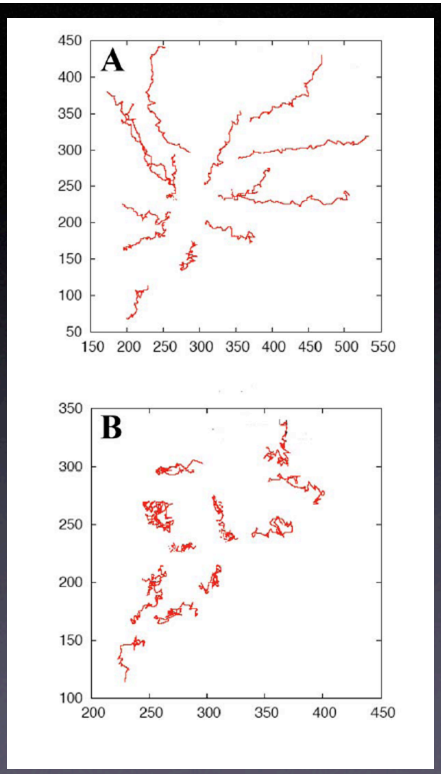
Vessel formation occurs de novo, i.e., vasculogenesis





Time-Lapse of Planar Allantois culture

Perryn et al, Developmental Biology 2008



Mesothelium spreading versus autonomous endothelial cell motion?

Perryn et al, Devel. Biol. 2008

The punch line:

Mouse allantoides make one artery and one vein if left in situ. In contrast, when placed in a planar environment the same tissue makes a hexagonal network.

There can be no GRN in mouse that governs allantoic morphogenesis under planar conditions — ispo facto, creation of a hexagonal pattern is EMERGENT

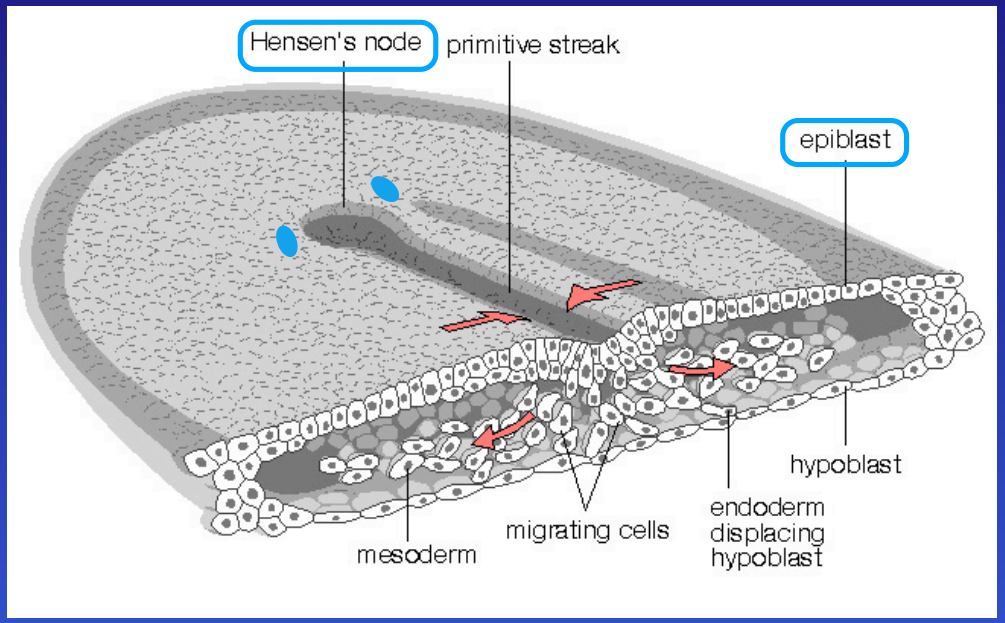
Relative Motion

To study the degree of relative motion between cells and the extracellular matrix it is important to understand that:

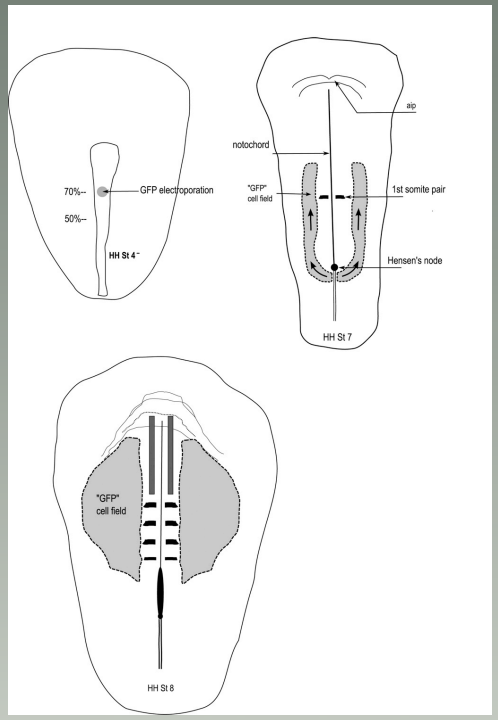
The most important day of your life is neither birth, nor death nor marrige, but is gastrulation

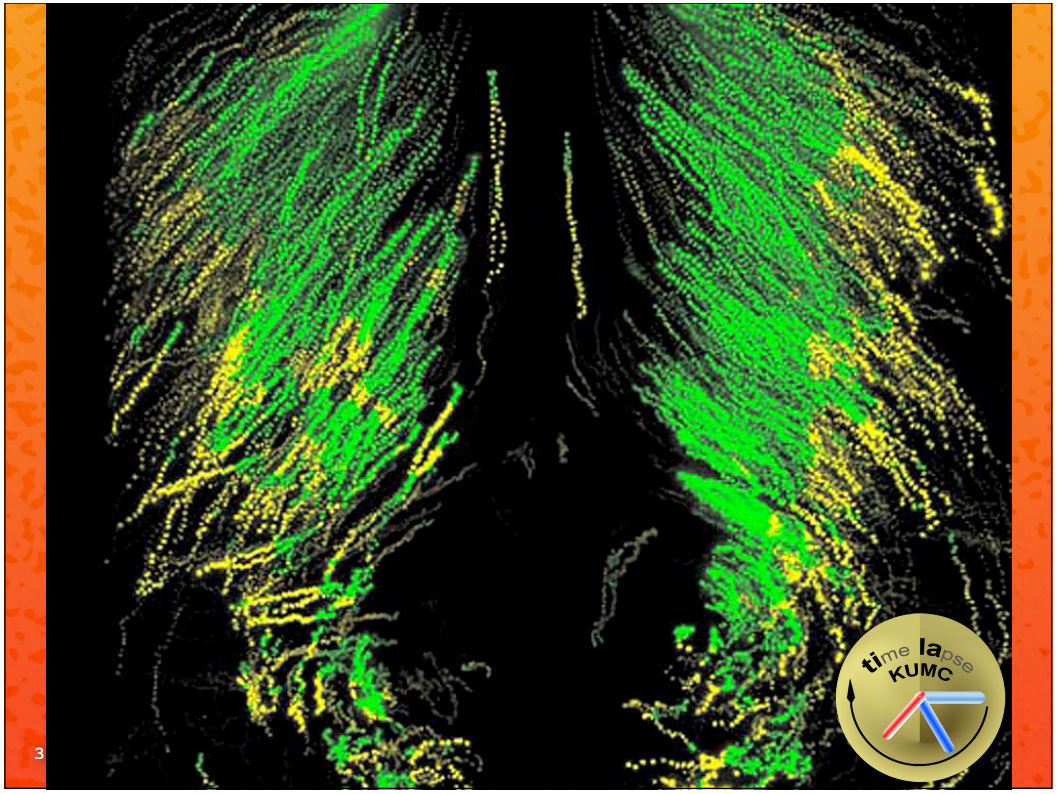
with apologies to Lewis Wolpert

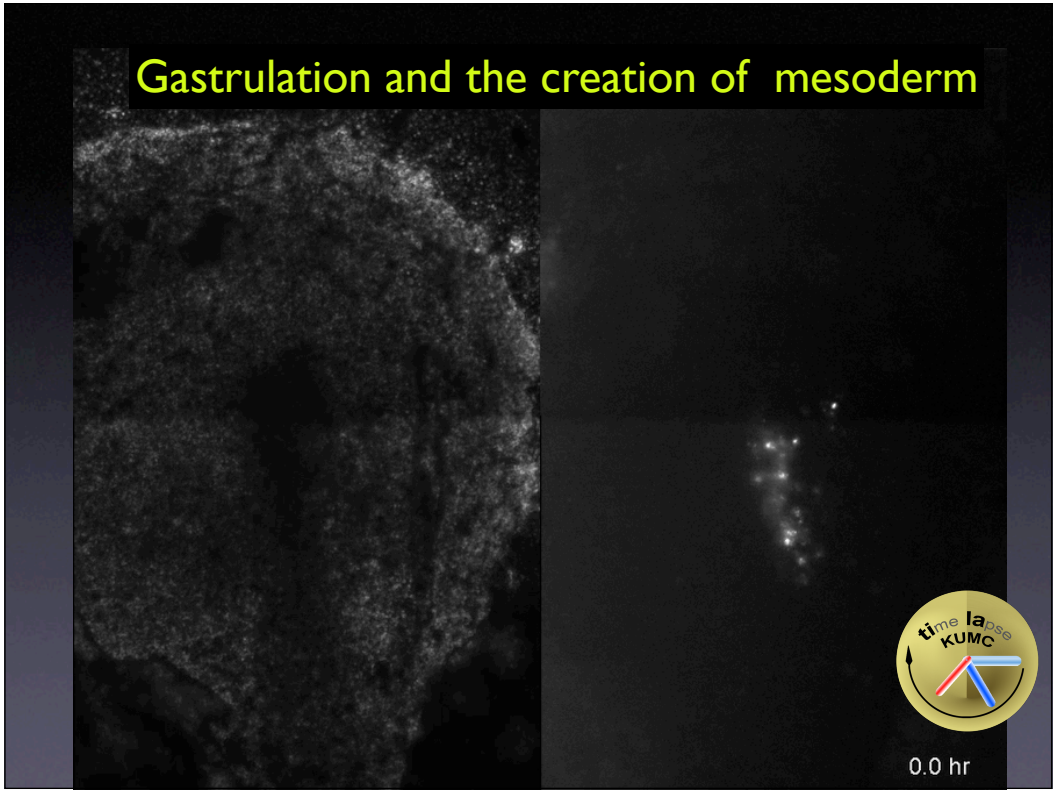
Target epiblast cells to left or right side of node



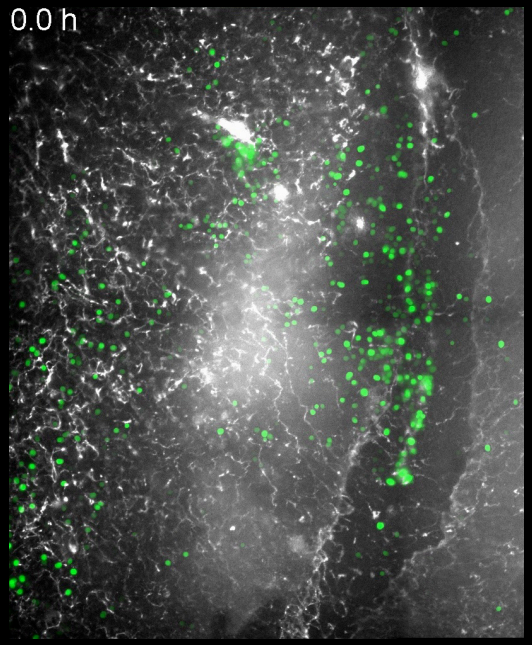
Positional fate maping of cells using electroporation




time lapse
KUMC


Gastrulation and the creation of mesoderm
time lapse
KUMC
0.0 hr

Shared large-scale motion pattern of ECM & cells

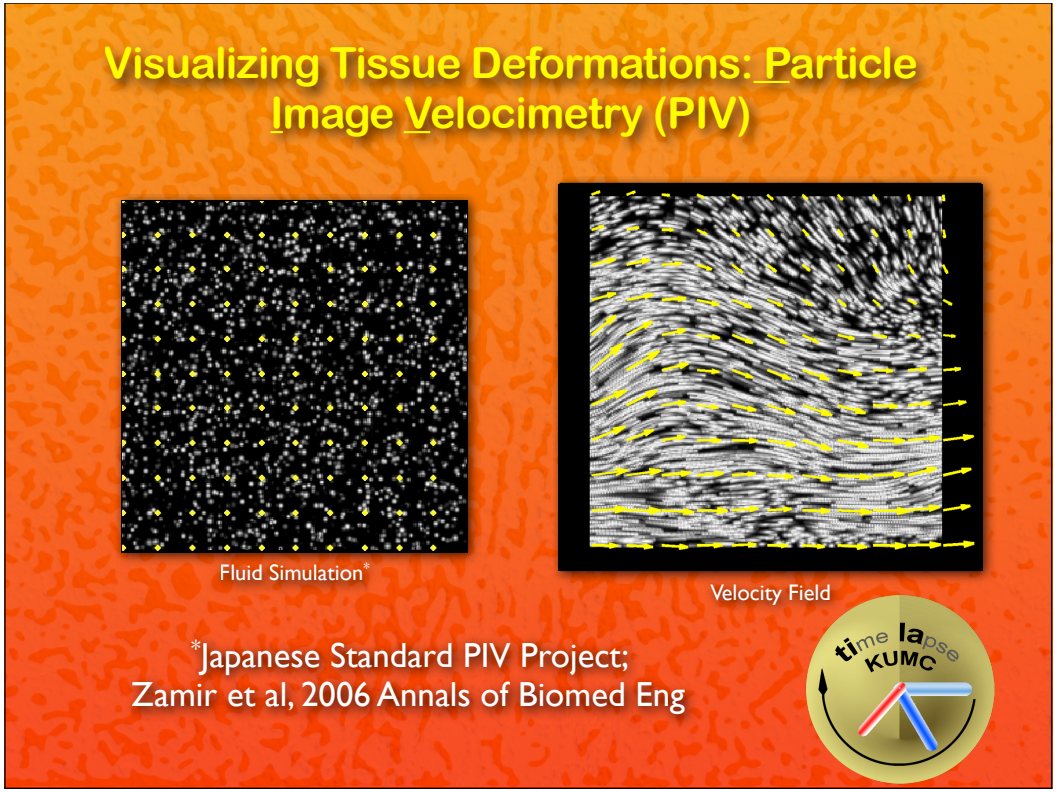


green - electroporated H2B :cells

white - mAb labeled FN

DNA Plasmids; provided by Rusty Lansford, CalTech

time lapse KU



To measure large-scale motions of the embryo, we use an engineering method called particle image velocimetry (PIV).

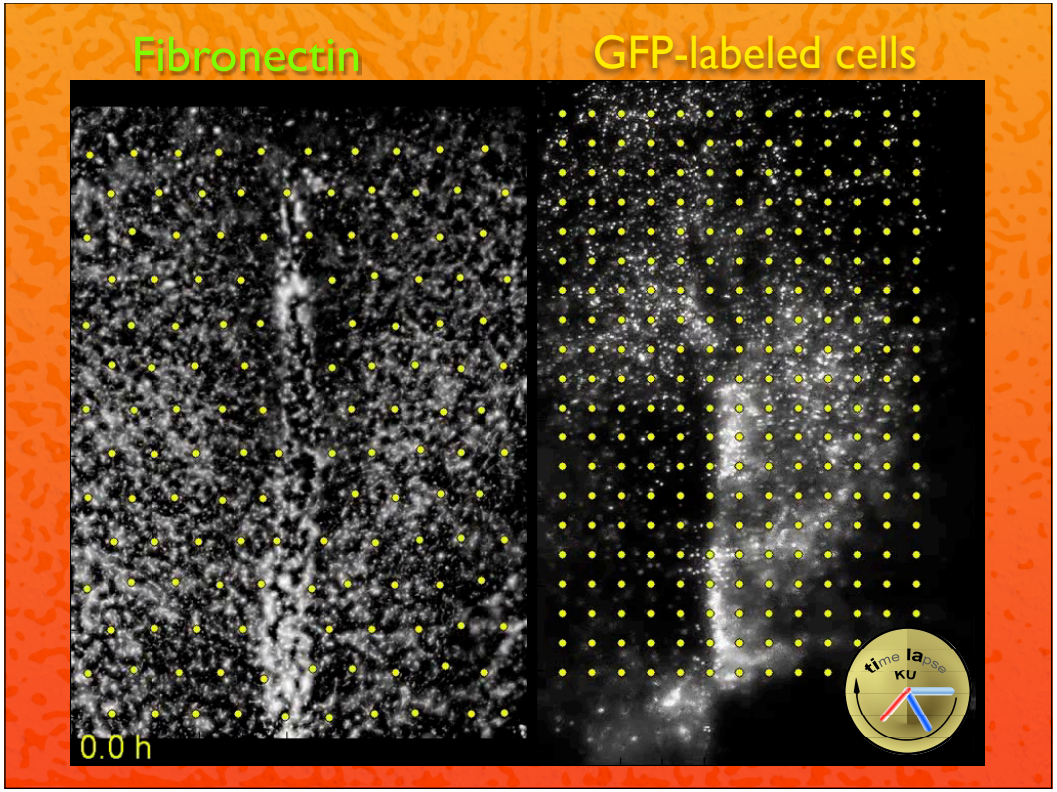
PIV is typically used for measuring flow dynamics (i.e. blood flow), but it can also be used to measure correlated motion caused by mechanical deformation of soft tissues.

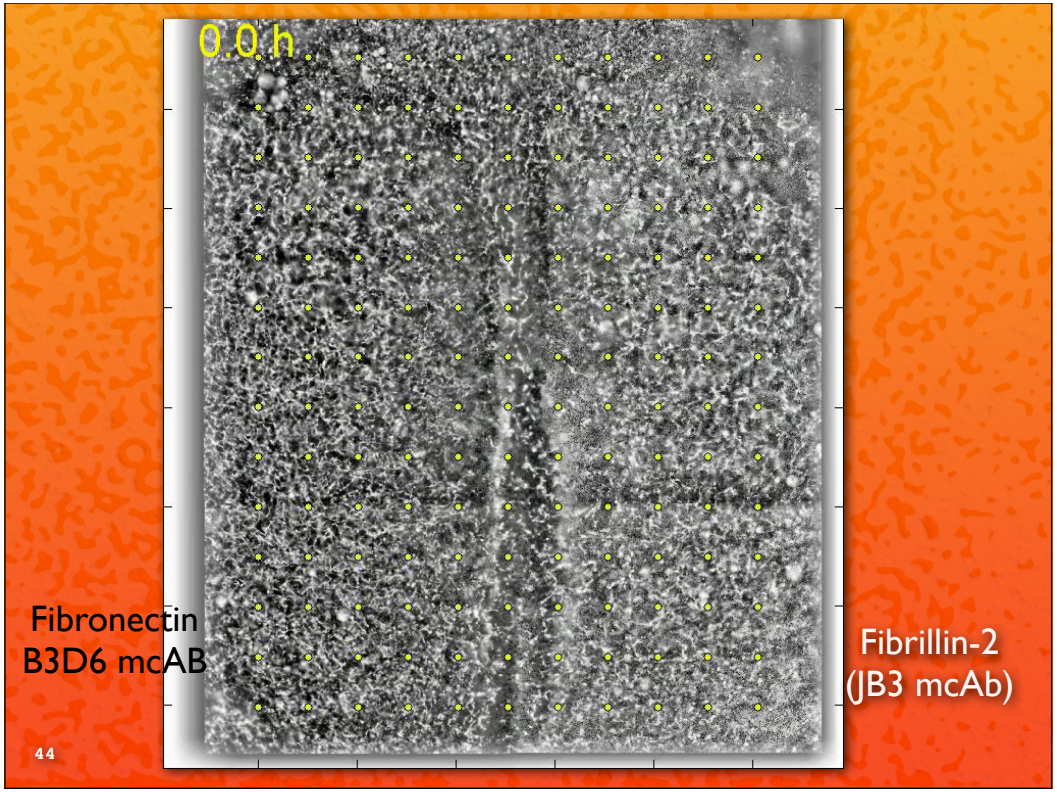
Here is a demonstration of our PIV algorithm on a standard test sequence of synthetic images simulating fluid flow with some arbitrary velocity profile. The motion of the yellow particles was determined by the PIV algorithm to best match the direction and velocity of the simulated flow, represented by the white particles.

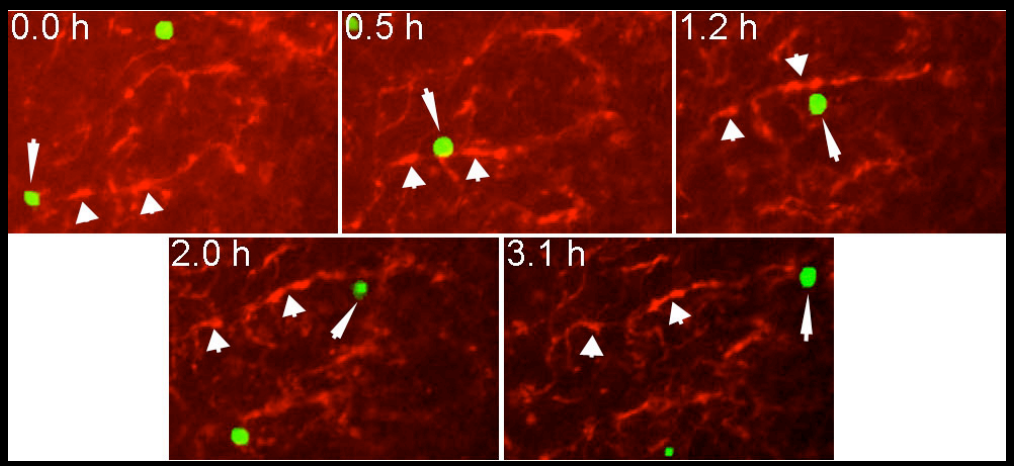
It may help to think of the yellow particles as virtual leaves that are float down a stream and passively follow or are convected by the changing current. The PIV method allows us to calculate the current automatically using only the pixel intensity information in the images, themselves.

At the right is shown an image of this test sequence projected over time, along with the computed velocity vectors for each PIV virtual particle. This represents the velocity field.

The main advantages of PIV are that it is a rapid automated technique capable of measuring motion fields very accurately, even for images or labels whose features would be too difficult to track by eye. The only real disadvantages of PIV are that it requires a sufficient amount of image information for a robust analysis.


Fibronectin
GFP-labeled cells
0.0 h
time lapse
KU

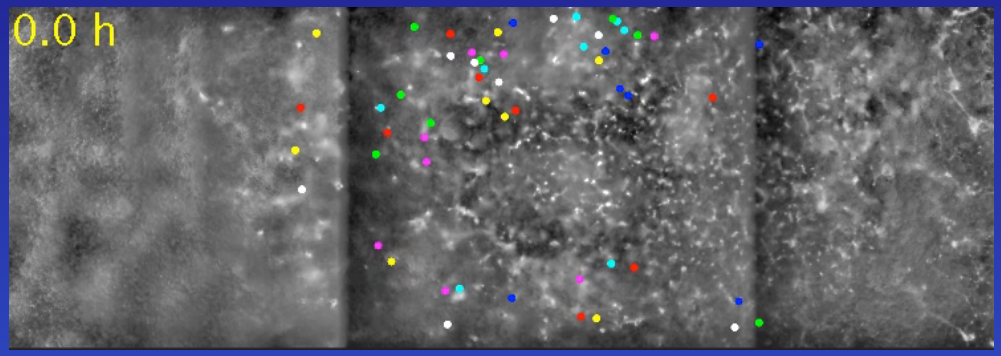

0.0 h
Fibronectin
B3D6 mcAB
Fibrillin-2
(JB3 mcAb)
44



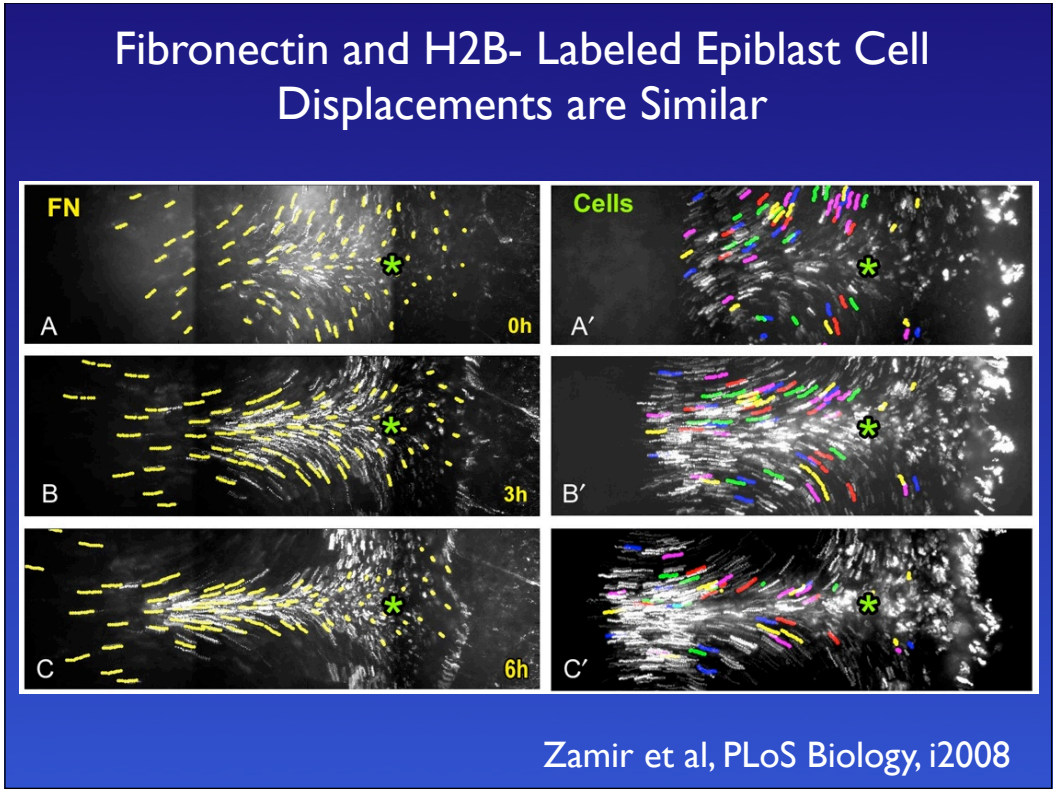
Cell-autonomous motility (green) with respect ot to the ECM (red)

Zamir et al, PNAS, 2007

Projections of Cellular Trajectories and Corresponding Fibronectin Motion



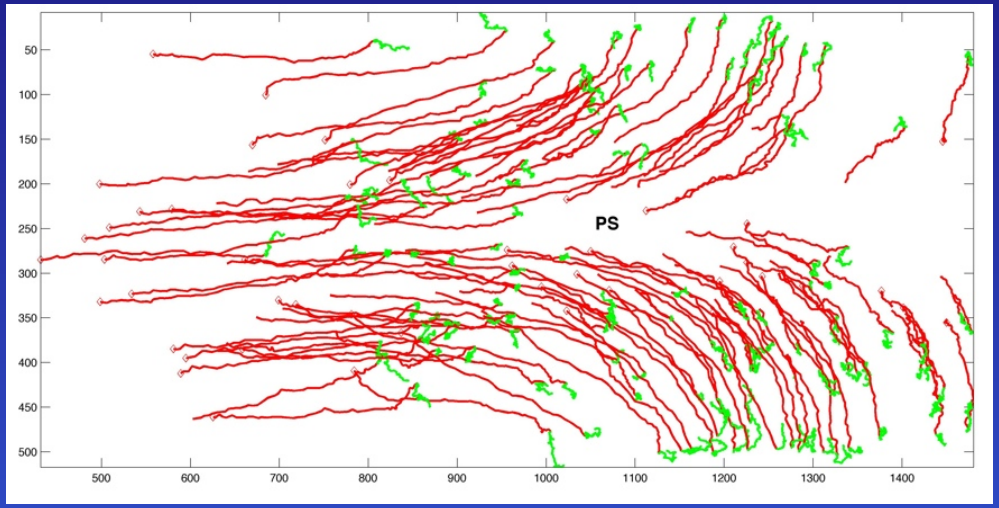
Zamir et al, PLoS Biology, 2008



left: fibronectin fibril displacments and VMP displacements

right: tracked cell displacements overlaying fluorescent total cell population

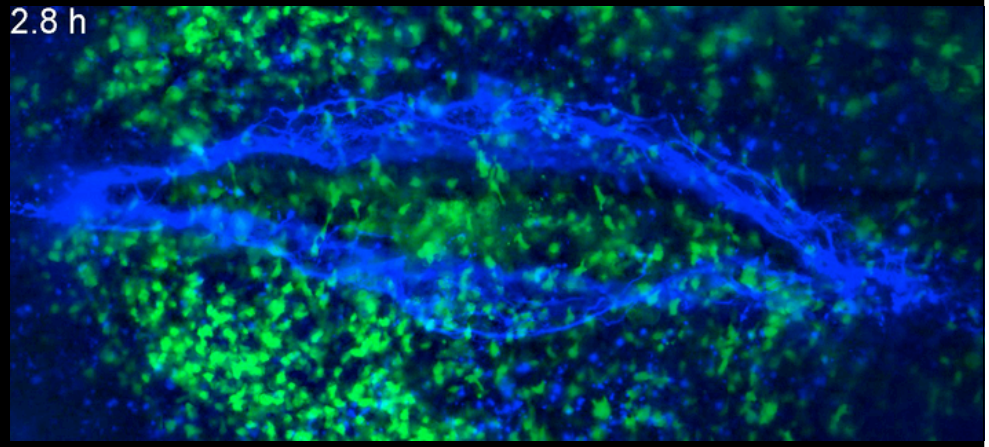
Cell Autonomous Motility is Much Less Than Tissue Motion



Cellular trajectories: total (red); autonomous (green);
Zamir et al, PLoS, 2008

near streak, CCA may be even less than further away from PS
next step, correlate individual cells and nearby FN fibrils
PIV is less accurate near edges and where FN label is not good.

Shared large-scale motion pattern of ECM & cells during gastrulation



Zamir et al, unpublished

green - electroporated H2B :cells

blue - mAb labeled FN

Deficiencies:

Lack of critical mass of experimental studies on cellular to tissue level lenght scales.

There is fifty years of reductionism describing genes and intracellular signaling pathways but relatively little work on how tissues form and move

Deficiencies:

No data regarding the material properties of the embryonic tissue; much less how the material properties change during morphogenesis .We therefore need some way of conducting time-lapse material properties measurements.

We simply do not understand the forces that shape the amniote embryo. Convergence likely occurs, but there are morphogenic forces in bird and mammal embryos that are completely unknown. For example, if local cell shape change is a morphogenic mechanism it appears to operate globally.

This is why a meaningful mathematical model and simulations would be so helpful.

Components of the system:

Cells
ECM
Physical forces much greater than a single cell or even a cluster of cells could generate

Cellular collectives or transitory tissues
- Some autonomous motility- independent agents
- Cell shape change as a collective force
- Shared cell displacements= tissue motion
- Creation of gradients between two states: autonomous motility v. coherent tissue

Assembly of the ECM fibers
- Motion dependancy
- Cellular autonomous motility driven or tissue displacement driven or both?

Deformations driven by “outside” forces
- Epipboly (radial spreading of a sheet under tension)
- Formation of rigid body the notochord?
- Stiffening of cell sheets and rods via cell:cell adhesions
- Stiffening of cell sheets and rods via actin/myosin cytoskeleton
- Stiffening of ECM via dehydration and crosslinking
- Loss of hydrated space-filling molecules, principally hyaluronan

Shear between cell layes
- Basement membrane as a lubricant?

"If you're not part of the solution

you're part of the precipitate"

Henry J. Tillman

Possible Talk Outline

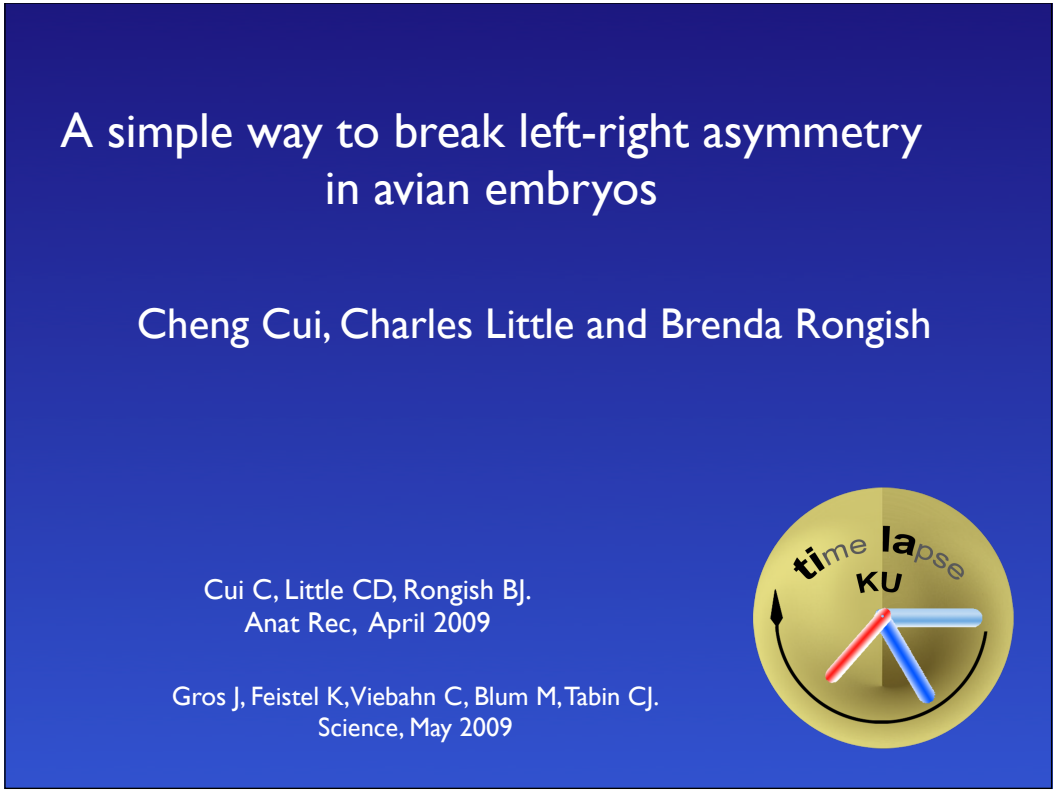
- Overarching Scientific/Medical/Engineering Context (1-2 slides).
- System Under Study (1-3 slides).
- Key Scientific/Medical/Engineering Questions Addressed (1-2 slides).
- Current Methodologies Employed (1-2 slides).
- System Components (Cell Types, ECM, …) (2-4 slides)
- Key Component Behaviors (2-4 slides).
- Deficiencies of Current Methods/Approaches, Needs (1-2 slides).
- Results, Focusing on HOW they are Expressed (1 slide).
- Possible Applications of CBO/CBMSL/Repository (1-2 slides).

Guidance

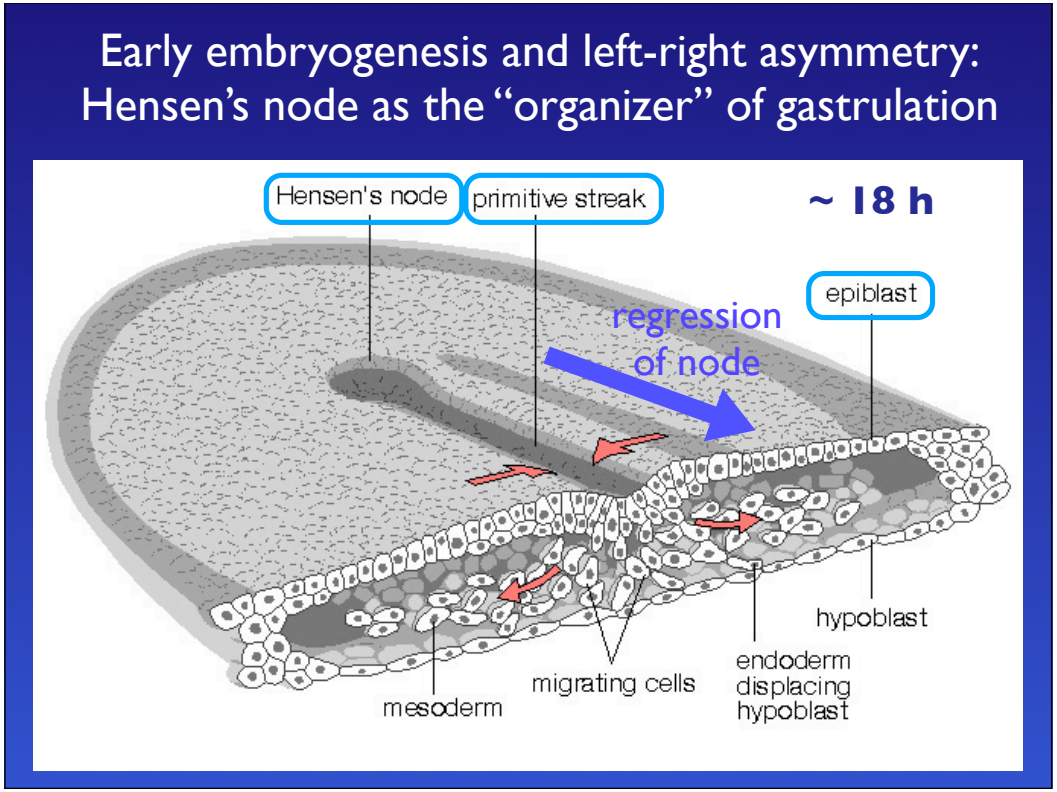
- Think top down.
- Think about how you Describe your Experimental Results/Simulations to Others.
  - What Components (Cell, ECM…) are Involved?
  - What Behaviors, Morphologies,…are **Crucial** to these Components in your Particular Problem?
    - Start as Generically as Possible.
    - Treat Cells Initially as Black Boxes.
    - Add Detail Hierarchically.
    - Separate Control (differentiation) from Behaviors.
    - Stop when you become Quantitative.
  - What Language do you Use to Describe Locations?
  - Do all Components (Cells) of a Given Type Share the Same Behaviors?

Requests for Talks and Talk Structure

- Focus on Goal of Identifying in Your Work:
  - Key Components.
  - Component Behaviors.
  - Collective Behaviors.
  - Commonality/Uniqueness of Components/Behaviors.
  - Key Scientific Questions.
  - Methodological Needs.
  - Utility/Applications of CBO/CBMSL/Repositories.
- How could simulations advance understanding (for experimentalists)?
- What experimental information is missing (for simulators)?

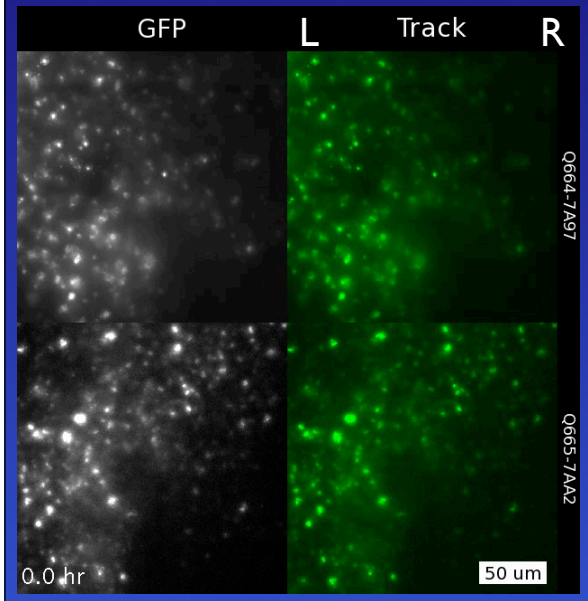


situs inversus leads to reversement of organ placement; may not be aware unless report with appendicitis with pain on your left side. portion due to defects in cilia



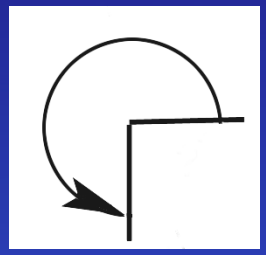
Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut; organizer in mice

Observed counterclockwise rotation of the "nodal" cells



dorsal view

electroporated *H2B-GFP: biased to embryo left

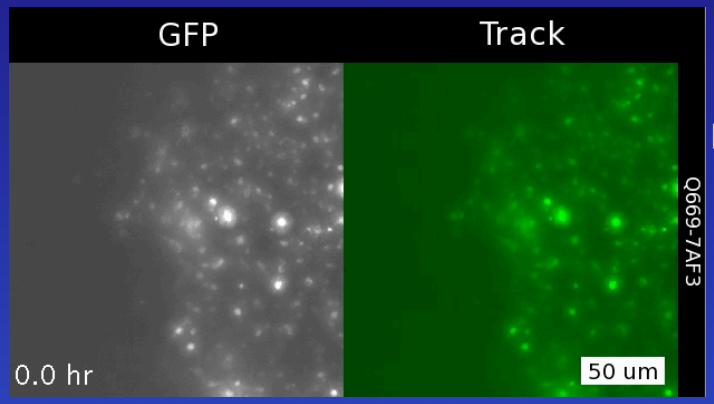


tracks are 20 consecutive frames;

green = most recent 5 frames

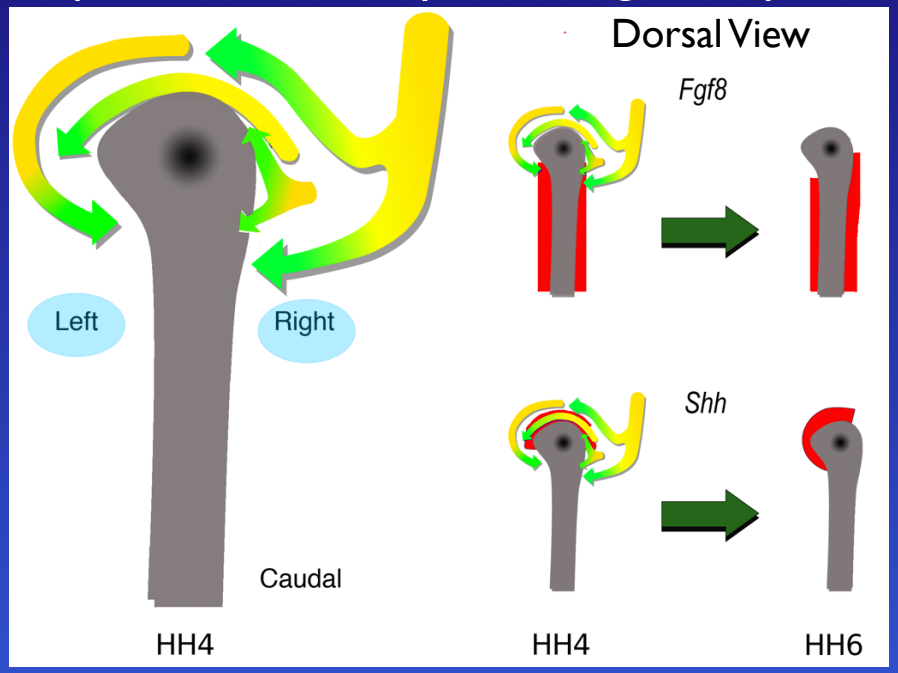
*courtesy of R. Lansford, Caltech

Clockwise rotation of the node in one sector; "fork" is observed 60-100 µm from node



electroporated
H2B-GFP
biased to embryo R

Proposed model of asymmetric gene expression



Conclusion

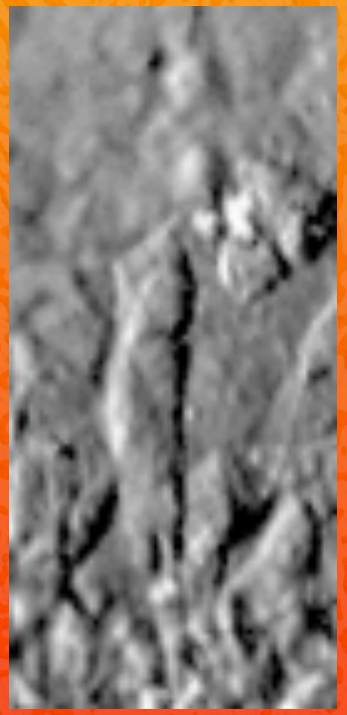
- The early tissue deformations of warm-blooded embryos are examples of emergent patterns —
- Such patterns are variable among individuals and no gene or small number of genes has been linked to the formation of any particular formed element.

SO WHAT?

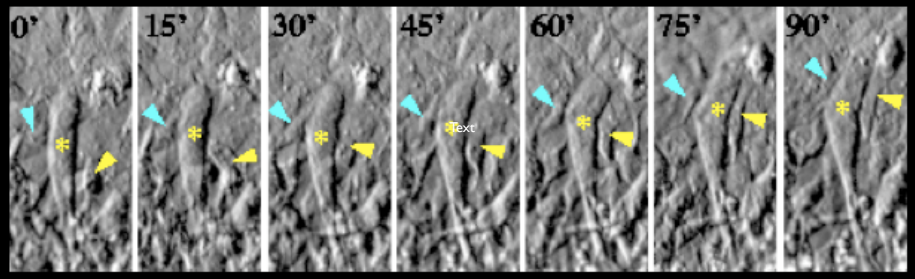
Vertebrate morphogenesis will not be understood until bioengineers, biomathematicians and biophysicists define the rules that integrate tissue motion with genetic and cellular signaling regulatory networks.

Andras Szabo
Grad Student with
Andras Czirok, Eotvos Univ.
Budapest

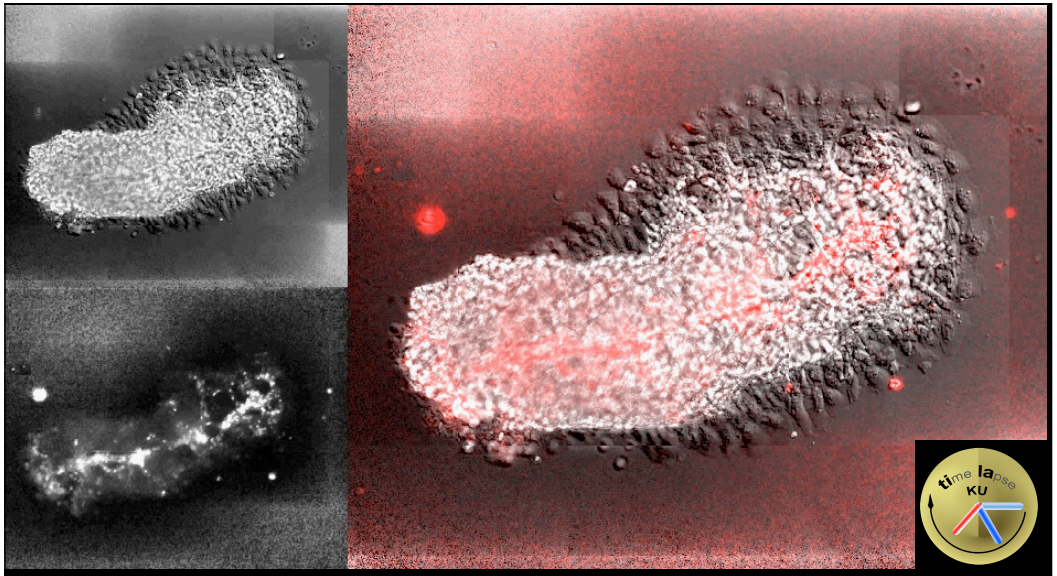
Simulations made using cellular "Potts" code according to Roland Merks and James Glazier



A presumptive tip cell "overtaking" the existing tip cell



This allows a multicellular sprout "keep up" with the constantly expanding bulk tissue

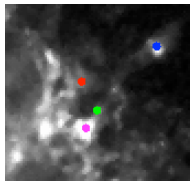


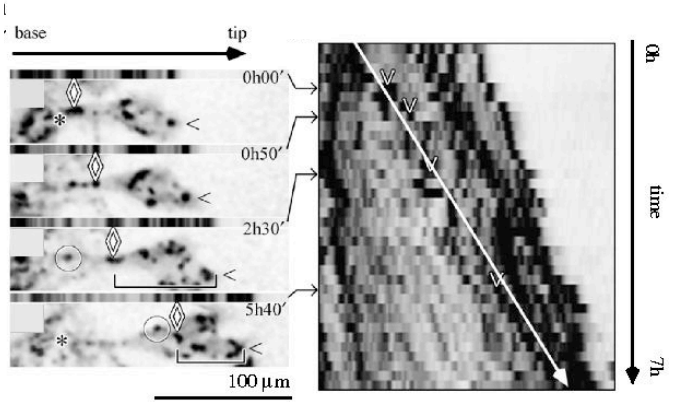
Time-Lapse of Planar Allantois culture

Perryn et al, Developmental Biology 2008

Mouse allantois explant

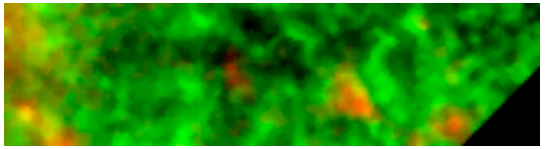
sprout formation

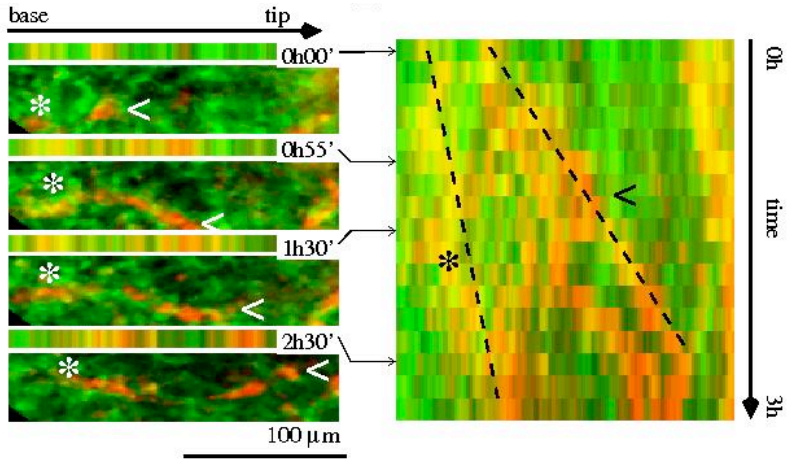




Vasculogenesis in a quail embryo

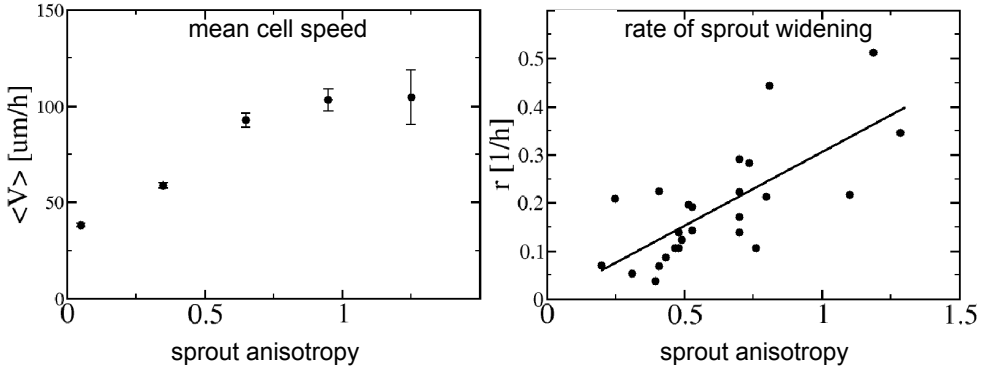
sprout formation





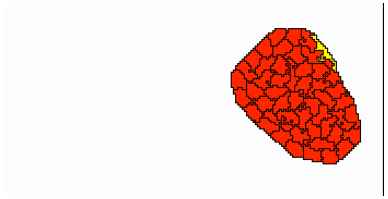
C6 cell culture

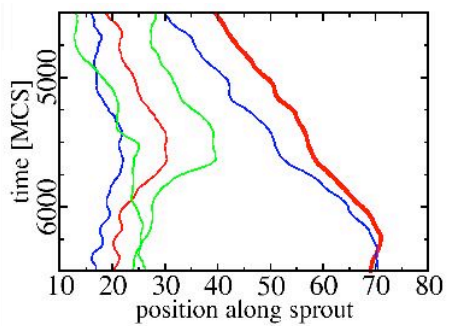
Preferential attraction to elongated cells



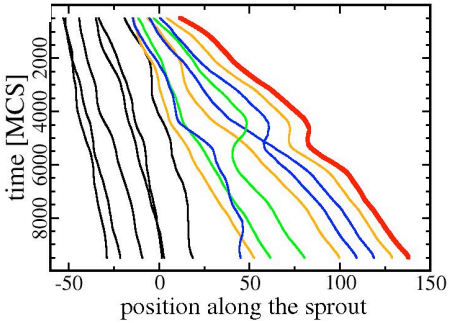
A leader cell and…

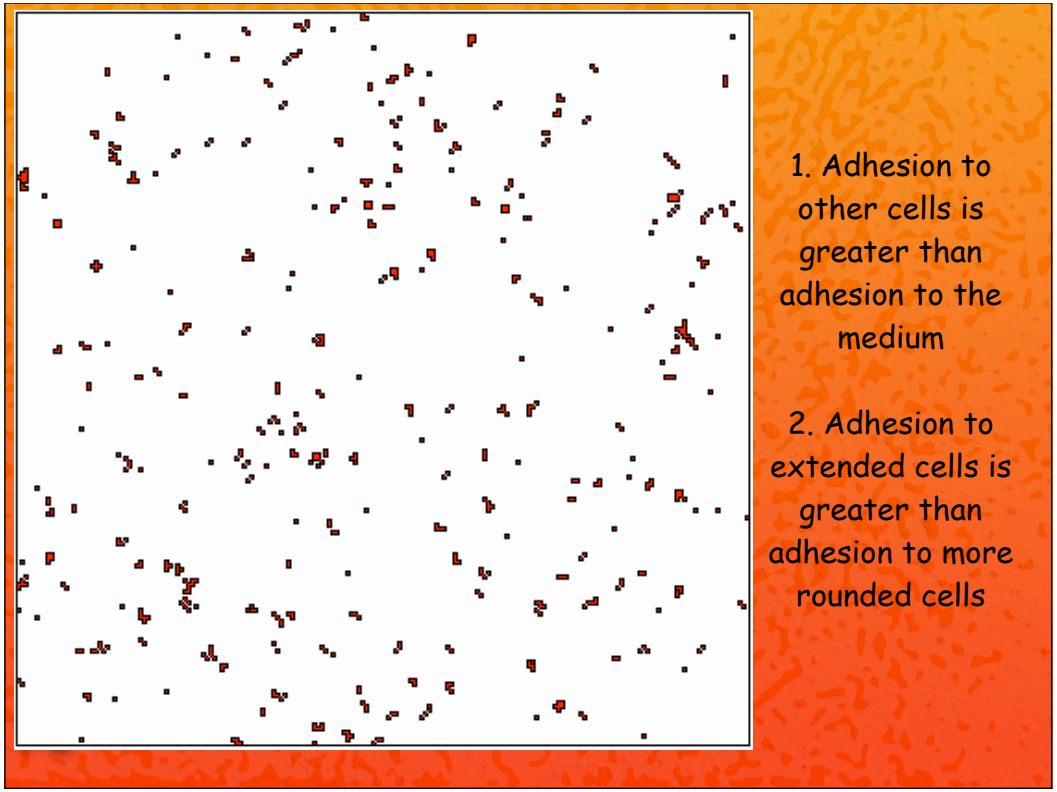
cell-cell adhesion only





preferential adhesion to elongated cells





1. Adhesion to other cells is greater than adhesion to the medium

2. Adhesion to extended cells is greater than adhesion to more rounded cells

Transgenic Quail

Constructed by Rusty Lansford, Senior Scientist, CalTech

Future Directions

TRANSGENIC QUAIL

Rusty's Transgenic Quail

- Tie1-GFP germline tranformed Japanese Quail
- The fluorescent reporter construct is a nuclear-directed H2B-GFP; Tie1 is a vascular endothelial cell marker

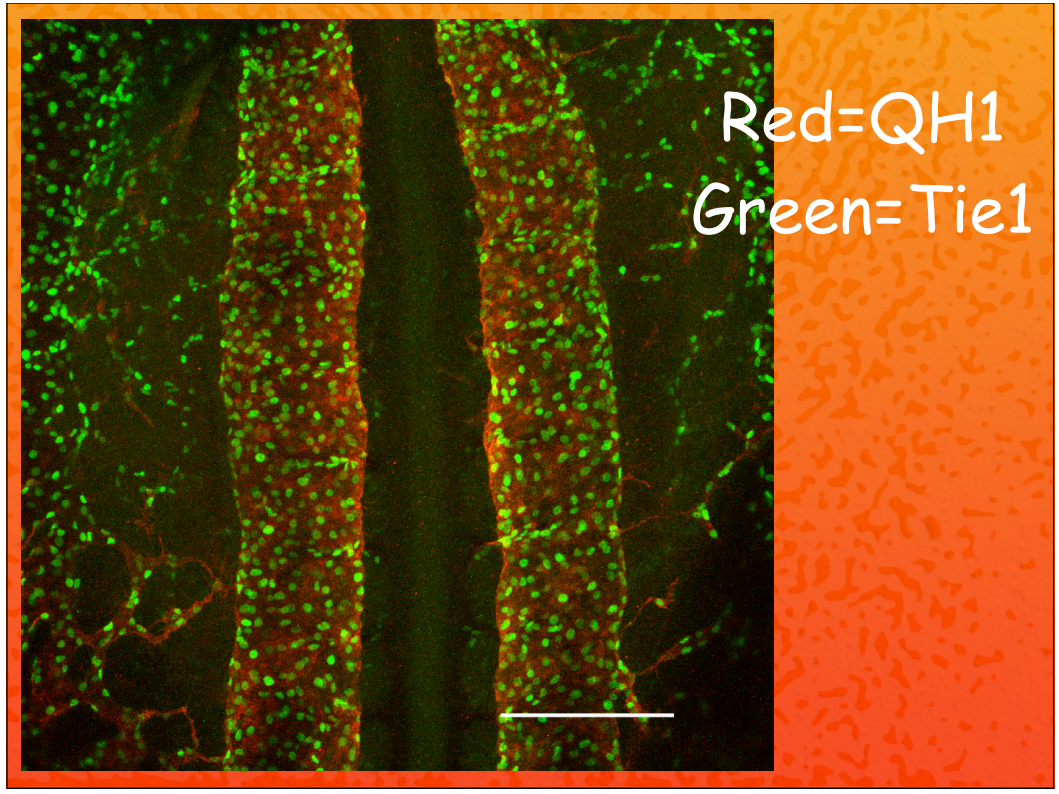
IN OTHER WORDS:

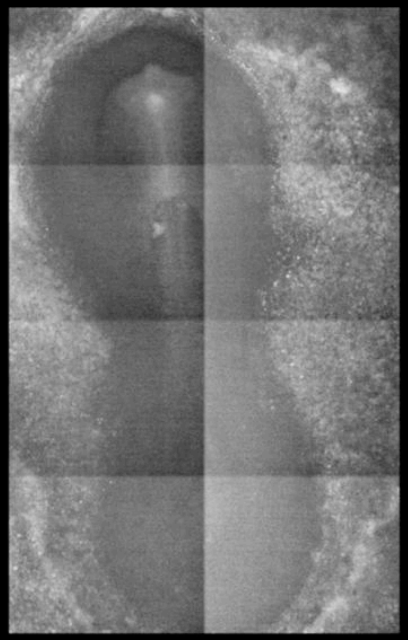
The grueling 6 years of work needed to make transgenic quails was accomplished by

Rusty Lansford

NOT

Charlie Little


Red=QH1
Green=Tie1



Tie1 nuclear GFP transgenic quail - approximately 40 hours of time-lapse

Empirically Determined Physical Rules of how to form vascular polygons*

1. Angioblasts are distributed randomly in the mesoderm
2. Primordial ECs extend protrusions in random directions
3. ECs will hold hands if possible, form stable protrusive contacts
4. ECs only hold hands with one or two neighbors
5. Autonomously motile ECs move with high persistence
6. The lowest free energy state of assembly is polygons
7. Excess tissue strin causes ECs to subdivide the polygonal pattern
8. ECs appear to preferentially adhere to rigid bodies (Czirok et al)
9. "New" ECs preferentially move on pre-existing vascular elements
10. Vascular sprout "base" cells move distally to form new tip cells.
11. At very high density endothelial tubes fuse their lumens.

* in warm blooded amniote embryos