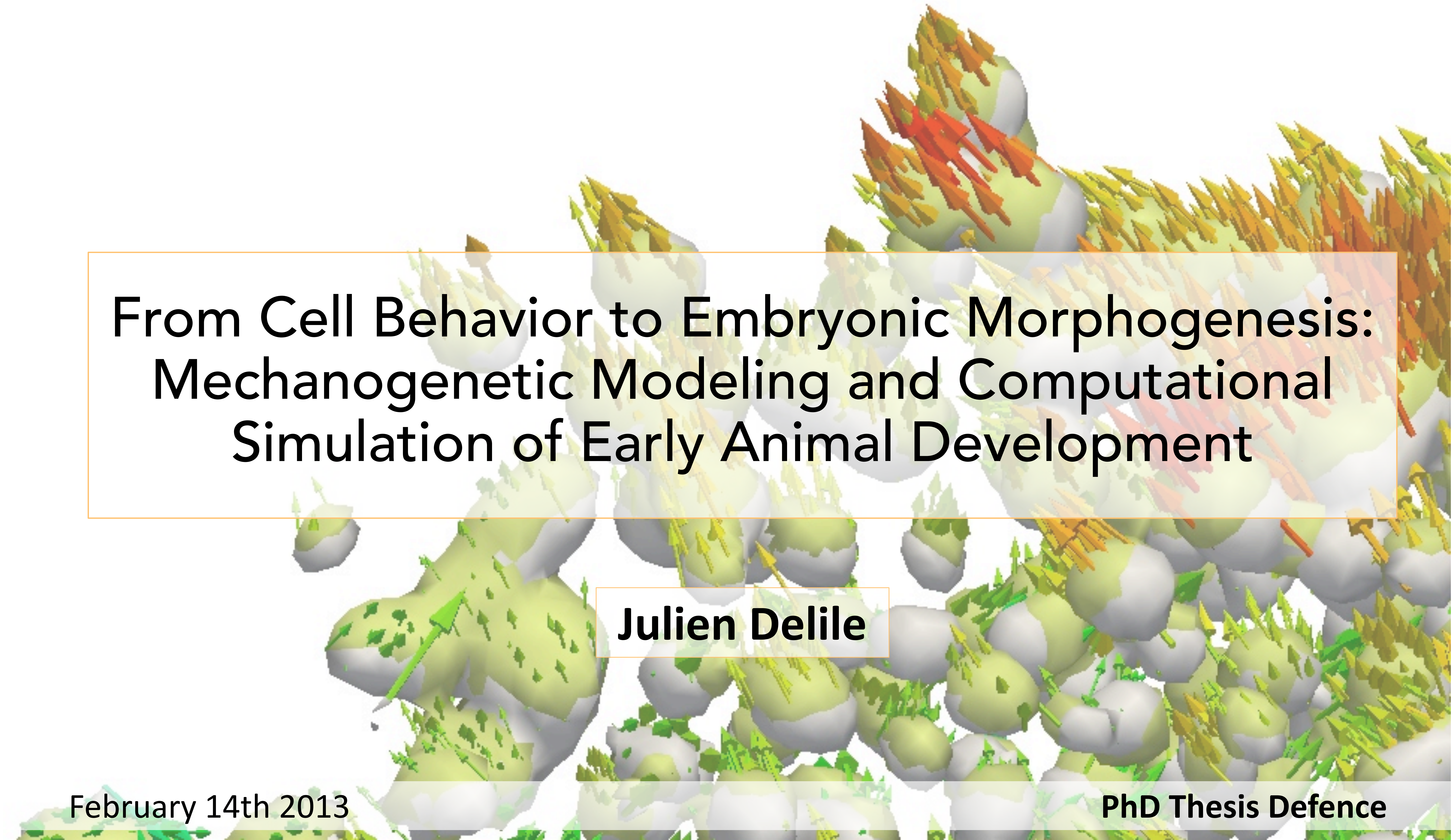
# From Cell Behavior to Embryonic Morphogenesis: Mechanogenetic Modeling and Computational Simulation of Early Animal Development

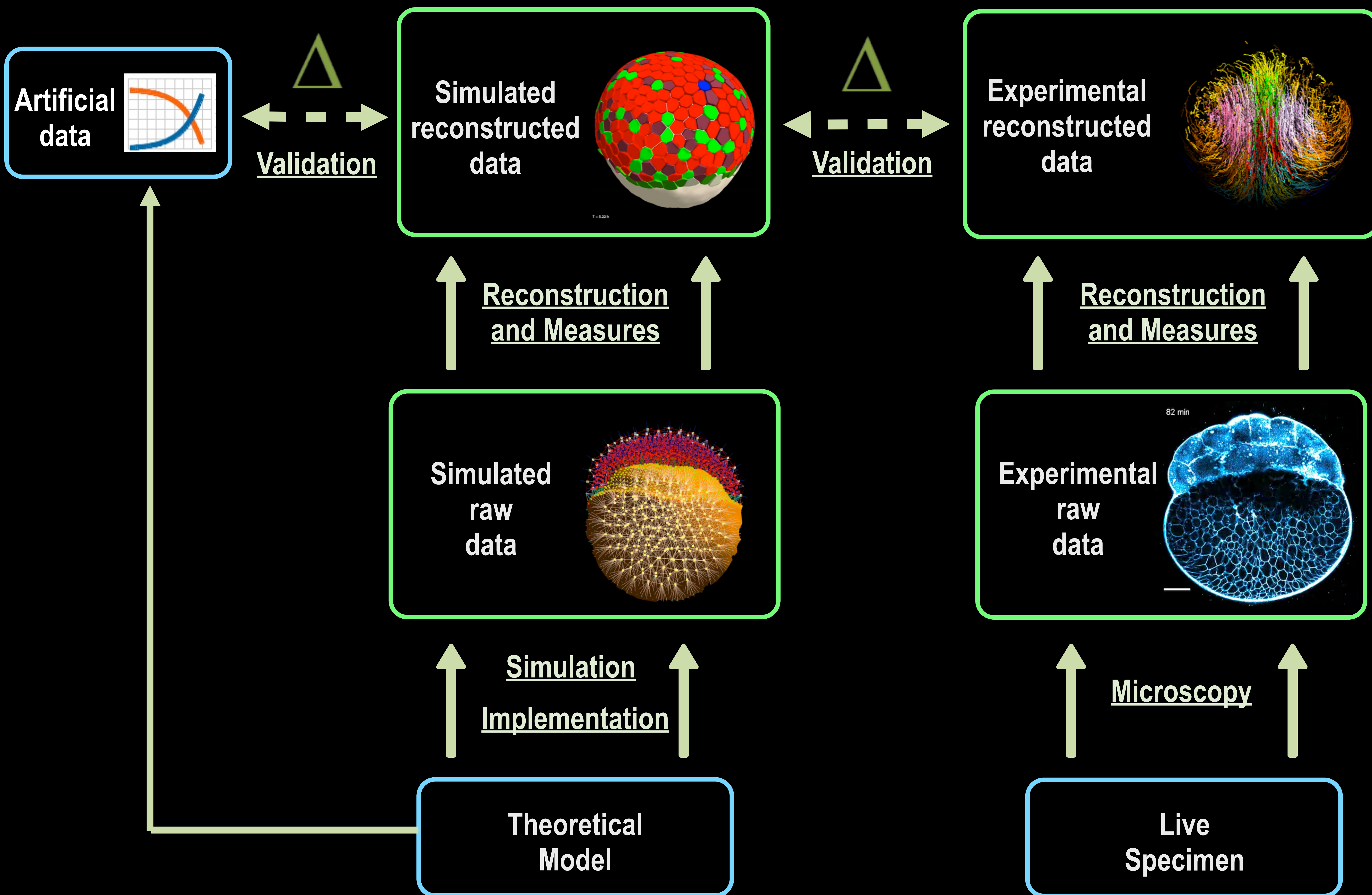


**Julien Delile**

February 14th 2013

**PhD Thesis Defence**

# A methodological framework to confront live and simulated data



# Outline

- **MECAGEN Model**
  - Cell Biomechanics
  - Molecular Interactions, Genetic Regulation and Cell Signaling
  - Coupling
- **Applications**
  - Zebrafish early development
  - Six case studies

# The components of the model

**Biomechanics (“MECA”)**

- spatial neighborhood
- attraction-repulsion potential
- protrusive forces

**Molecular and Genetic Regulation and Signaling (“GEN”)**

- gene/protein interactions
- cellular transduction
- extracellular diffusion

# Particle-based physics: 1 particle per cell

Newton's second law

$$m_i\vec{a}_i = \sum \vec{F}_{\text{applied on } i}$$

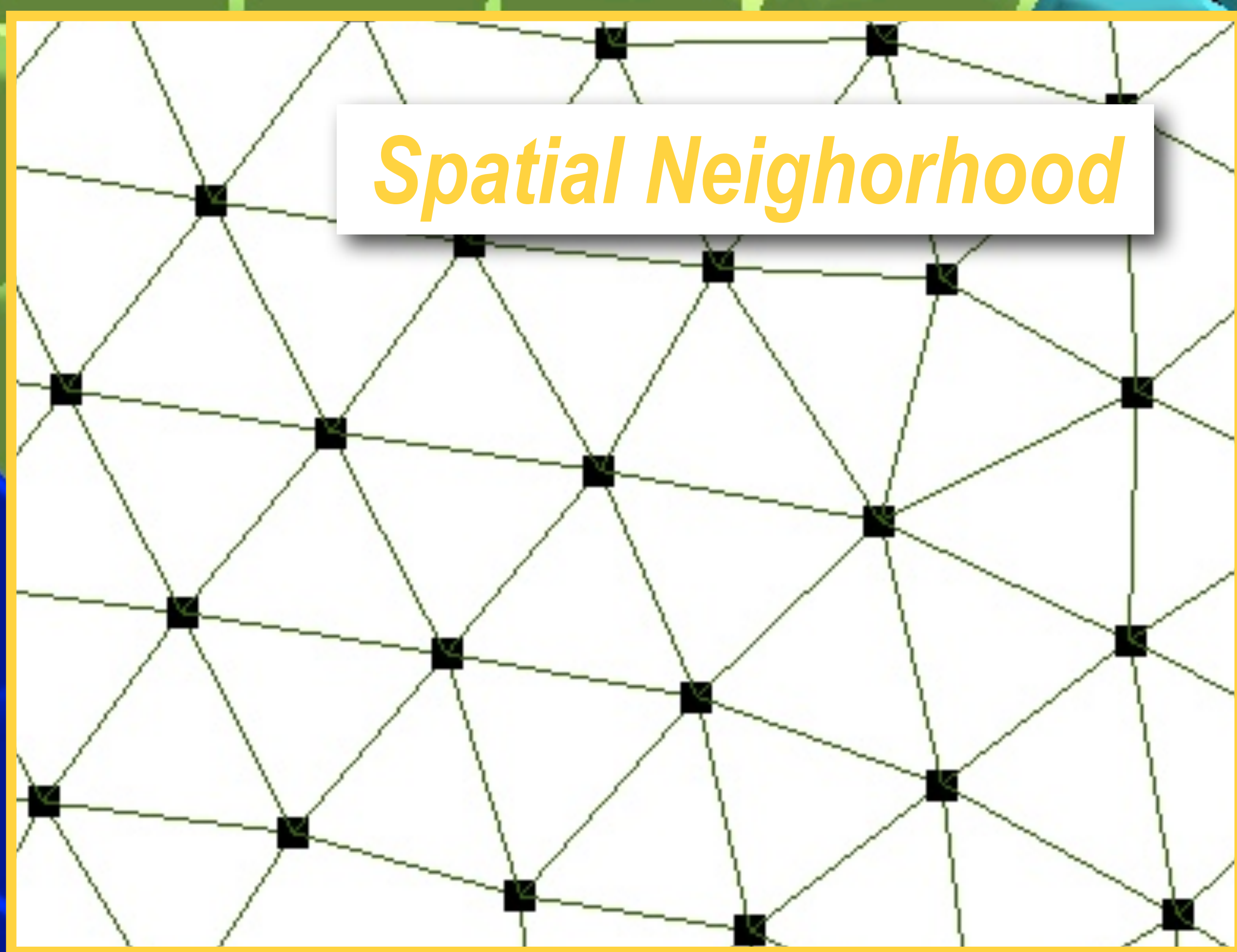
$$m_i\vec{a}_i = \vec{P} + \vec{F}_{visc} + \sum_{j\in\mathcal{N}_i} \vec{F}^{\text{cell}}_{ij}$$

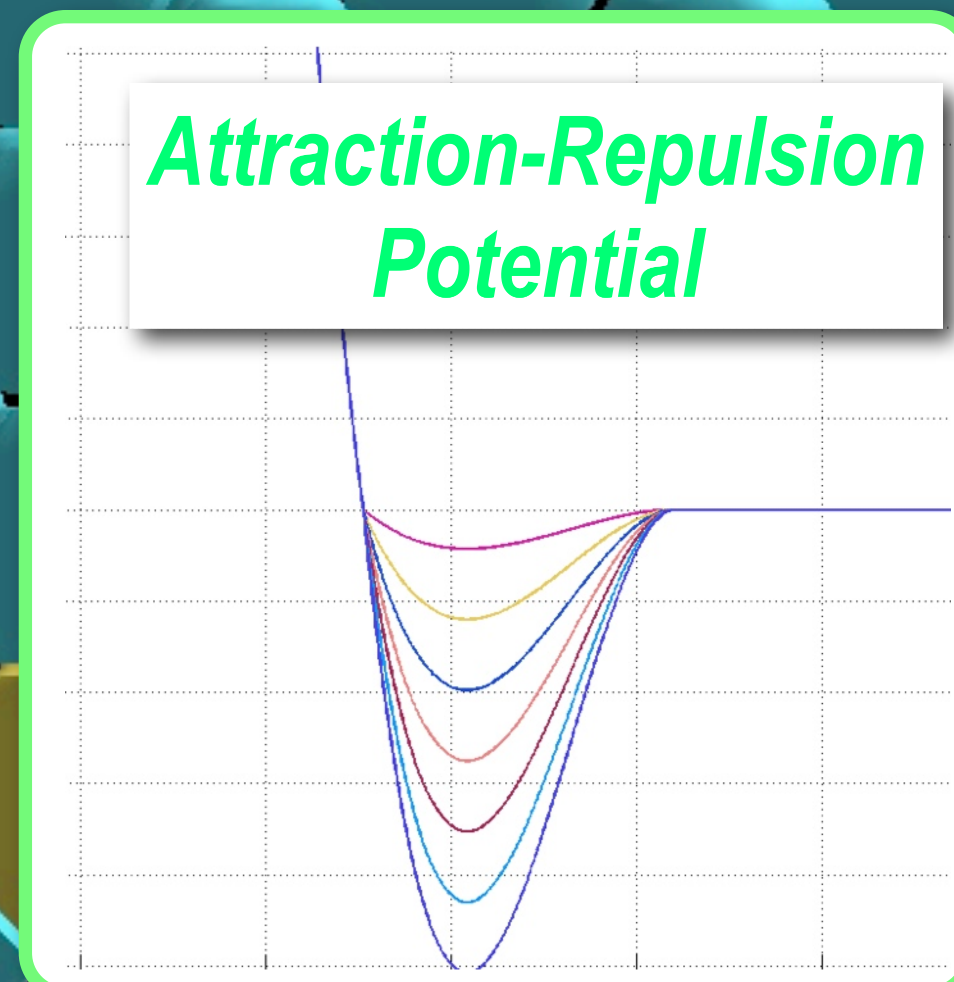
*Inertia and weight forces are neglected*

$$\cancel{m_i\vec{a}_i} = \cancel{\vec{P}} - \lambda_i\vec{v}_i + \sum_{j\in\mathcal{N}_i} \vec{F}^{\text{cell}}_{ij}$$

$$\vec{v}_i = \frac{1}{\lambda_i}\sum_{j\in\mathcal{N}_i} \vec{F}^{\text{cell}}_{ij}$$

# The three mechanical components

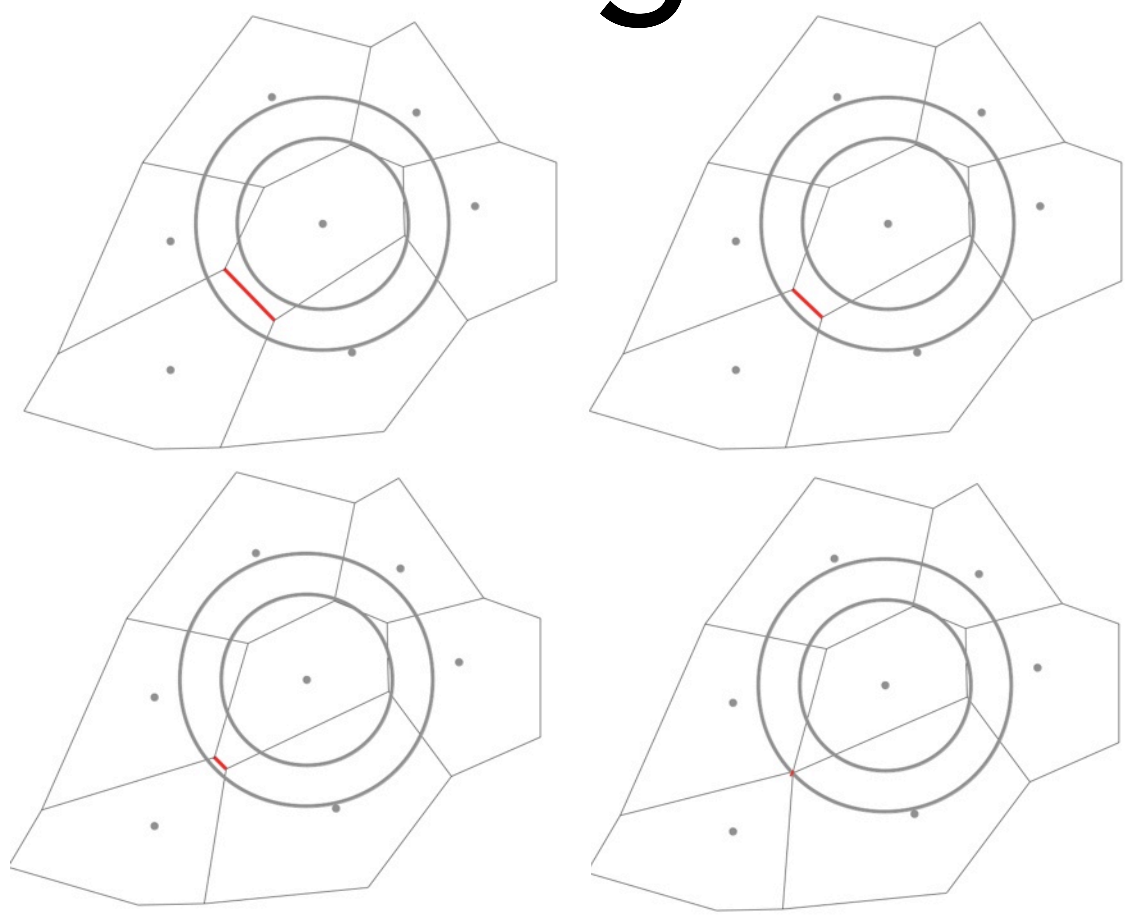


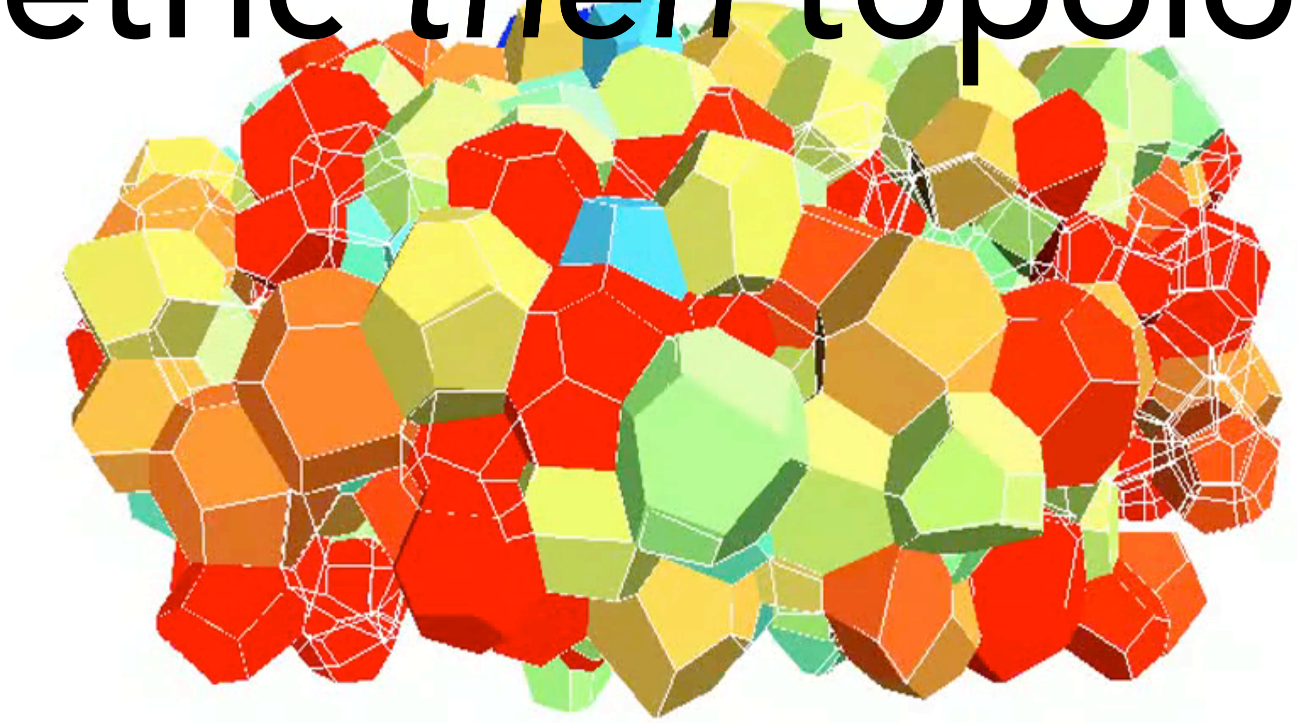





$$\vec{v}_i = \frac{1}{\lambda_i} \sum_{j \in \mathcal{N}_i} \vec{F}^{\text{cell}}_{ij} = \frac{1}{\lambda_i} \sum_{j \in \mathcal{N}_i} \left( \boxed{\vec{F}^{P}_{ij}} + \boxed{\vec{F}^{A,\text{int}}_{ij} + \vec{F}^{A,\text{ext}}_{ij}} \right)$$

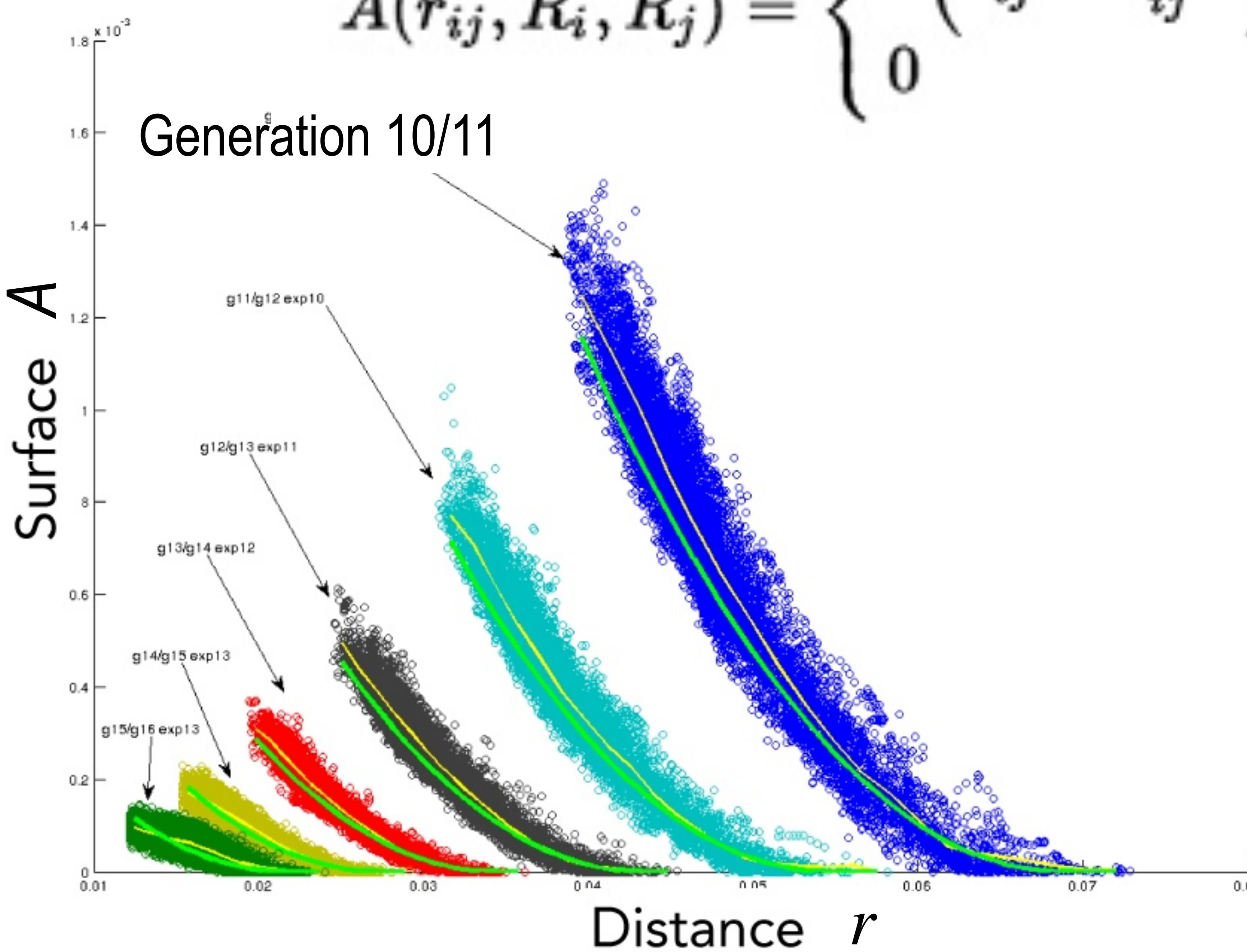
# Spatial neighborhood: metric *then* topological

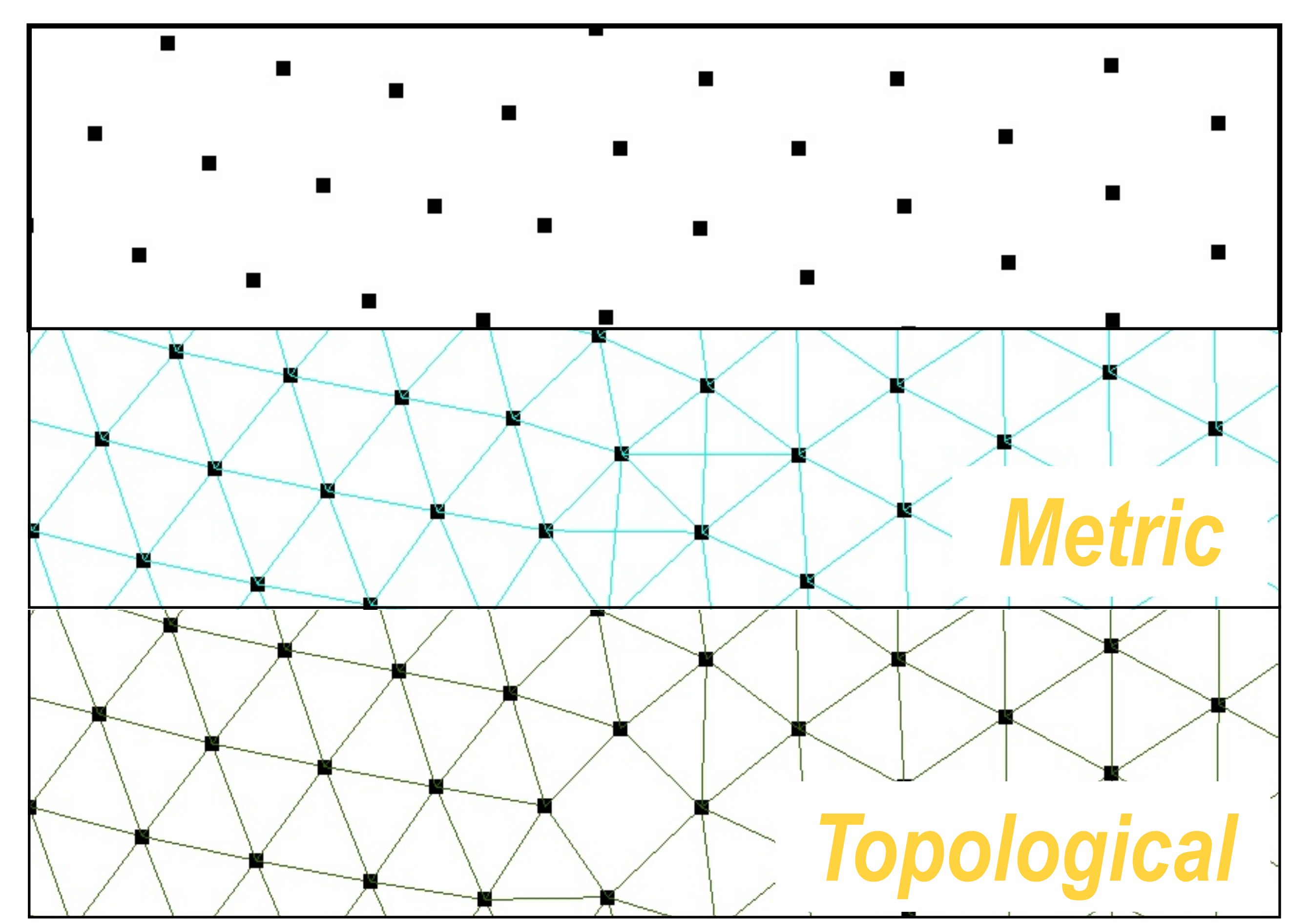




Surface:

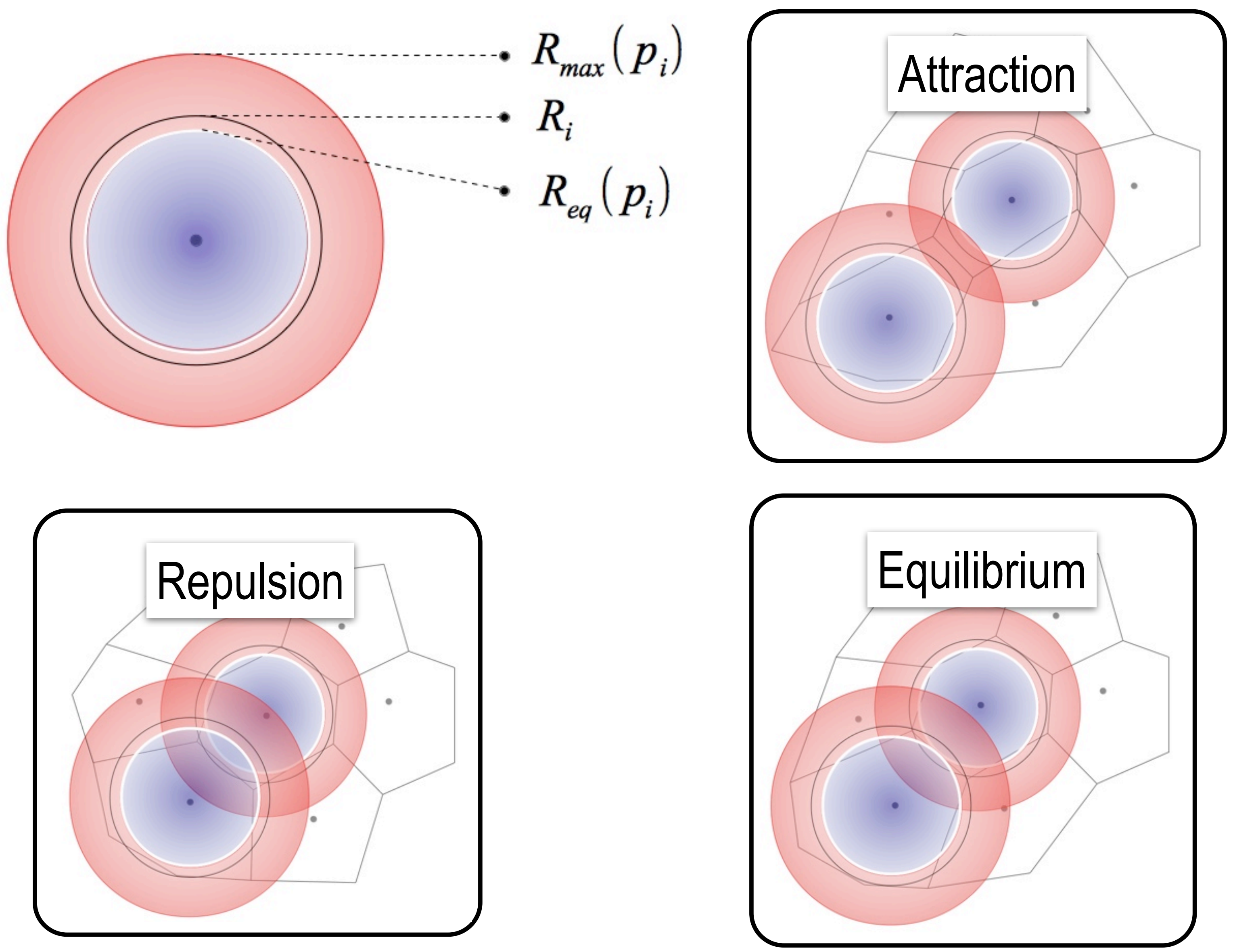
$$A(r_{ij}, R_i, R_j) = \begin{cases} a\left(r_{ij} - r_{ij}^{\max}\right)^2 & \text{if } r_{ij} < r_{ij}^{\max} \\ 0 & \text{if } r_{ij} \geq r_{ij}^{\max} \end{cases}, \quad \text{where} \quad r_{ij}^{\max} = c_{\max}(R_i + R_j)$$



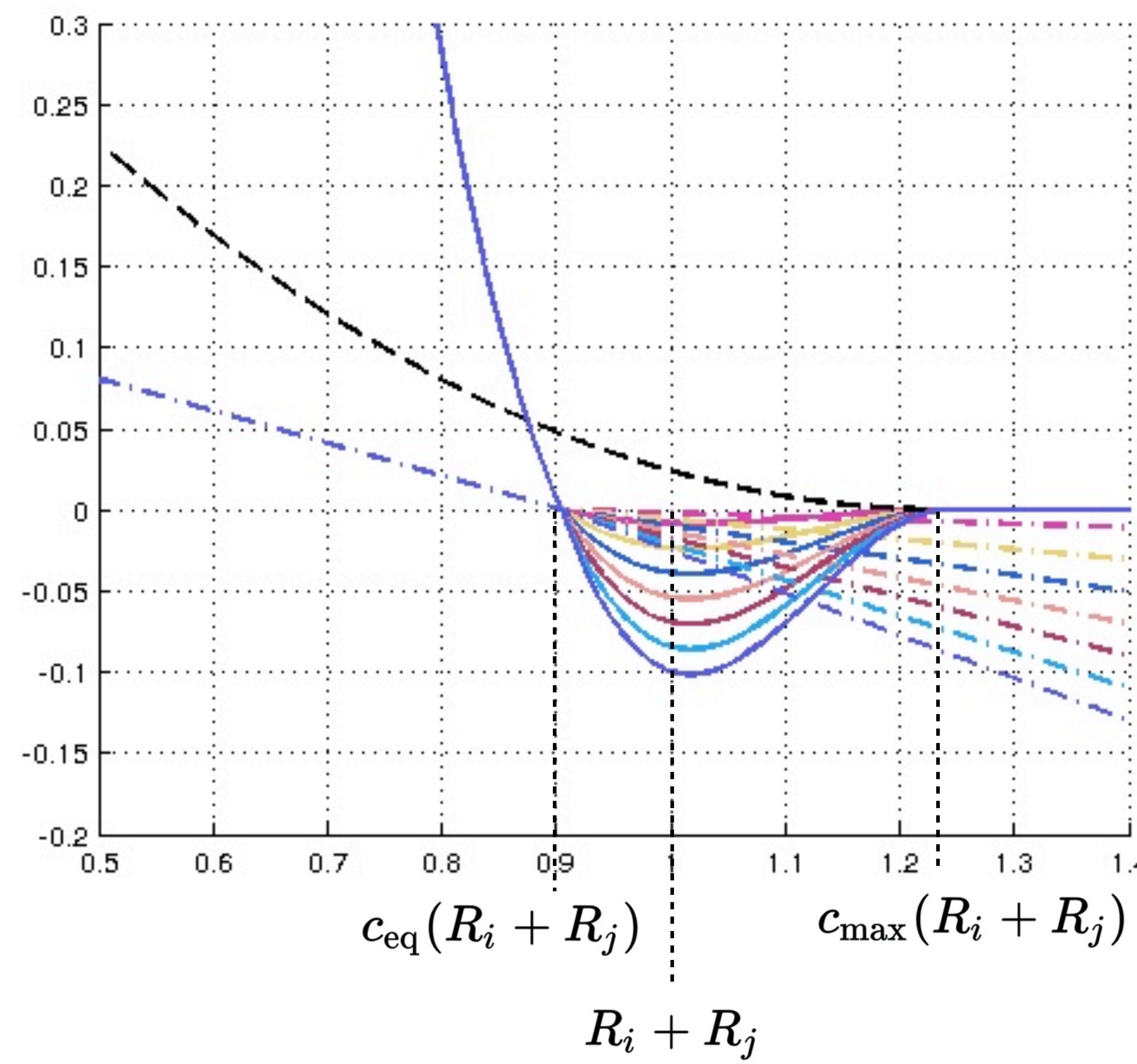



# Passive interaction: attraction-repulsion force

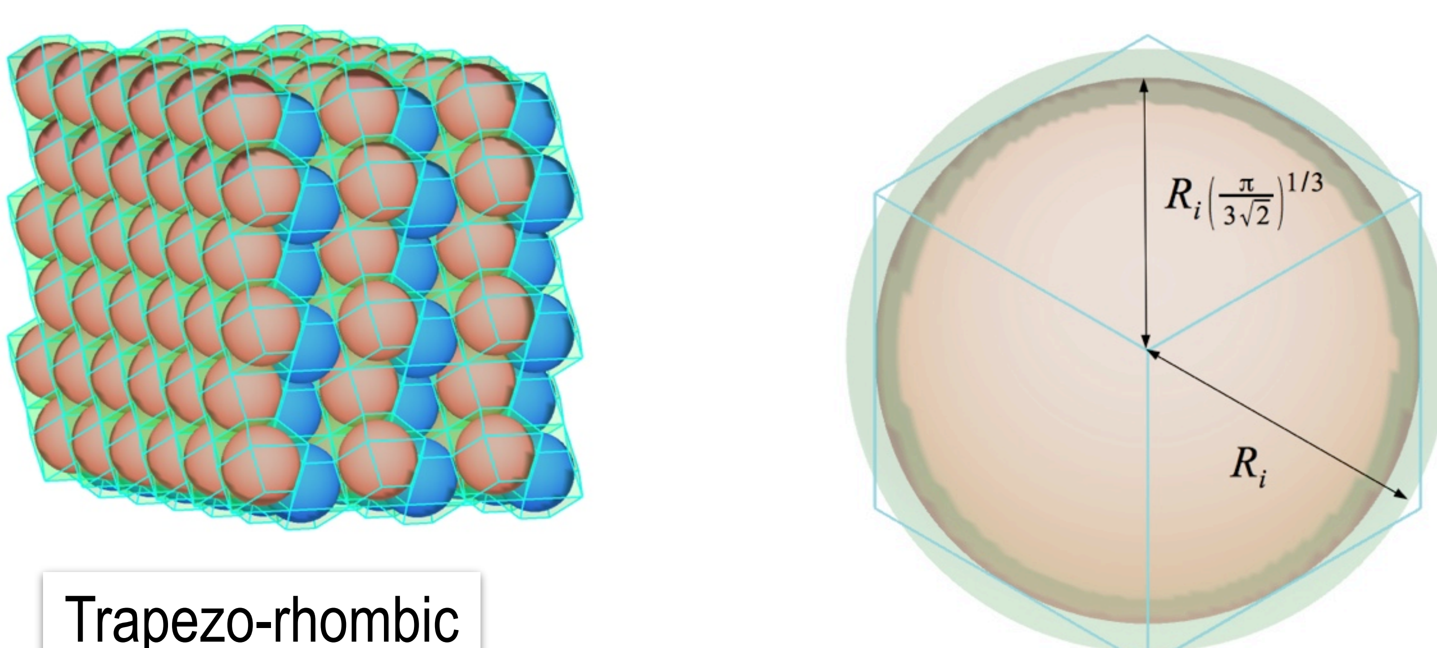
$\vec{F}^P$




## Linear spring-like force scaled by the surface of contact

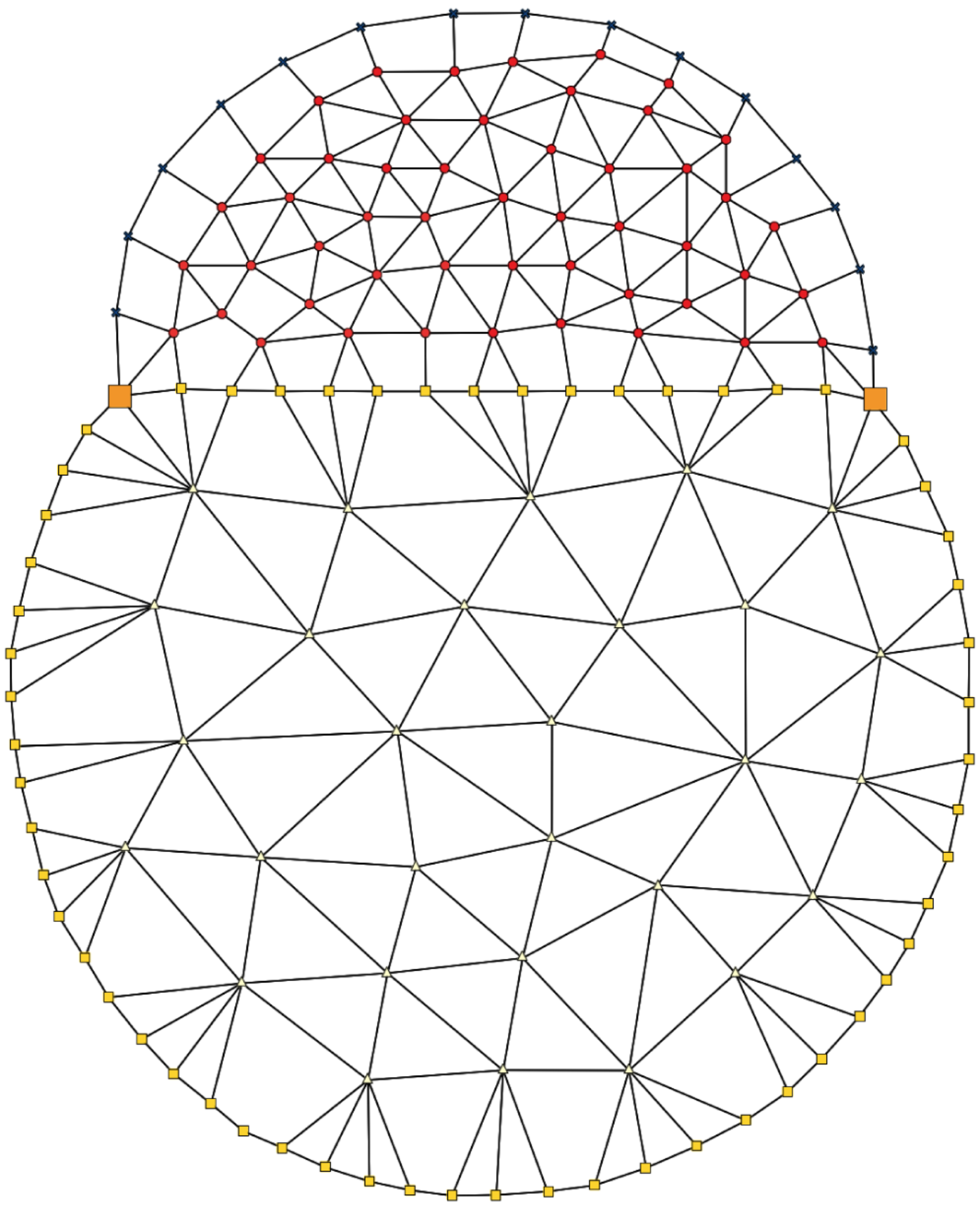



## Equilibrium distance



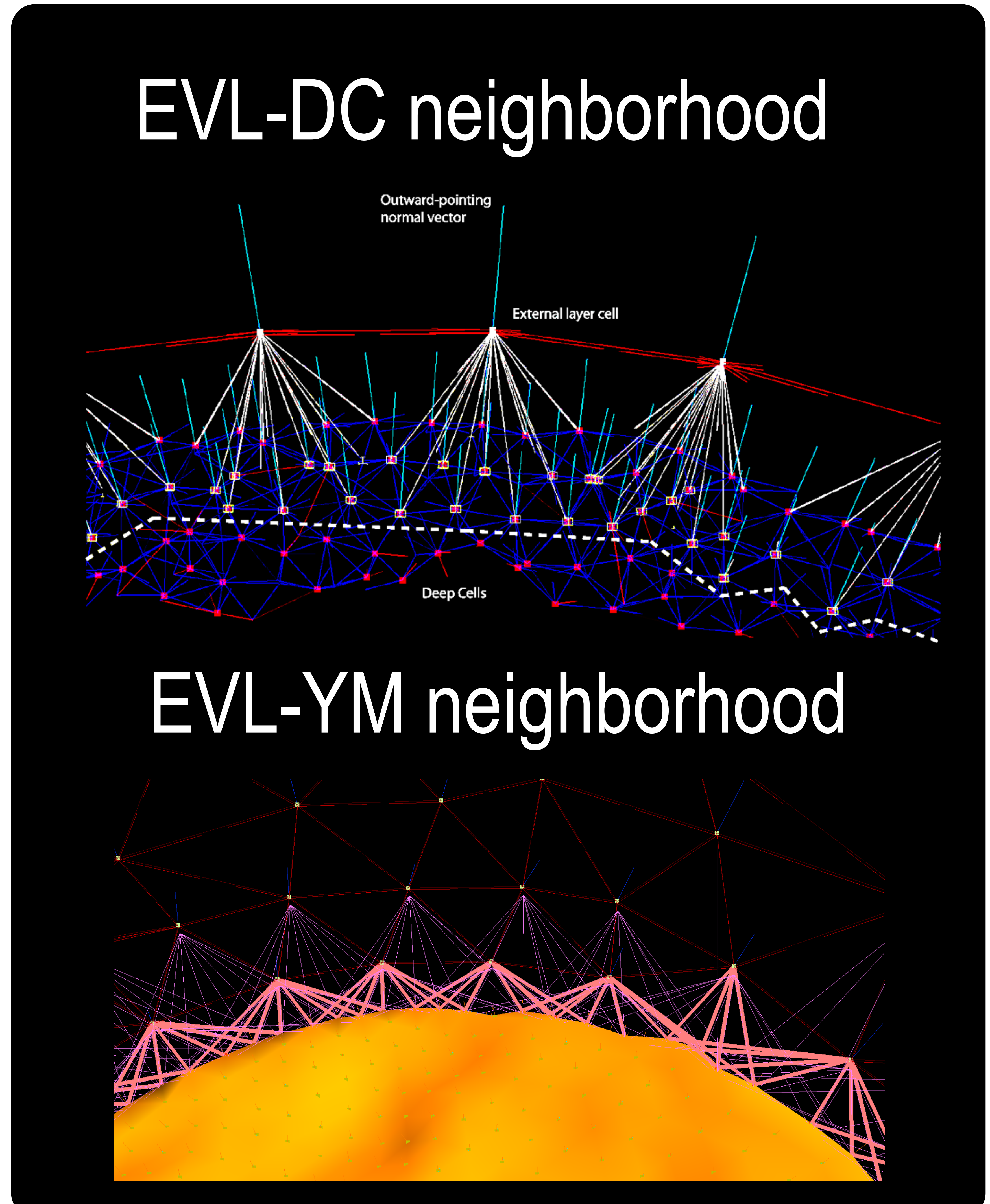

Trapezo-rhombic dodecahedron

# Customization for the Zebrafish embryo



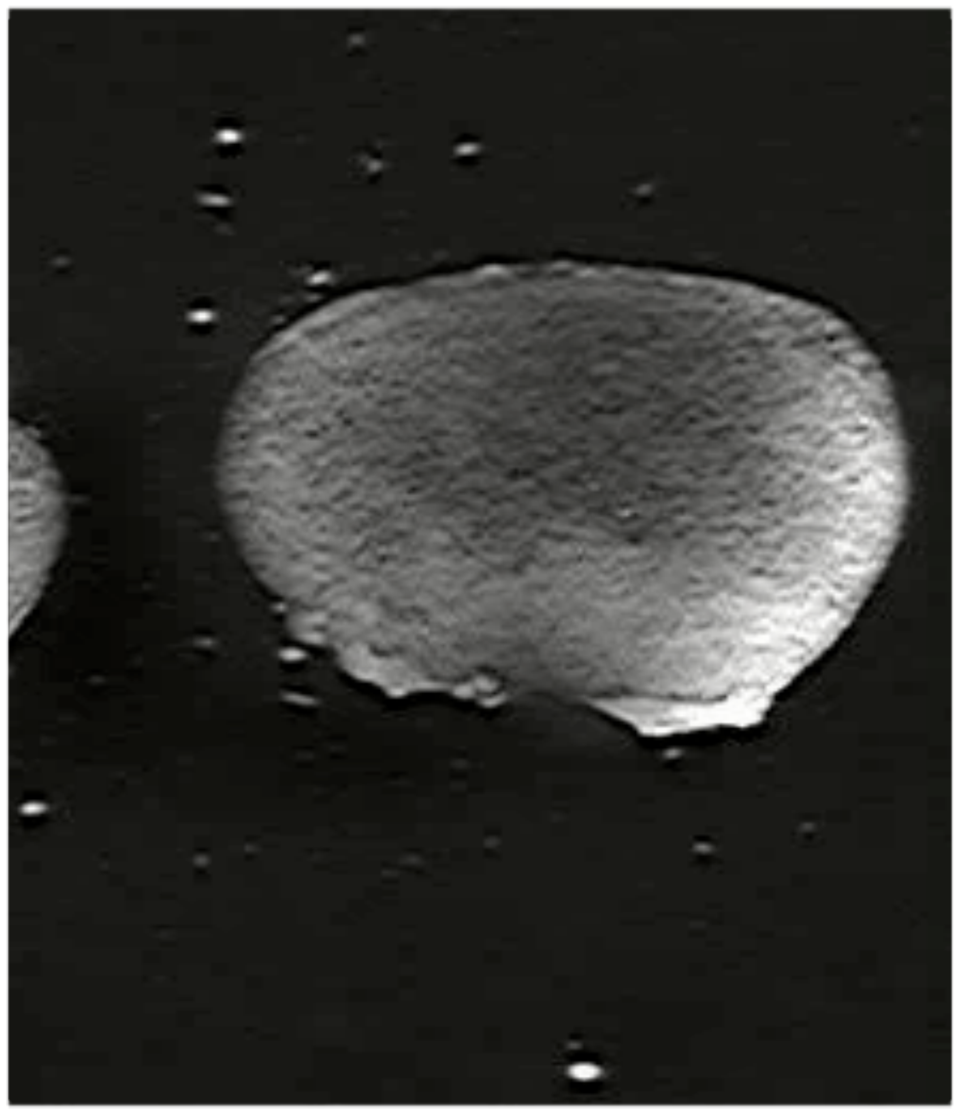
4 particle types:

- Deep Cells (DC)
- Yolk Interior (YI)
- Yolk Membrane(YM)
- EVL Cells (EVL)

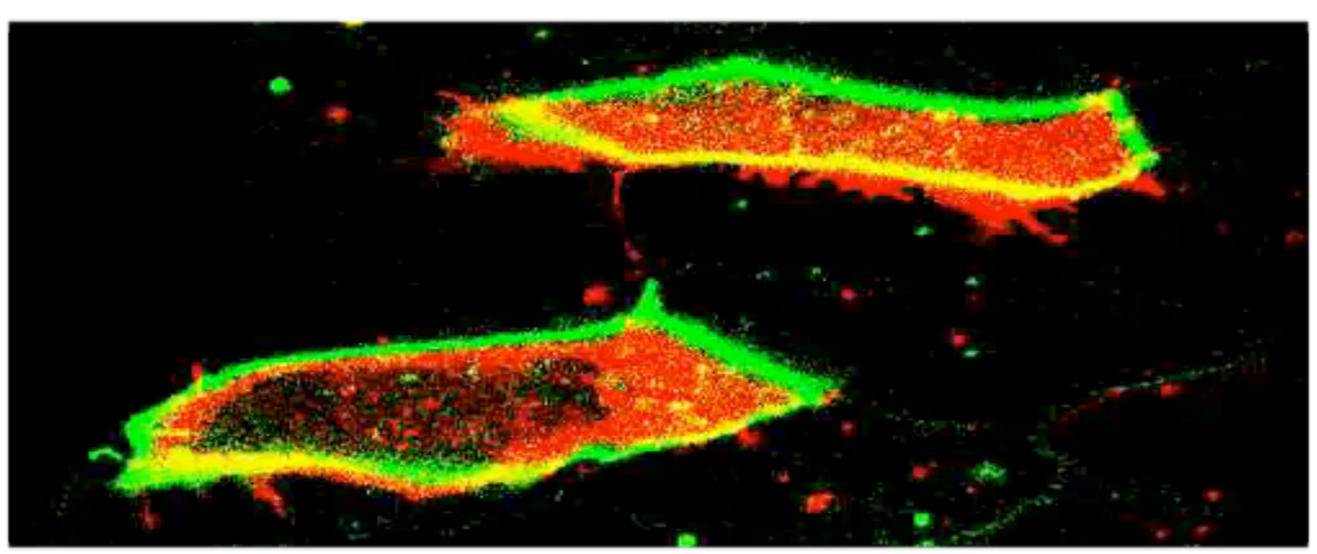


# Behavioral forces: protrusive activities

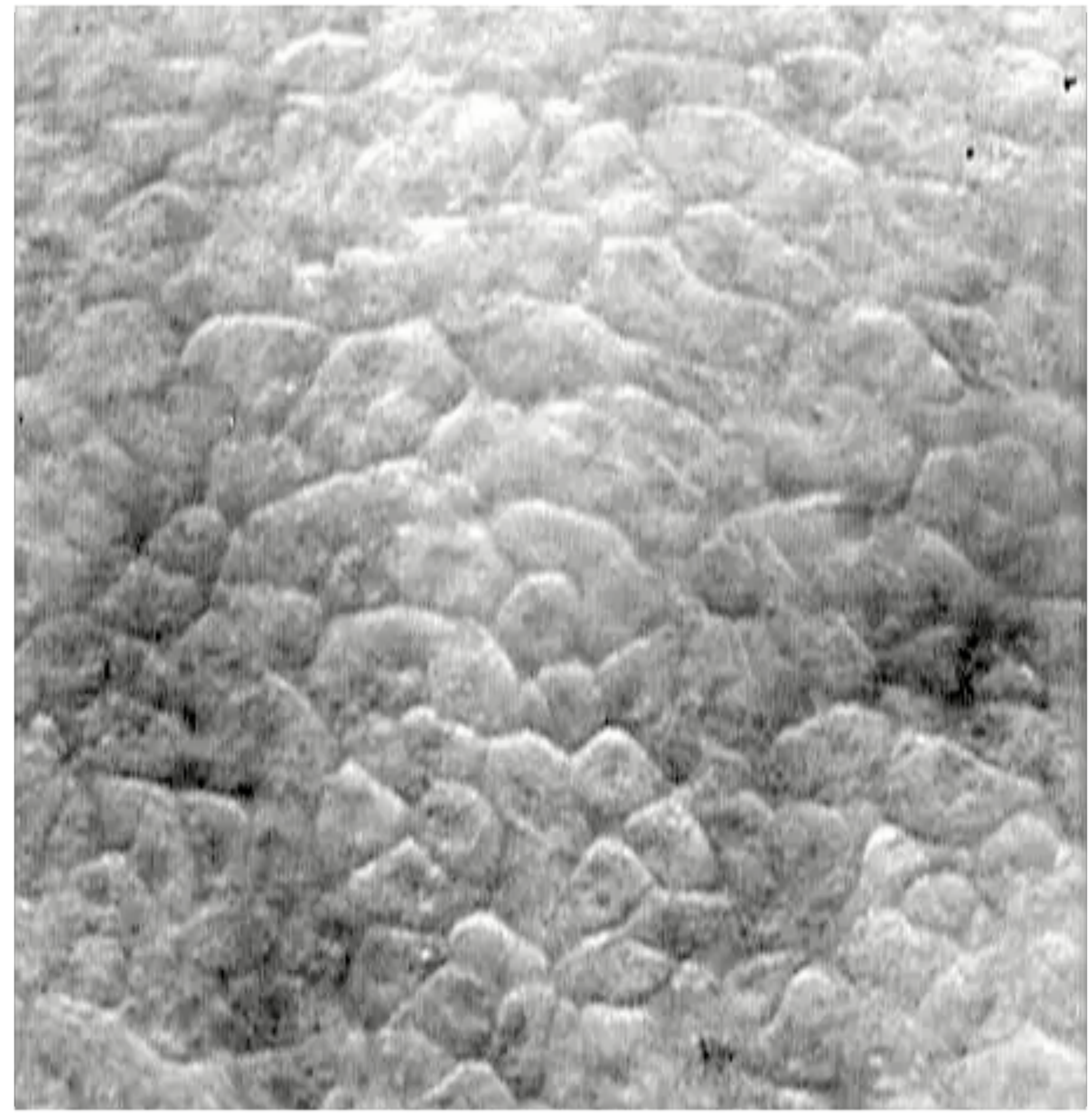
$$\vec{F}^{A,\mathrm{int}} + \vec{F}^{A,\mathrm{ext}}$$



Cultured explant of the dorsal part of the early gastrula of *Xenopus*



Somitic mesodermal cells polarized in the mediolateral axis

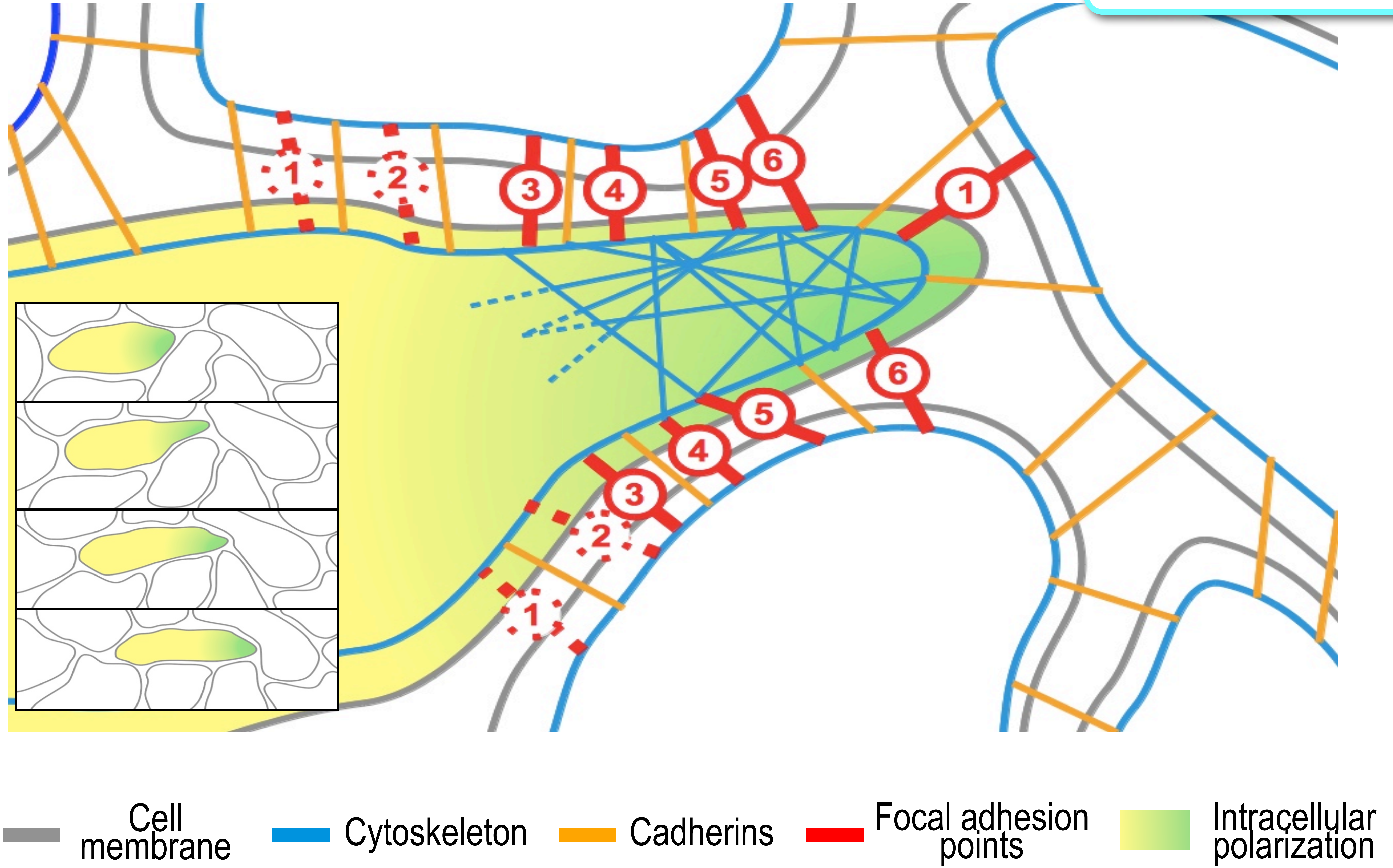


Mediolateral intercalation of mesodermal cells in the *Xenopus* explant

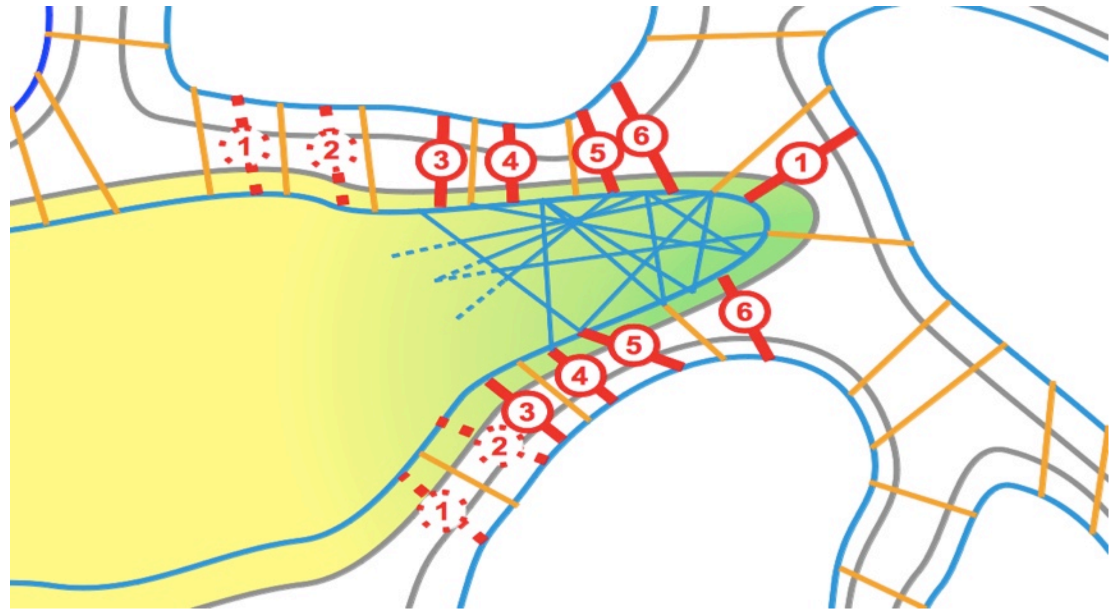
Keller. Shaping the vertebrate body plan by polarized embryonic cell movements. Science (2002)

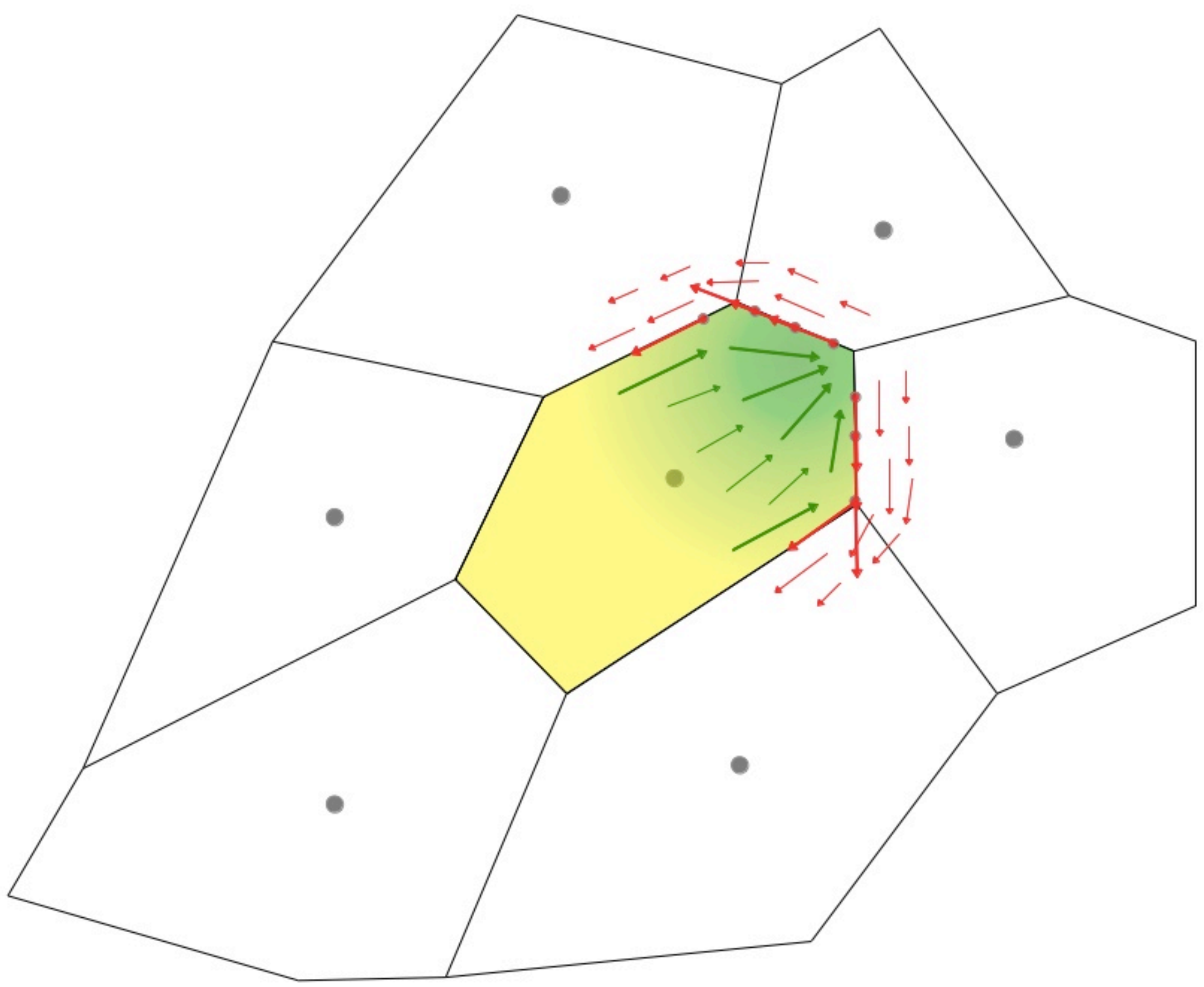
# Schematic of protrusion mechanisms

$$\vec{F}^{A,\text{int}} + \vec{F}^{A,\text{ext}}$$

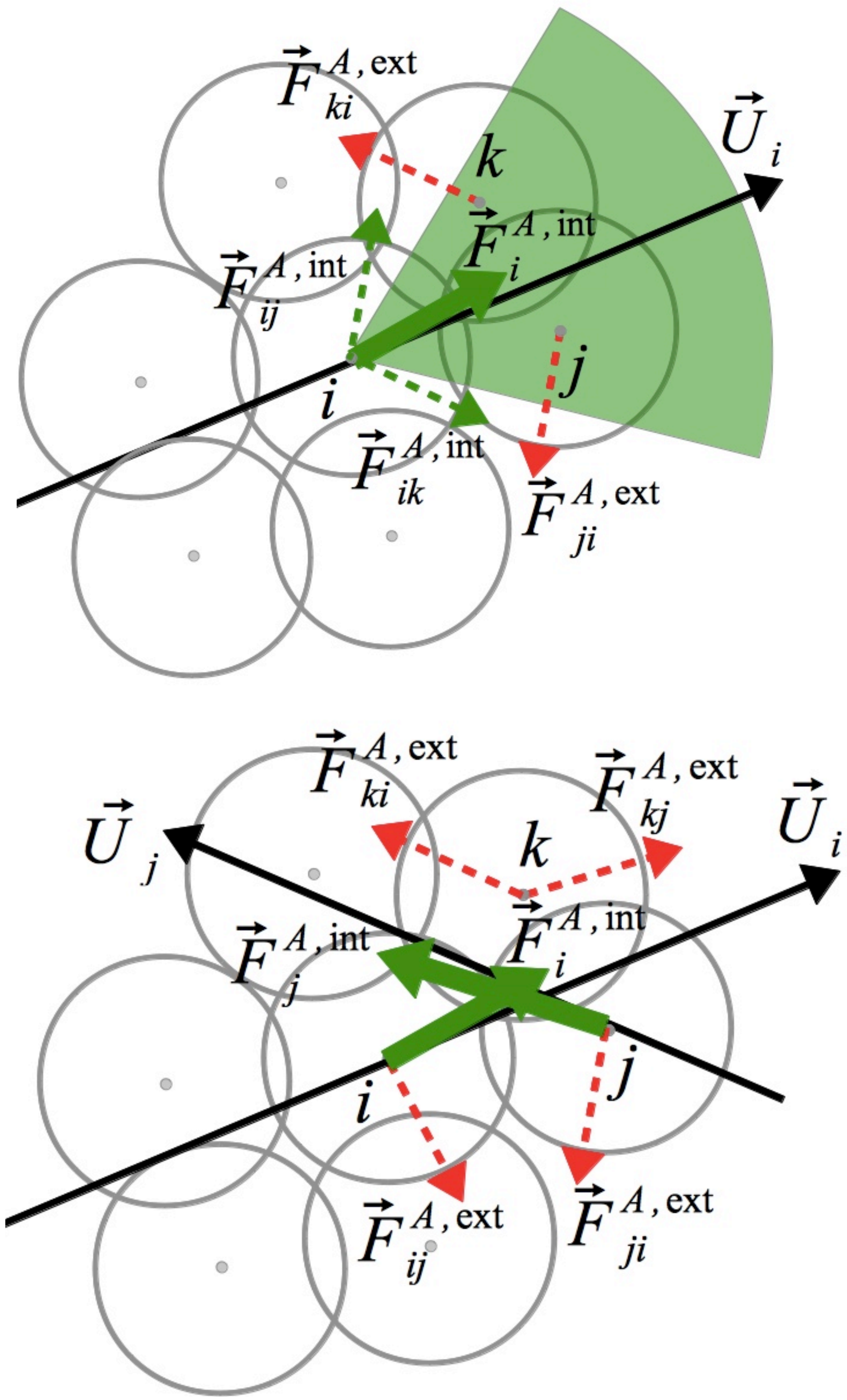


# Toward an "active" protrusion force schema





$$\vec{F}^{A,\text{int}} + \vec{F}^{A,\text{ext}}$$



# The three molecular and genetic components

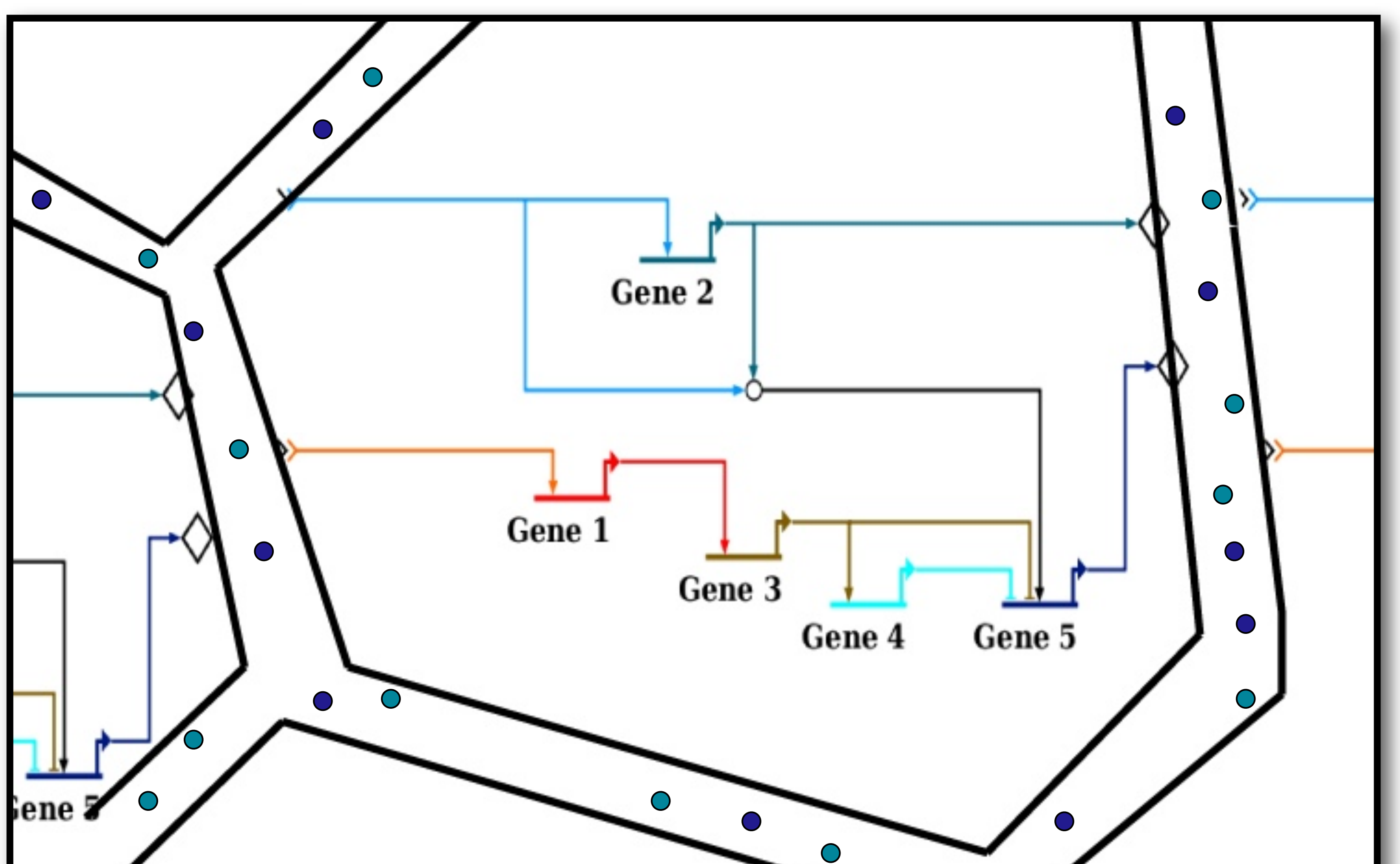
- **Intracellular gene/protein reactions (ODEs + Boolean logic)**

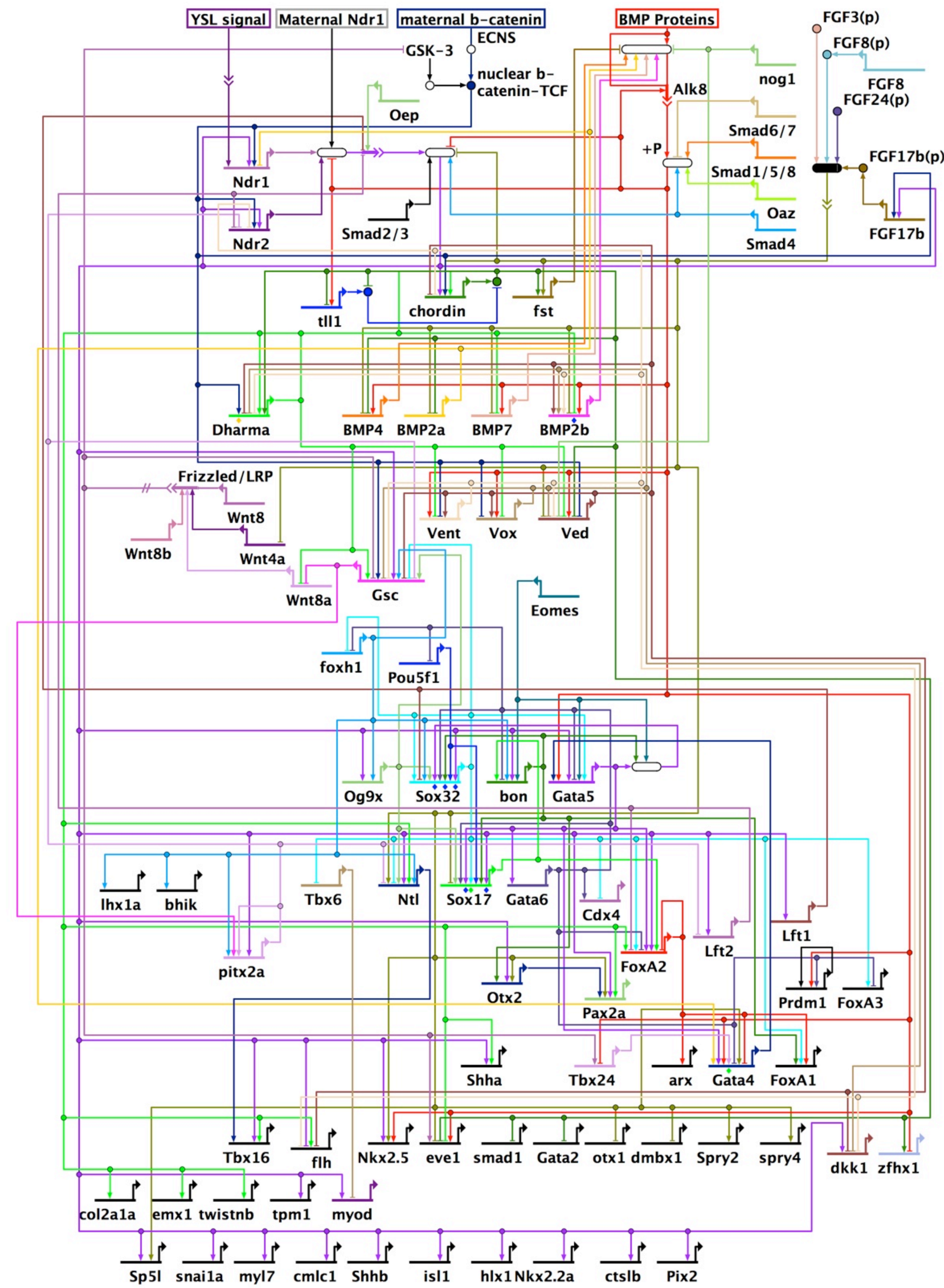
$$\Gamma_{ab}(t) = \begin{cases} 1 & \text{if } P_a \curvearrowright G_b \text{ and } p_a(t) \geq \theta_{ab} \\ 0 & \text{if } P_a \curvearrowright G_b \text{ and } p_a(t) < \theta_{ab} \end{cases}$$

- **Cellular secretion and transduction (ODEs)**

- **Extracellular reactions, transport & diffusion (PDEs)**

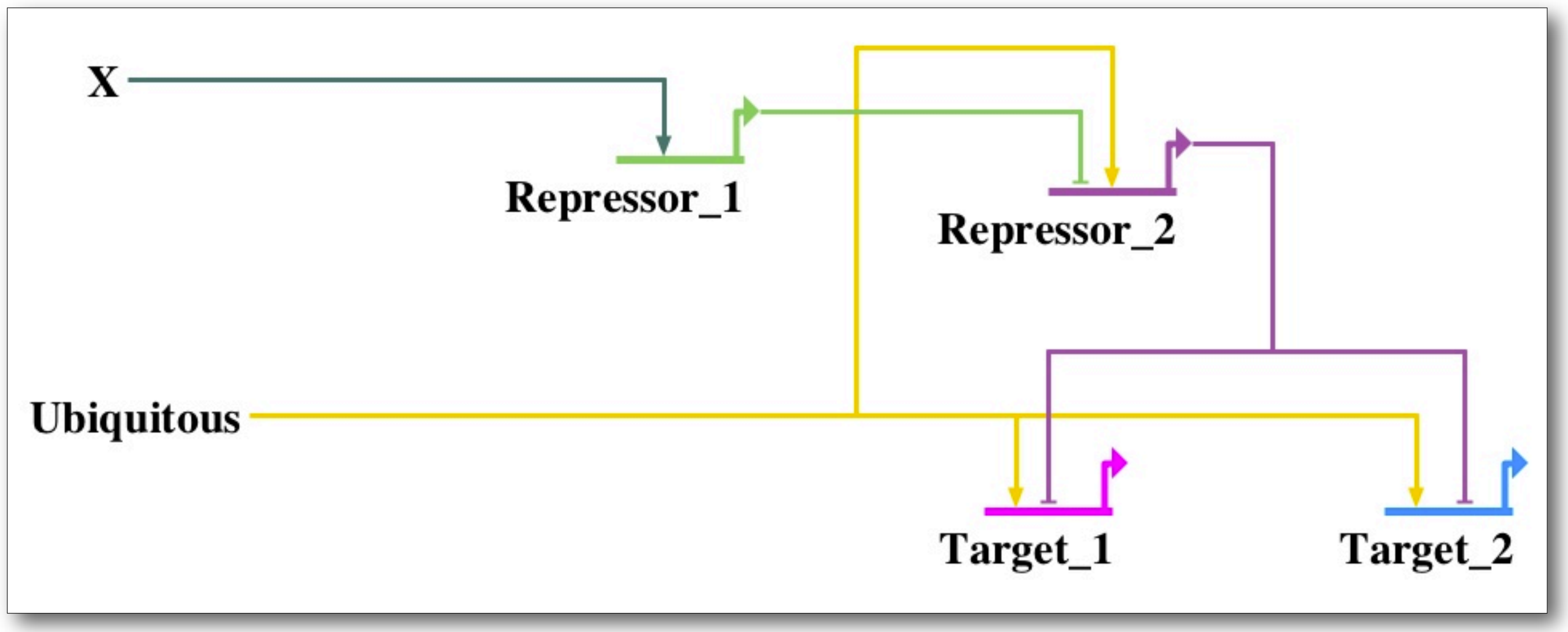
$$\frac{\partial q_a}{\partial t} + \iint_O \vec{J}_a . d\vec{A} = s_a + d_a$$

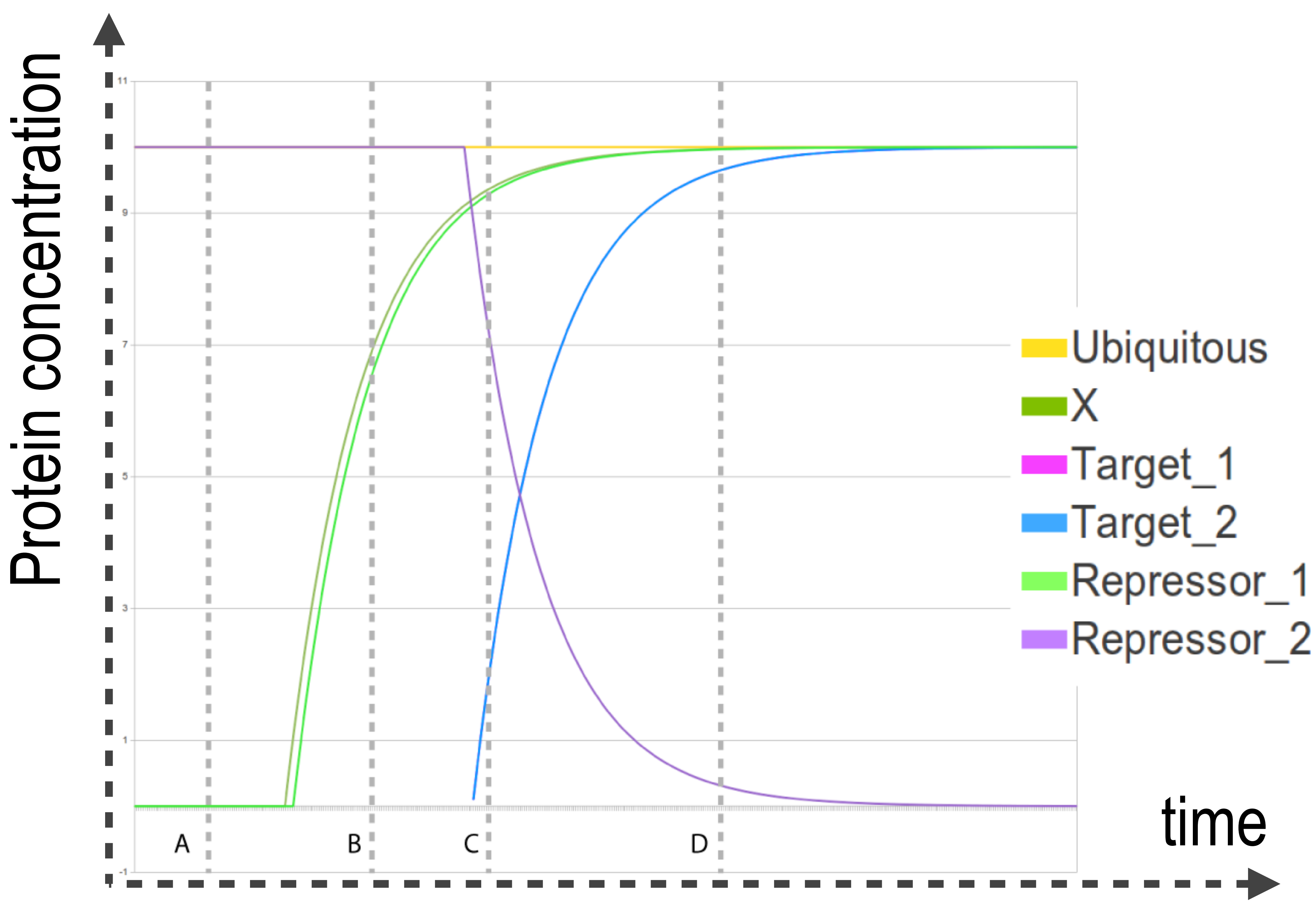


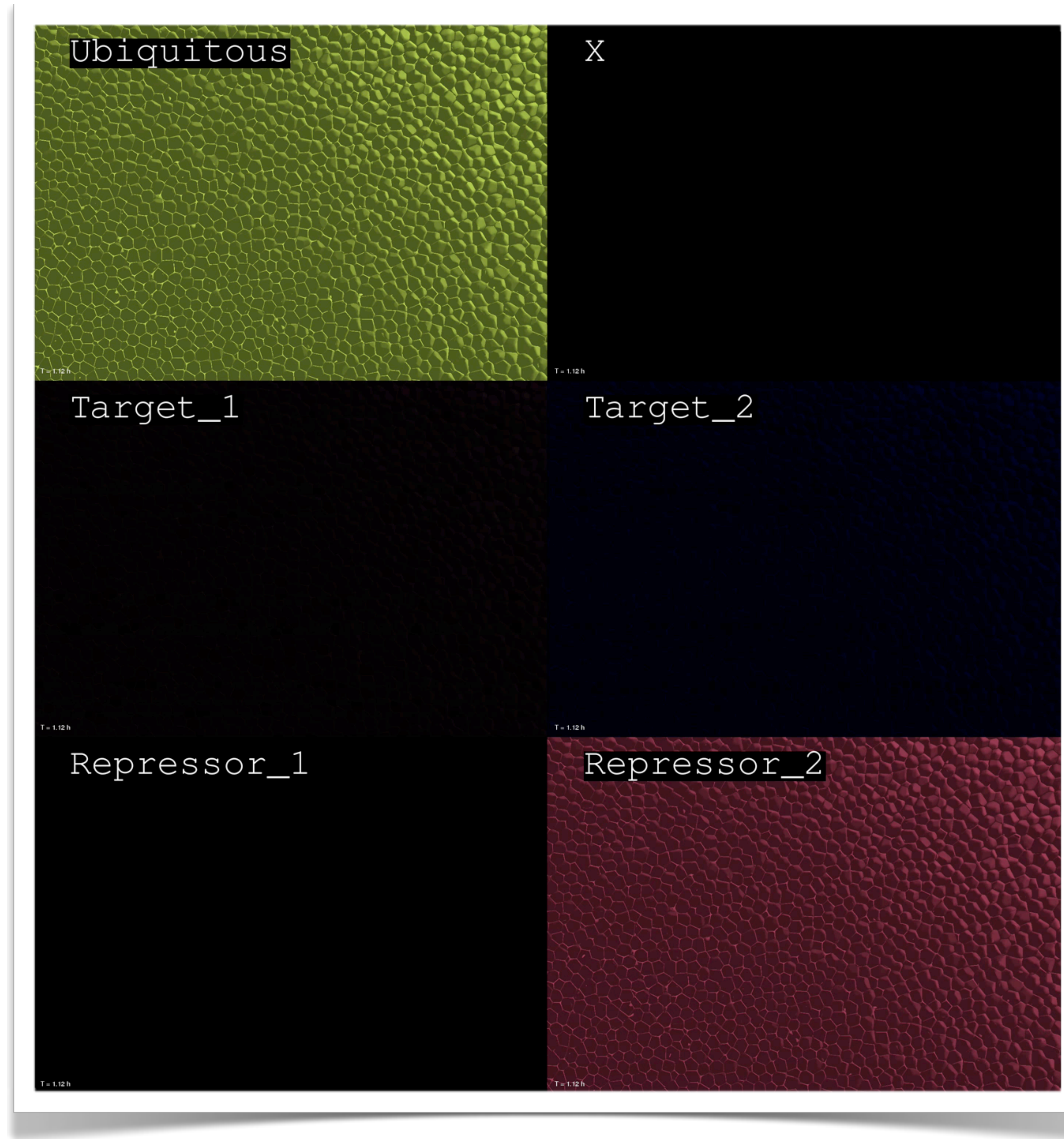




Chan et al., Developmental gene regulatory networks in the zebrafish embryo. 2009

# The case of the double negative gate motif

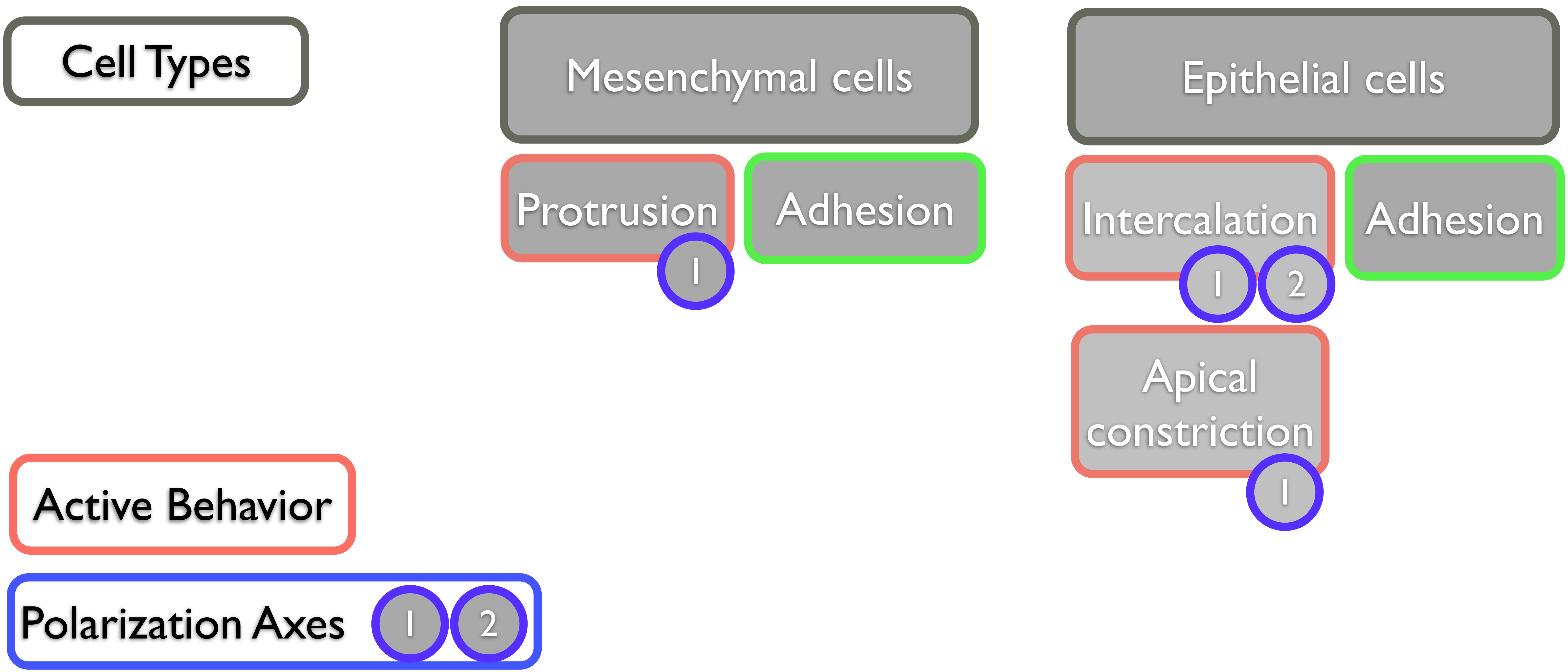


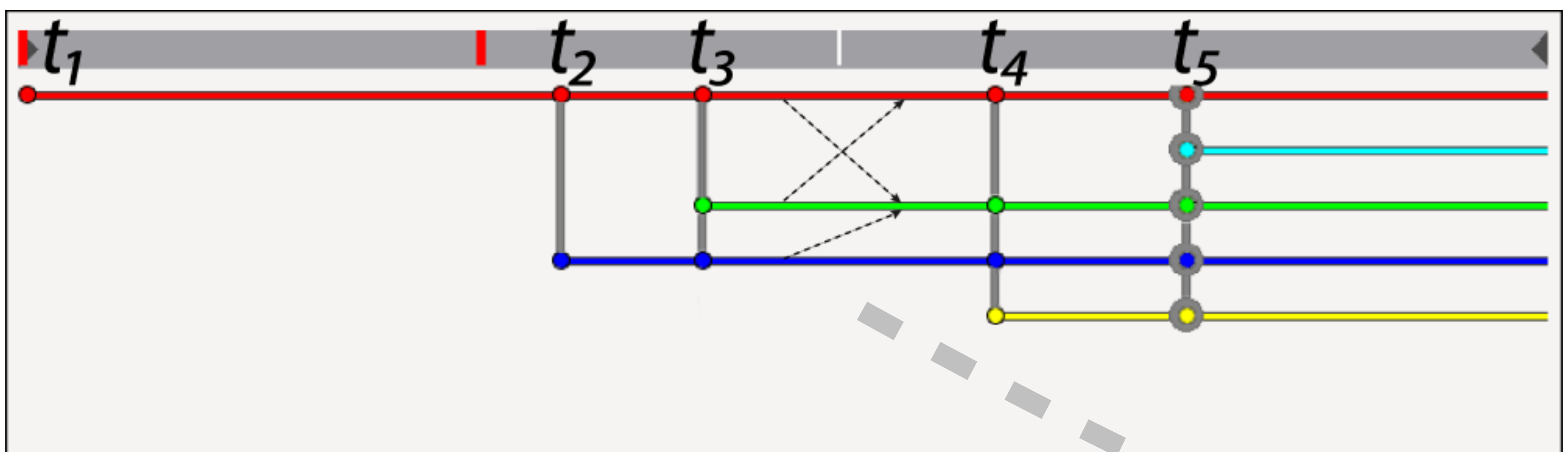

Davidson, E.H., 2010. Emerging properties of animal gene regulatory networks. Nature

# Toward a Cell Behavior Ontology (CBO)

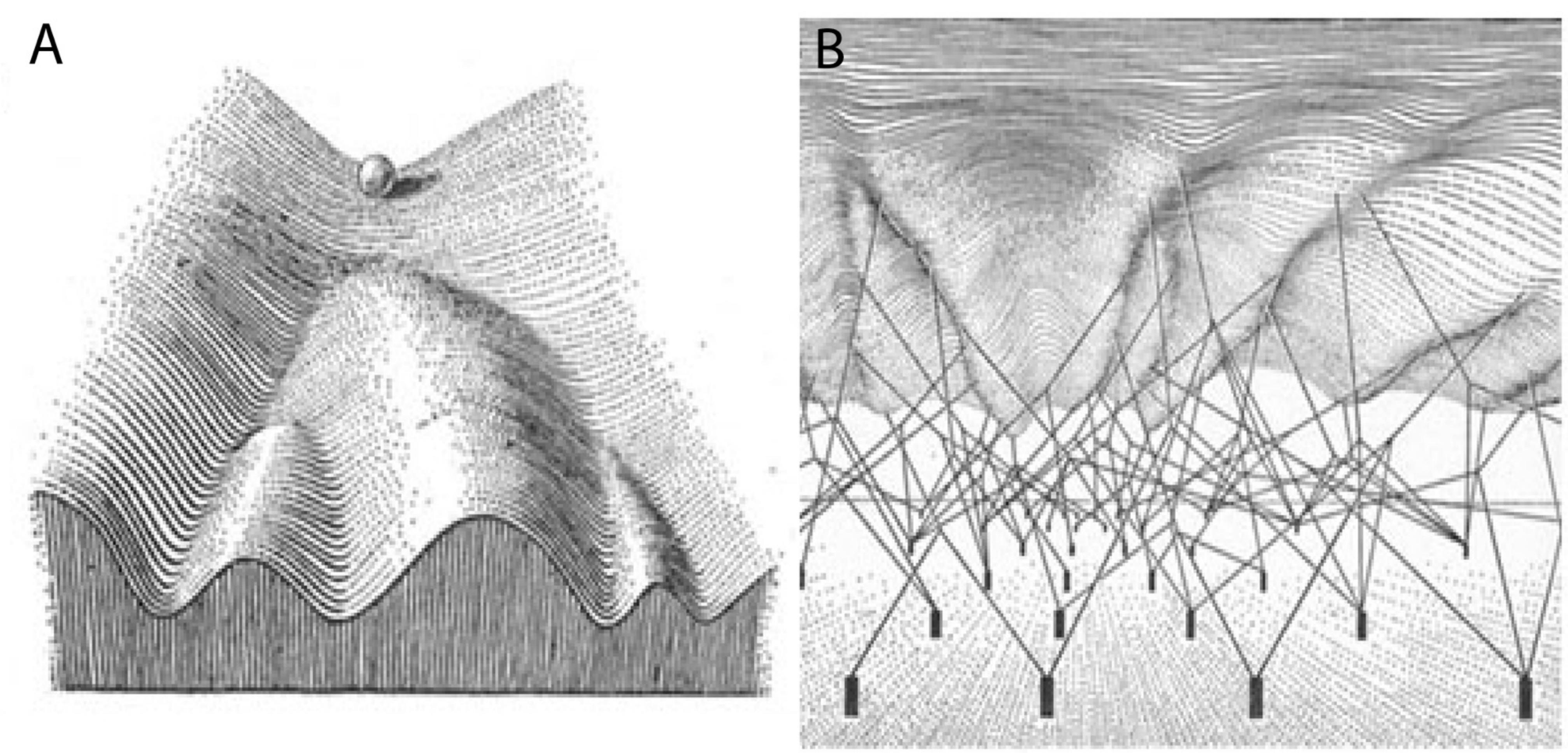


# MECAGEN: Coupling

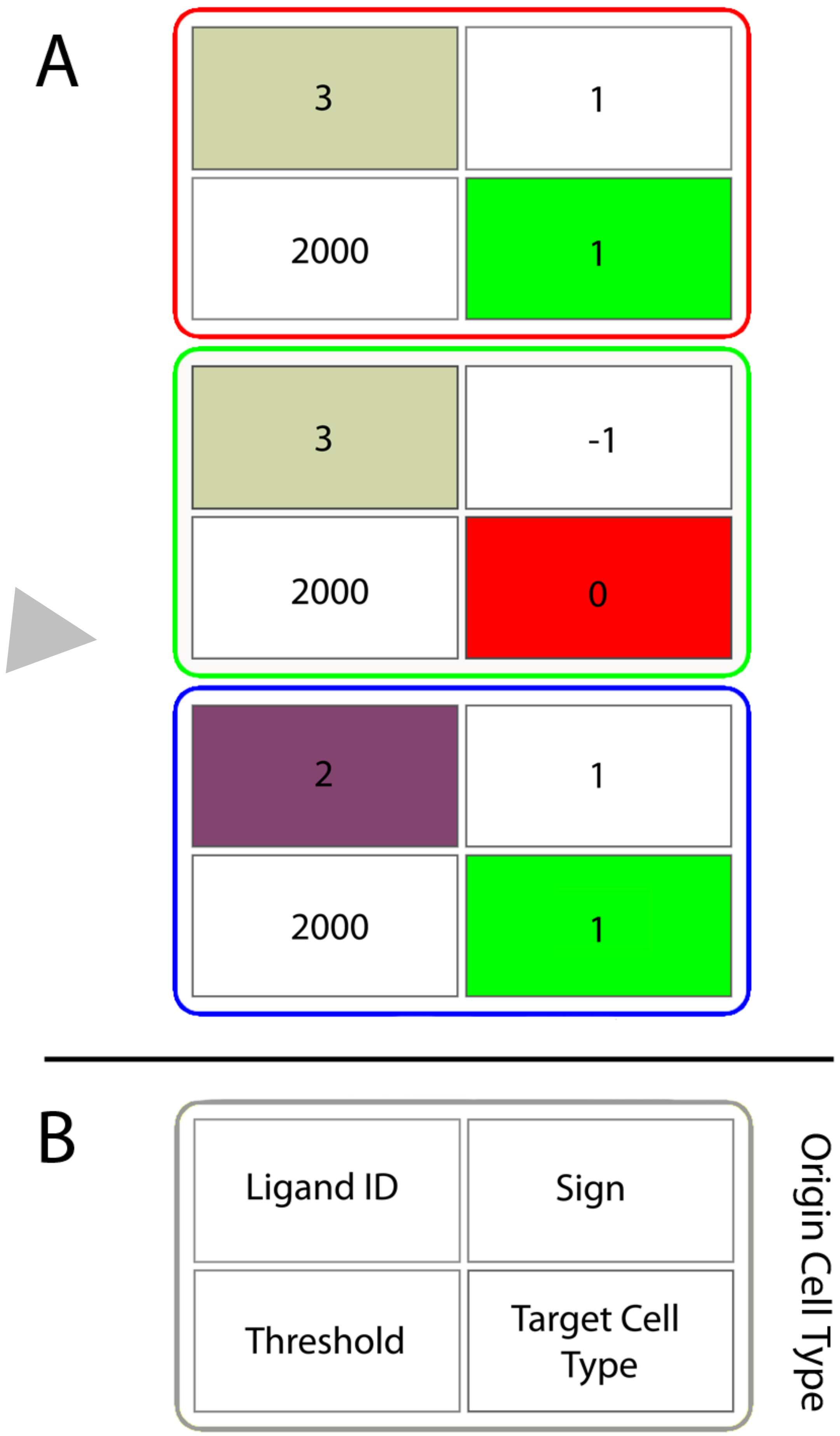
## Waddingtonian Timeline Specification




Waddingtonian Timeline GUI module



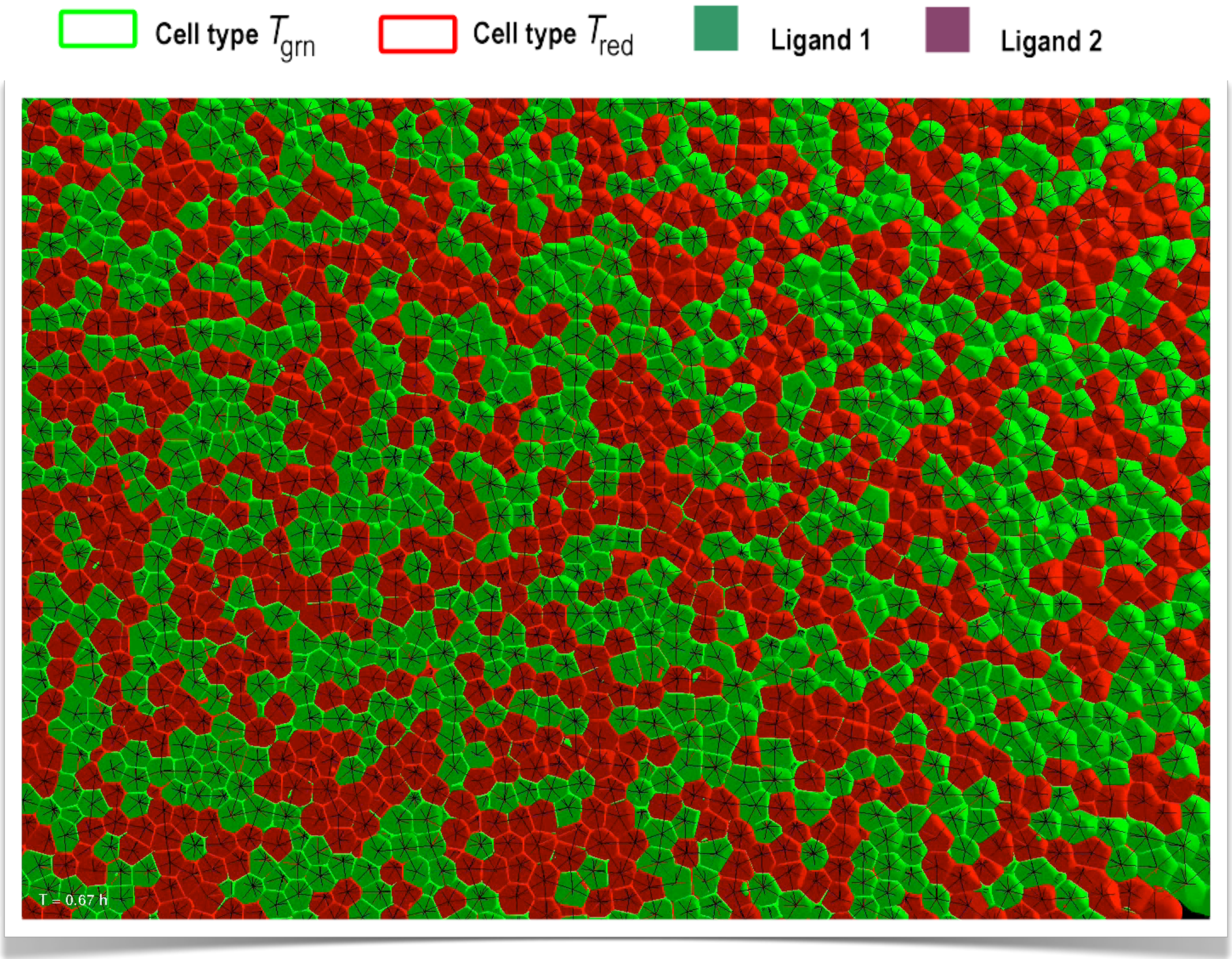

Jonathan M W Slack. Conrad Hal Waddington: the last Renaissance biologist?
Nature Reviews Genetics, 2002

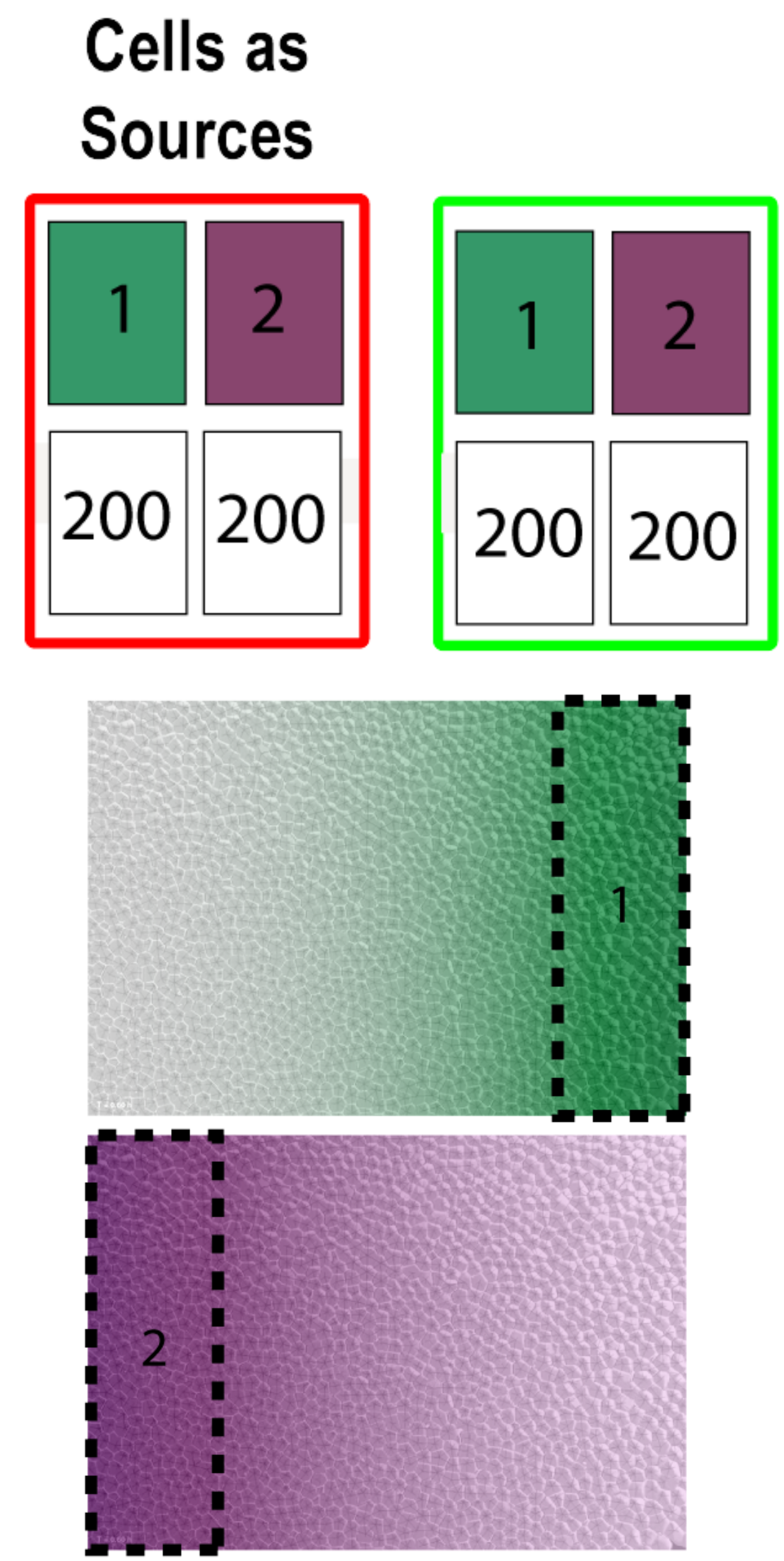



Differentiation (type-transition) GUI module

# MECAGEN: Coupling

## Cell sorting experiment




**Ligand-based heterotypic protrusion, planar diffusion sources**

# Case Studies

- 1. Investigating the **yolk** biomechanical properties

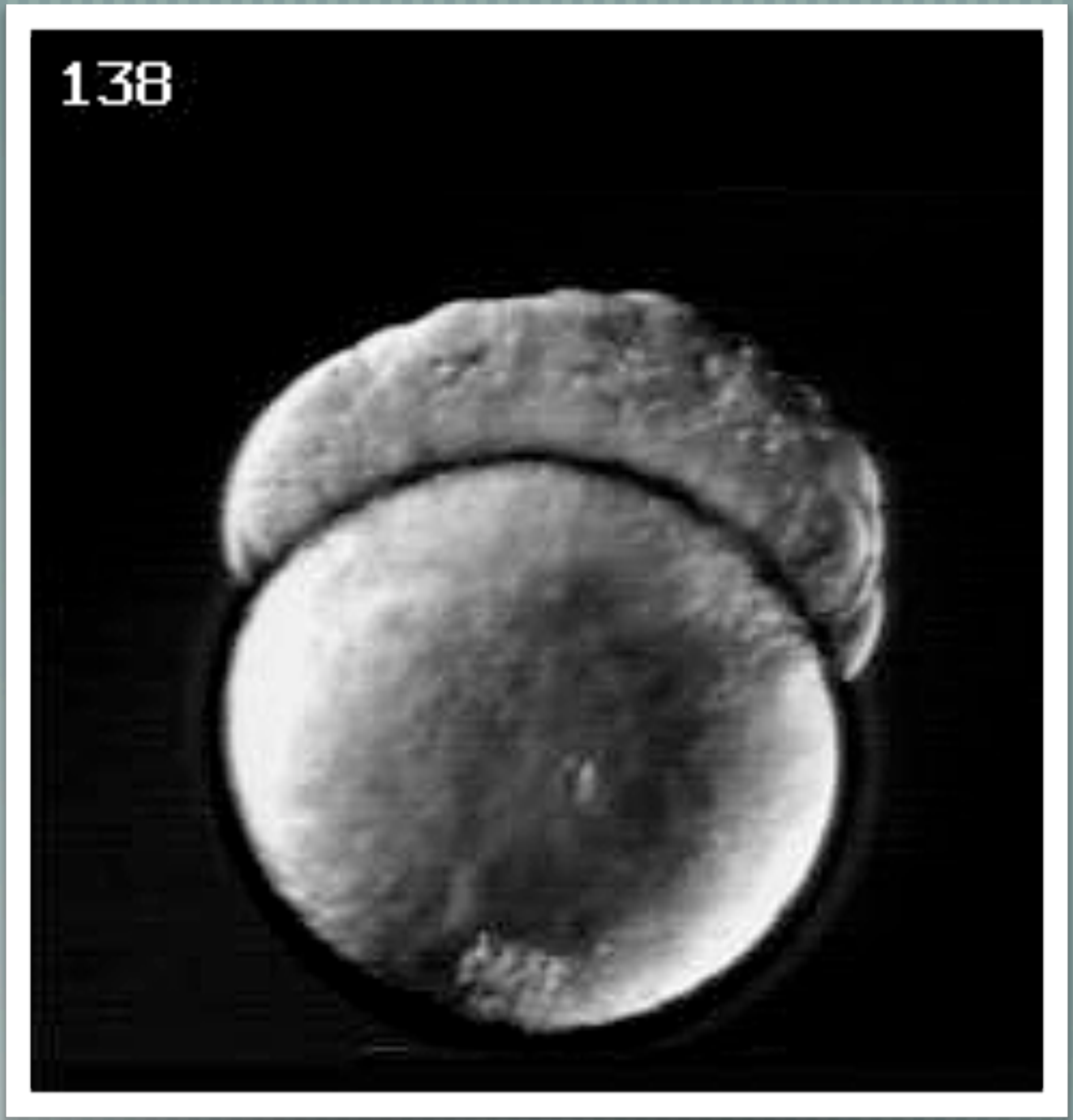
- 2. Cell **proliferation** rate along the cell lineage

- 3. Shaping the Zebrafish **blastula**

- 4. Cell behaviors in the **enveloping cell layer** compartment

- 5. **Intercalation** patterns

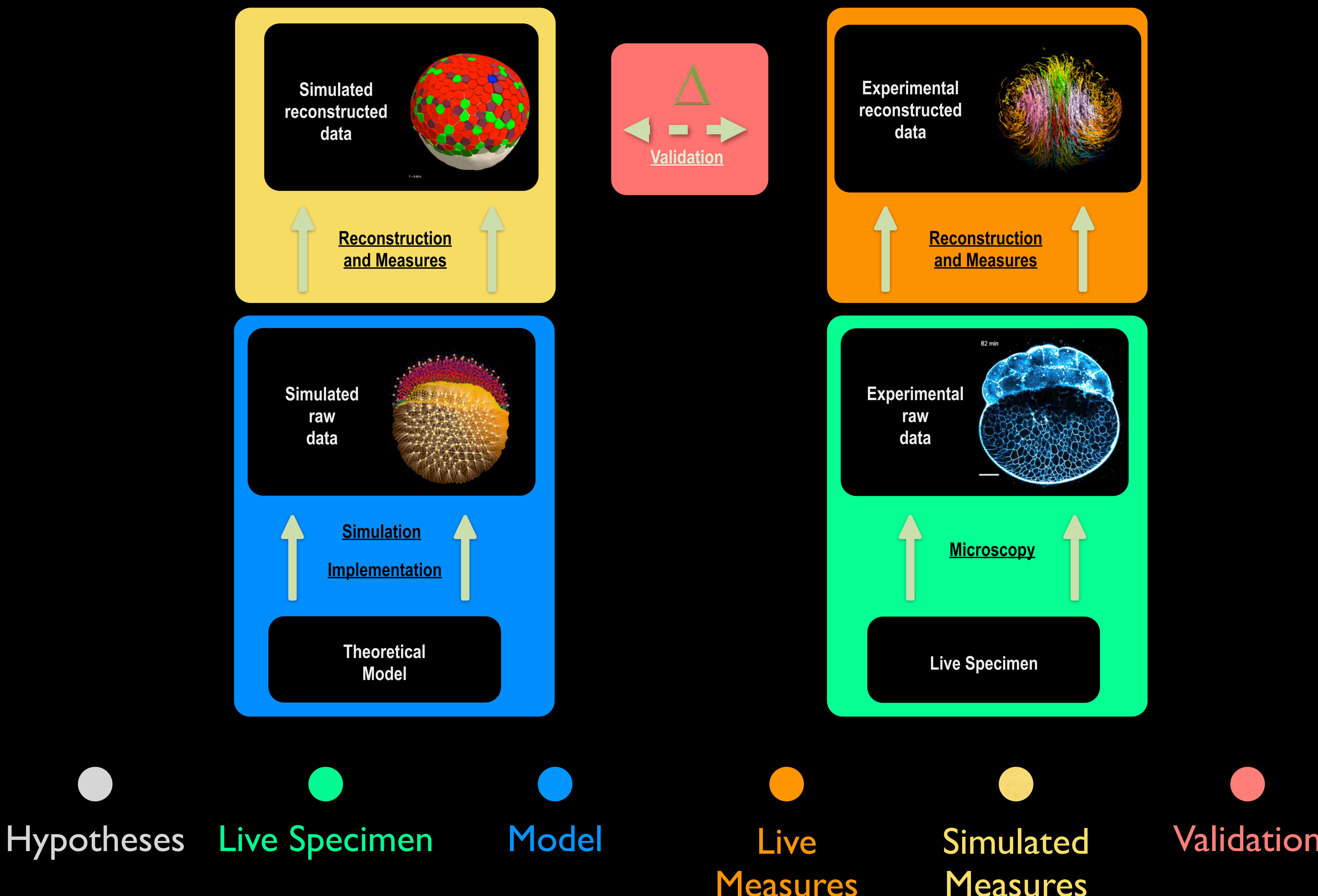
- 6. Cell behaviors during **gastrulation**



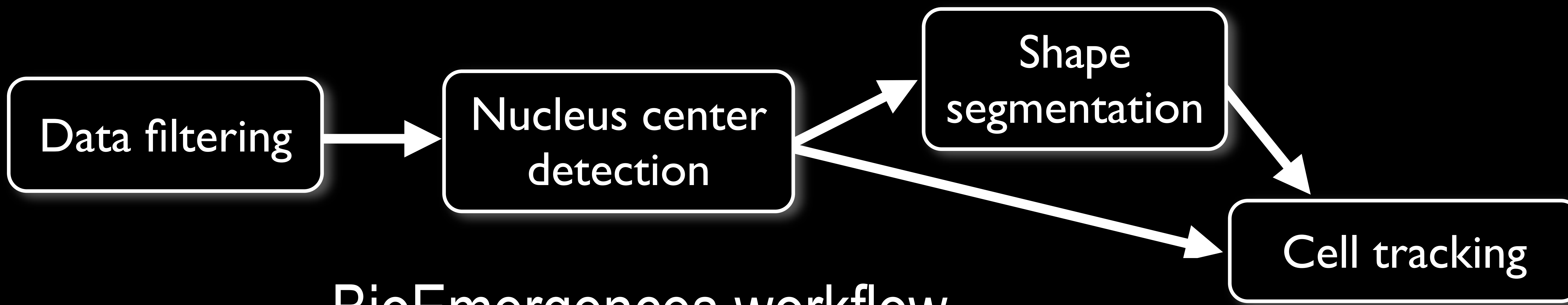

Karlstrom, R.O. & Kane, D.A., 1996. A flipbook of zebrafish embryogenesis. Development

completed case studies

# A methodological framework to confront live and simulated data

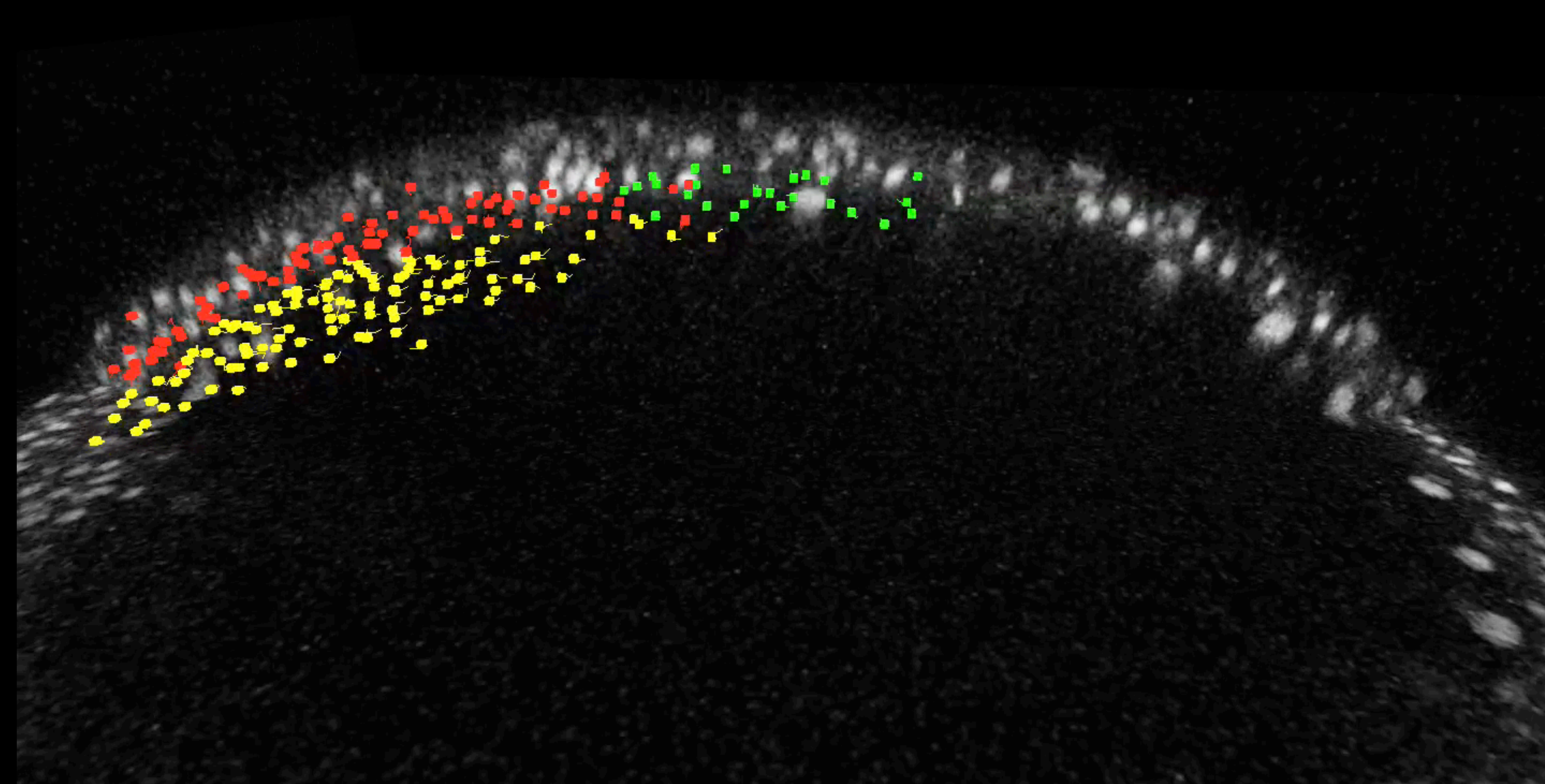


# Quantitative data from live imaging




BioEmergences workflow

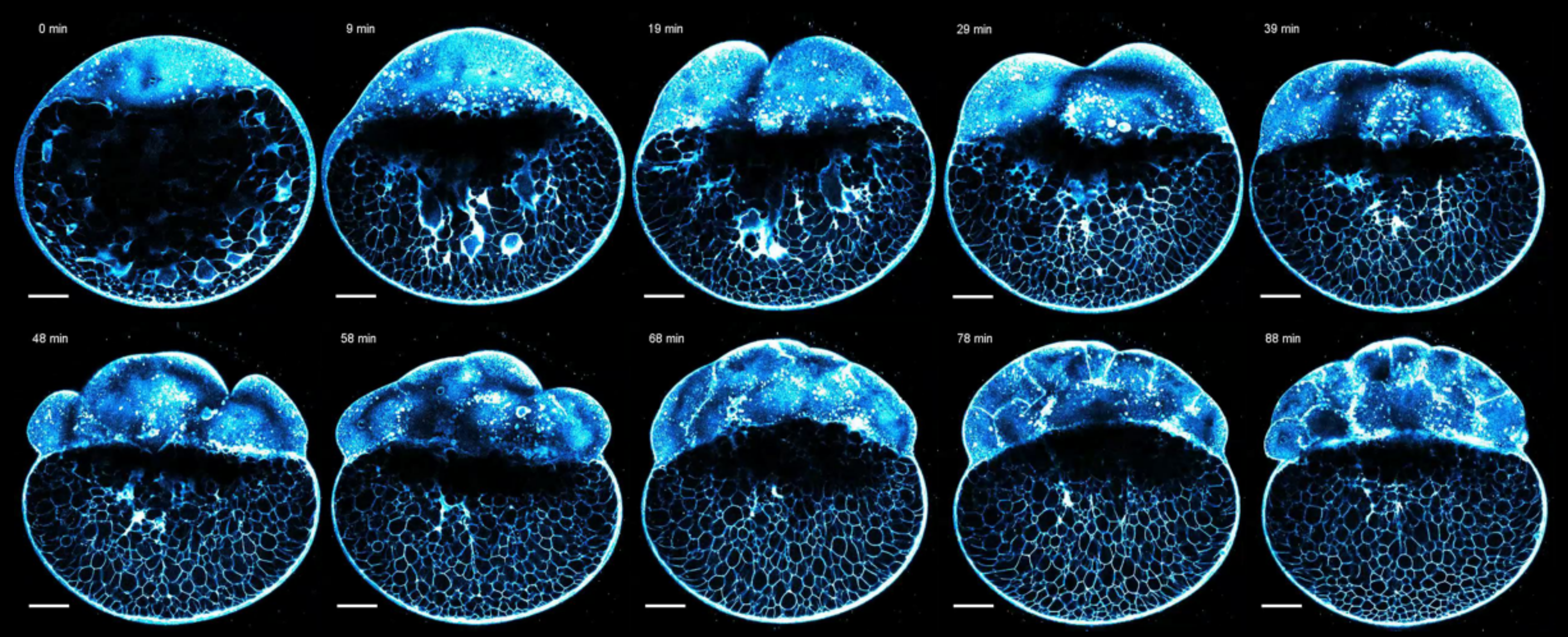
- hypoblast
- presumptive hypothalamus
- presumptive ventral telencephalon



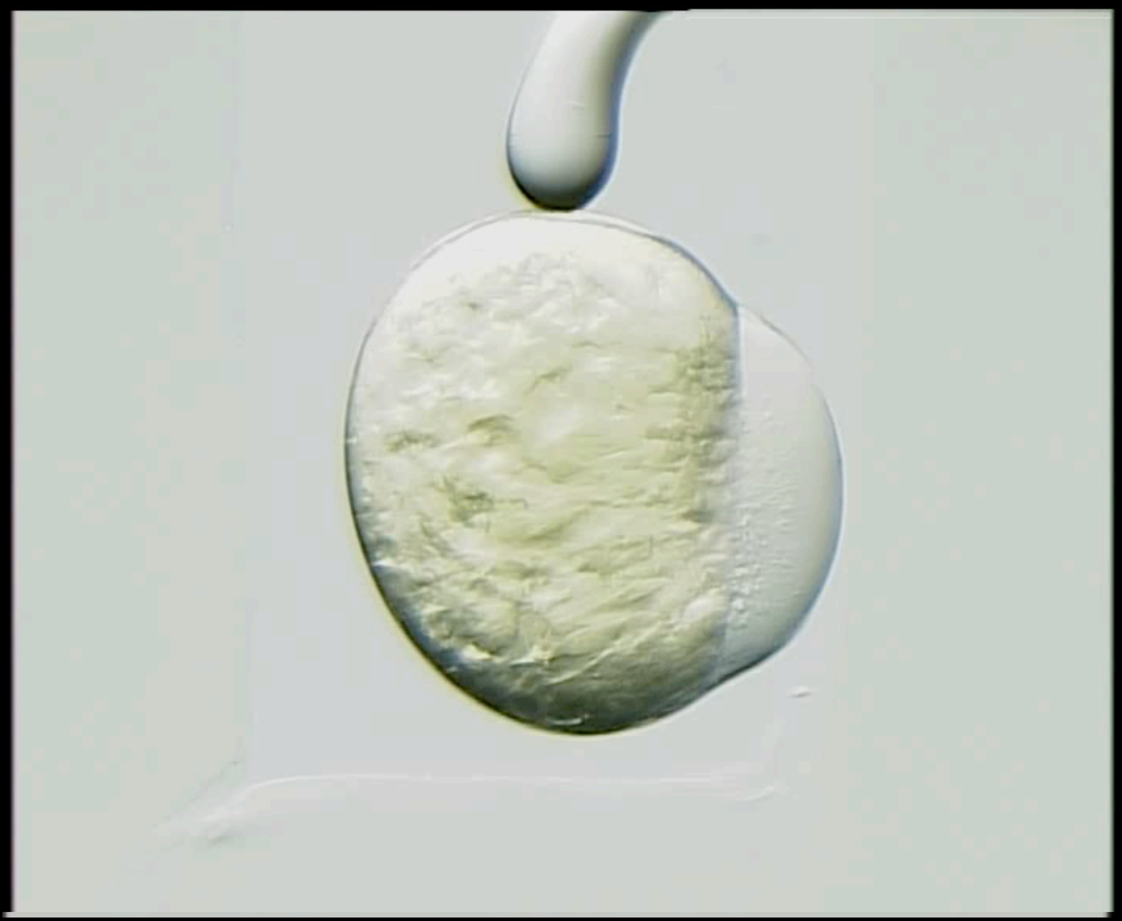
Dataset ID 070418a between 7 and 12 hpf. Relative cell displacement at the midline in a digital specimen. (Movie from the BioEmergences team.)

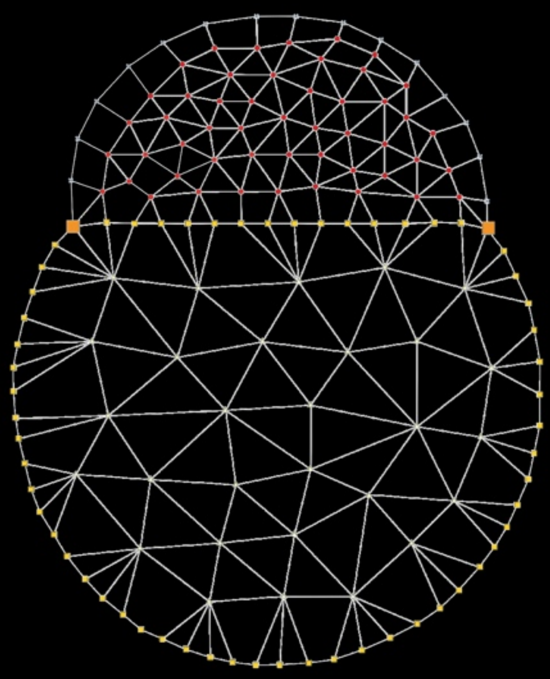
# 1. Investigating the Yolk Biomechanical Properties

## *Can we simulate the yolk's elasticity?*



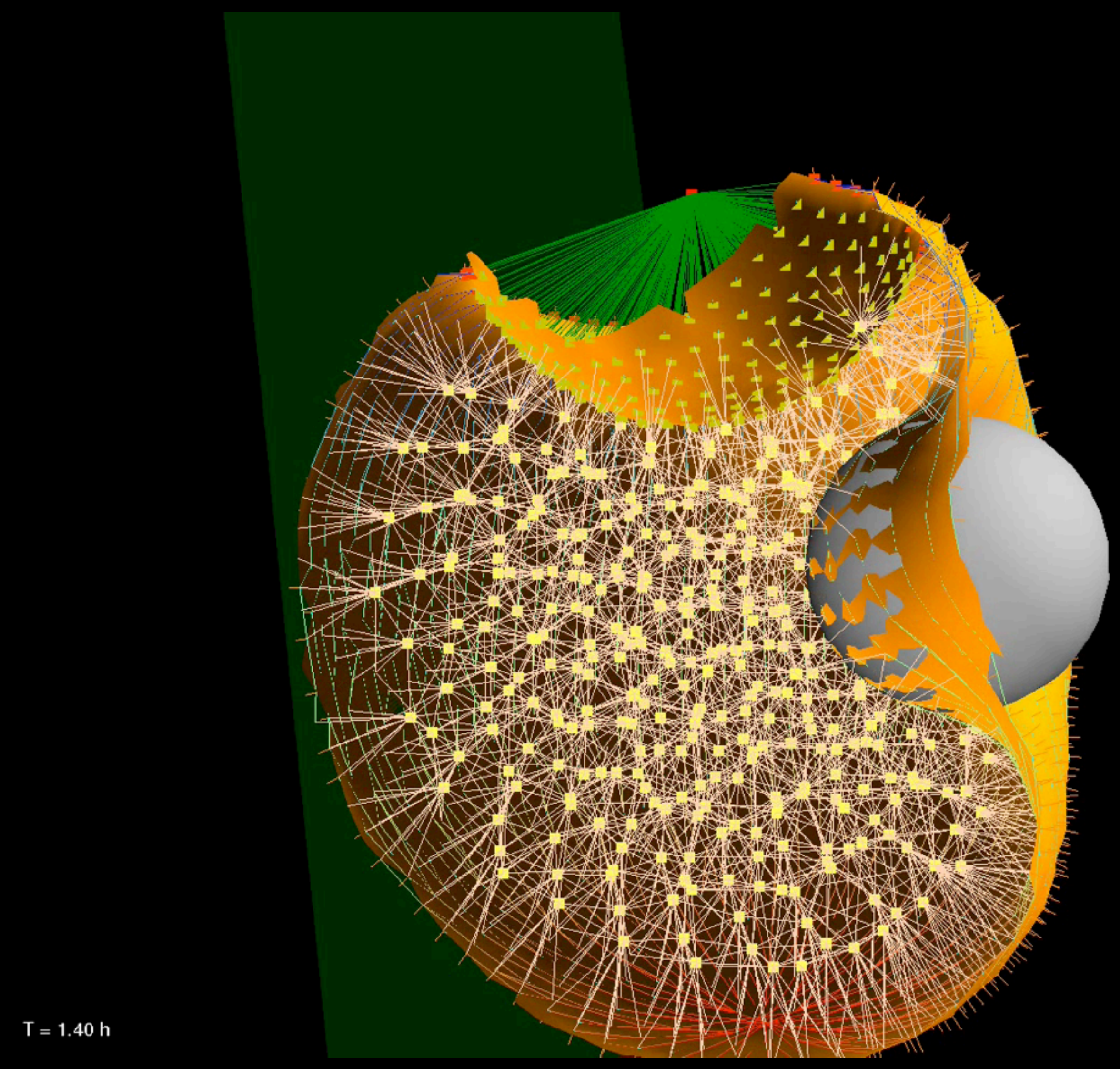

Third harmonic generation (THG) imaging of the zebrafish embryo.
Olivier et al., 2010.





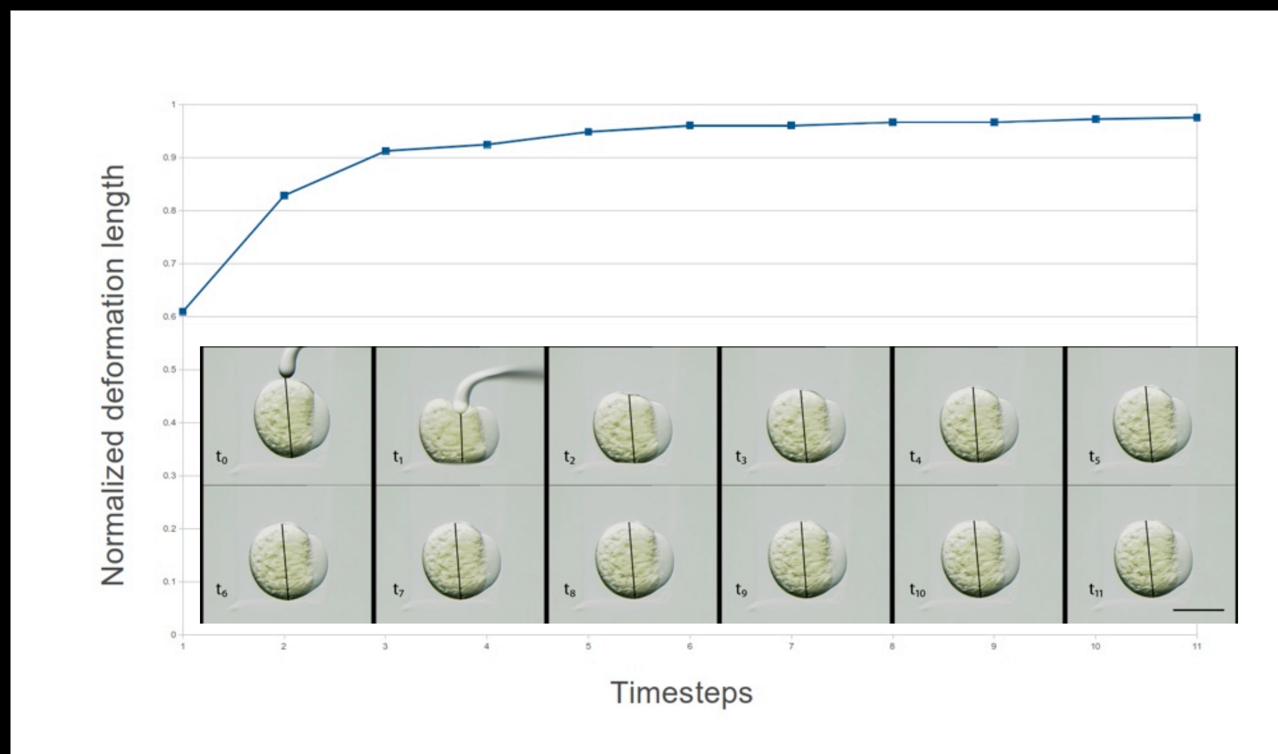
4 parameters :

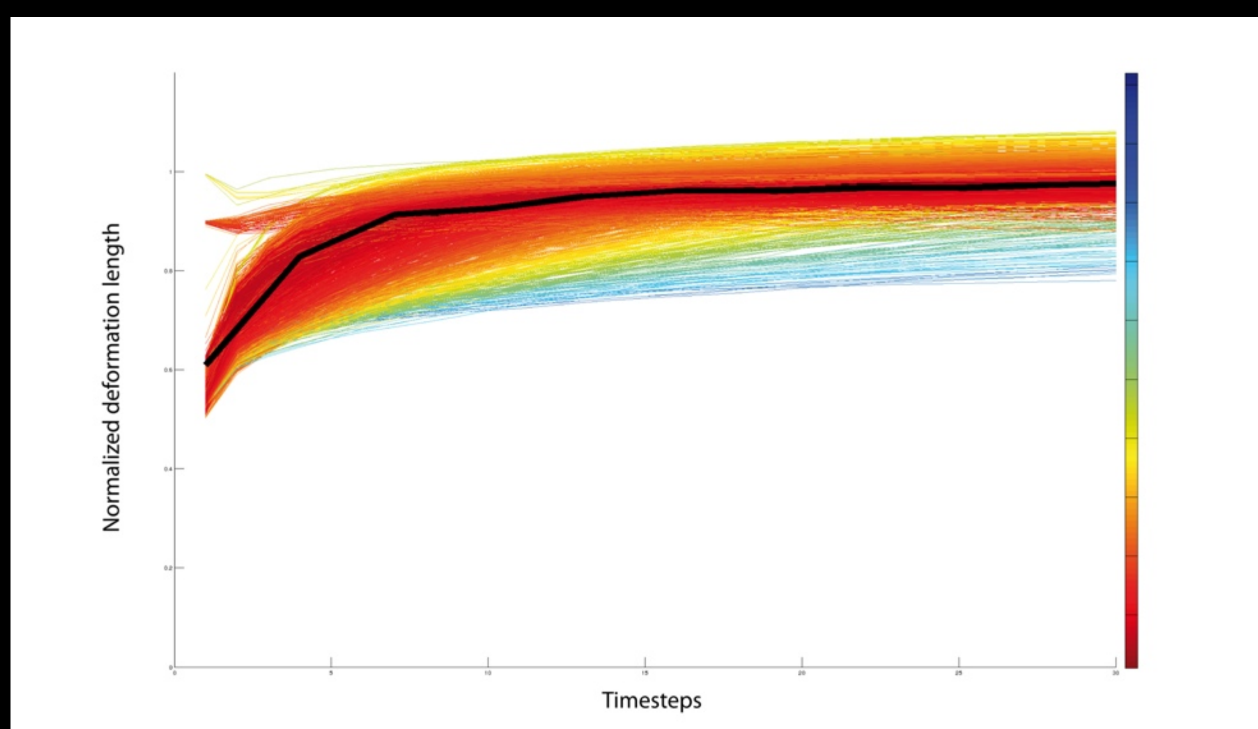
- yolk interior particle repulsion coefficient $\overline{w}^{y}_{\mathrm{rep}}$
- yolk interior particle attraction coefficient $\overline{w}^{y}_{\mathrm{adh}}$
- yolk membrane stiffness coefficient $\overline{k}_{\mathrm{my}}$
- yolk surface scaling parameter $c_r$




Hypotheses Live Specimen Model

# The best match is too computationally costly




| | Min. | Max. | Cardinality | Unit |
|---|---|---|---|---|
| $\overline{w}^{y}_{adh}$ | 0 | $3.7\ 10^{-5}$ | 4 | $s^{-1}$ |
| $\overline{w}^{y}_{rep}$ | $3.7\ 10^{-6}$ | $5.55\ 10^{-5}$ | 10 | $s^{-1}$ |
| $\overline{k}_{my}$ | $3.7\ 10^{-4}$ | $3.7\ 10^{-3}$ | 10 | $m^2.s^{-1}$ |
| $c_r$ | 0.5 | 1.0 | 10 | |

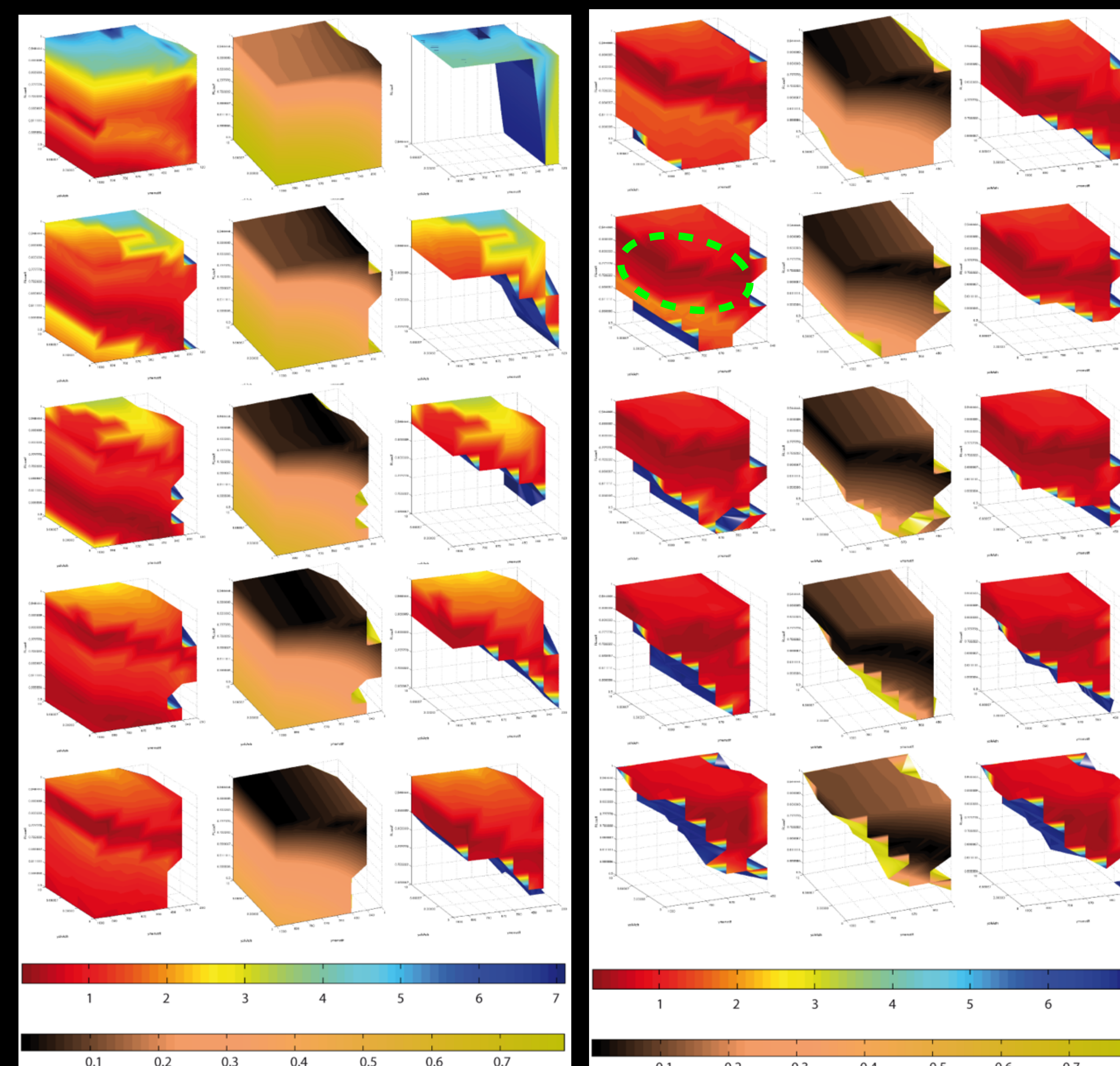
Fitness function

Normalized volume distance (NVD)

NVD < 20%

$\overline{w}^{y}_{rep}$

$c_r$ $\overline{k}_{my}$ $\overline{w}^{y}_{adh}$



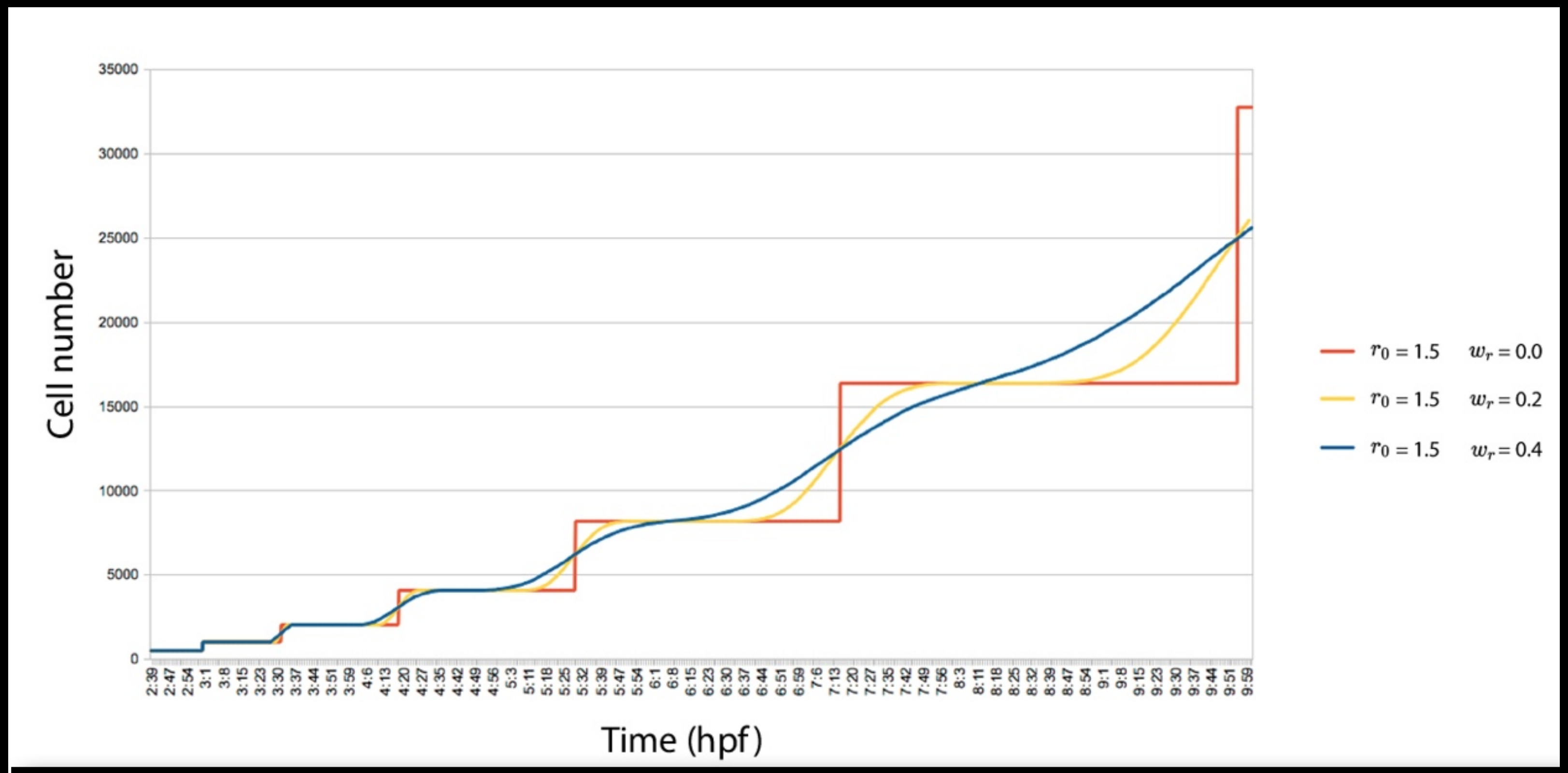

Live Measures

Validation

# 2. Cell Proliferation Rate Along the Cell Lineage

## *What is the law of cell cycle lengthening and desynchronizing?*

Dataset ID 071222bF between 32-cell stage
(1.75 hpf) and gastrulation.




Cell number as a function of the cell cycle length
(Geometric progression)

Geometric progression

$$\forall n \in [n_{\text{start}}, n_{\text{end}}], L_{\text{cc}}(n+1) = r \cdot L_{\text{cc}}(n)$$

$$r = \mathcal{U}(r_0 - \frac{w_r}{2}, r_0 + \frac{w_r}{2})$$

OR

Arithmetic progression

$$\forall n \in [n_{\text{start}}, n_{\text{end}}], L_{\text{cc}}(n+1) = d + L_{\text{cc}}(n)$$

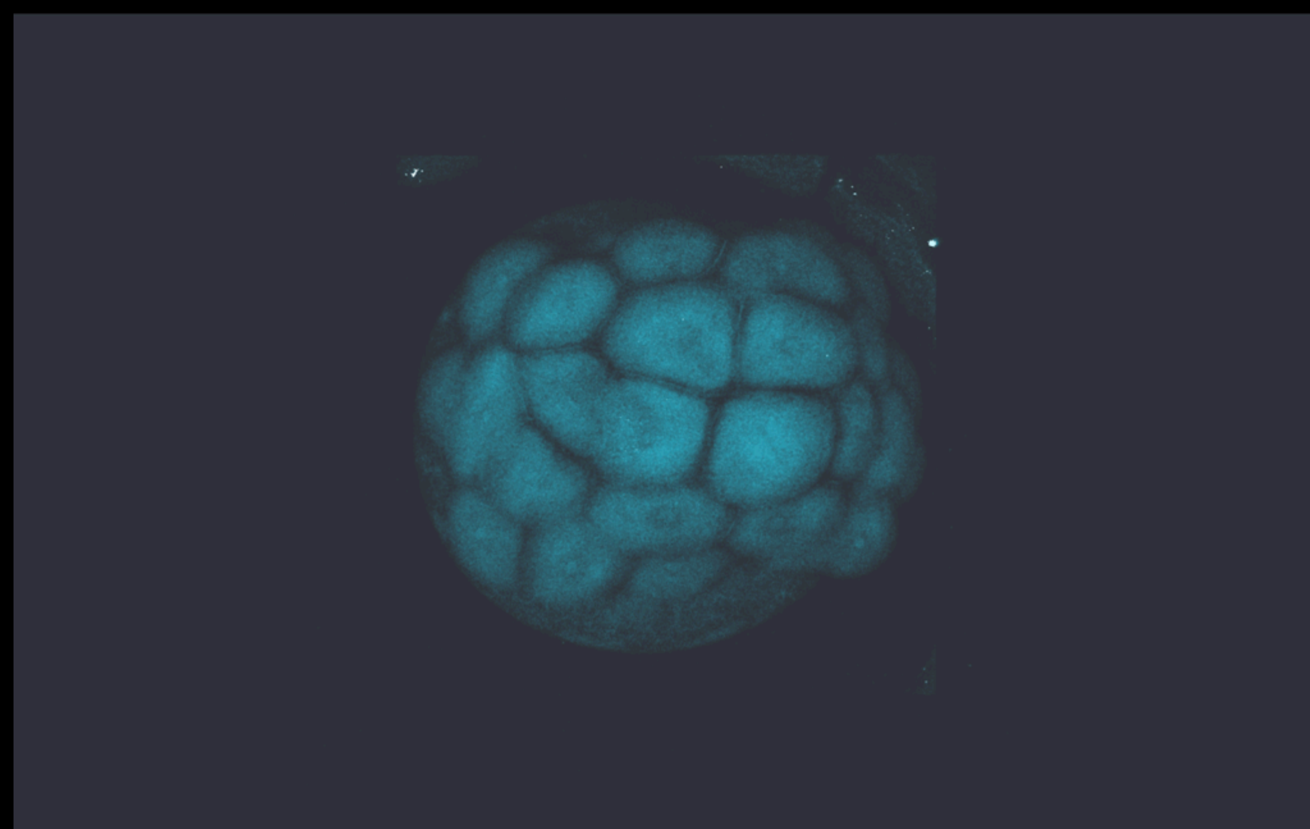
$$d = \mathcal{U}(d_0 - \frac{w_d}{2}, d_0 + \frac{w_d}{2})$$

Live Specimen

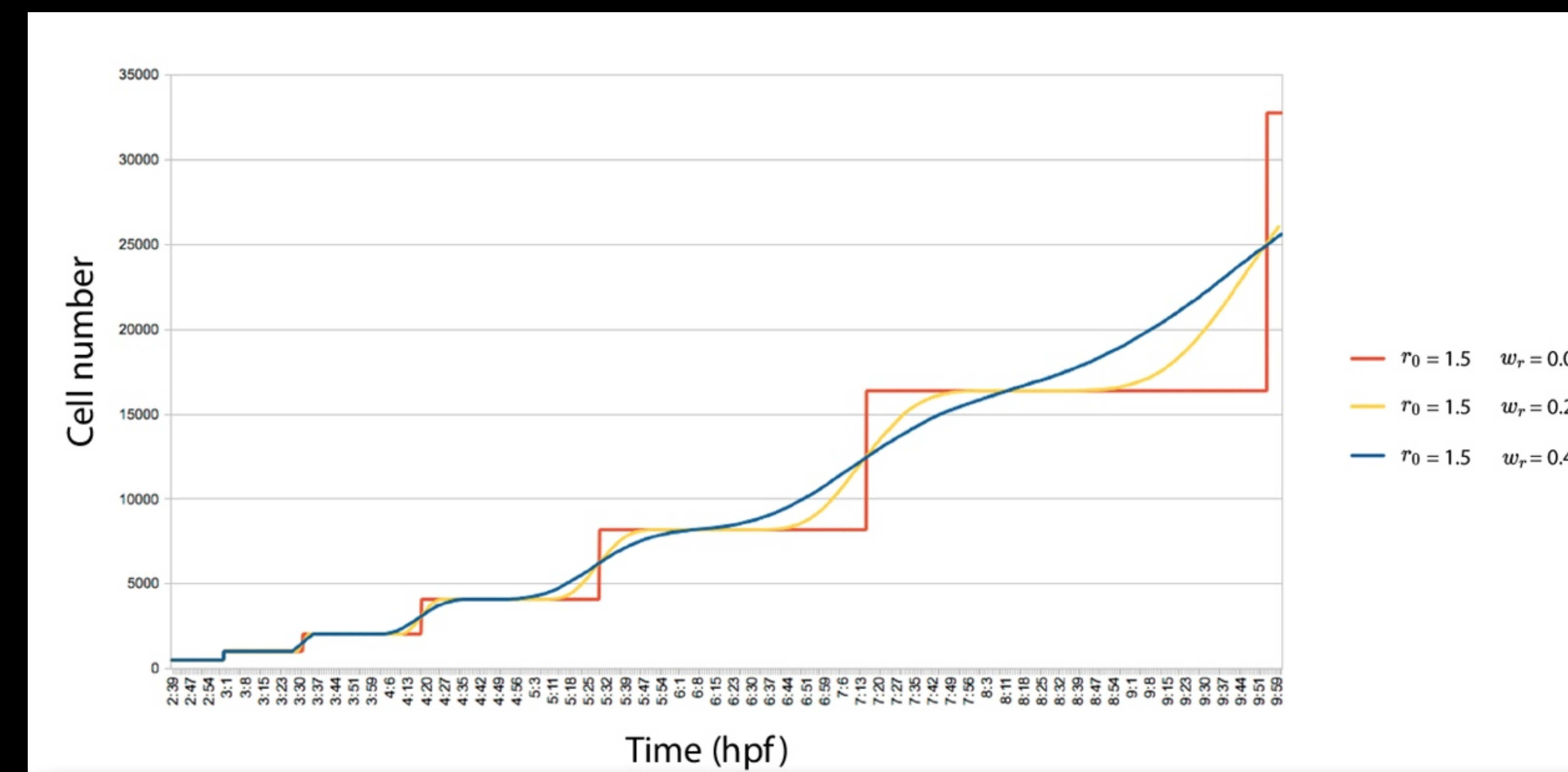
Model

# 2. Cell Proliferation Rate Along the Cell Lineage

## *What is the law of cell cycle lengthening and desynchronizing?*



Dataset ID 071222bF between 32-cell stage (1.75 hpf) and gastrulation.




Cell number as a function of the cell cycle length (Geometric progression)

Geometric progression

$$\forall n \in [n_{\text{start}}, n_{\text{end}}], L_{\text{cc}}(n+1) = r \cdot L_{\text{cc}}(n)$$

$$r = \mathcal{U}(r_0 - \frac{w_r}{2}, r_0 + \frac{w_r}{2})$$

OR

Arithmetic progression

$$\forall n \in [n_{\text{start}}, n_{\text{end}}], L_{\text{cc}}(n+1) = d + L_{\text{cc}}(n)$$

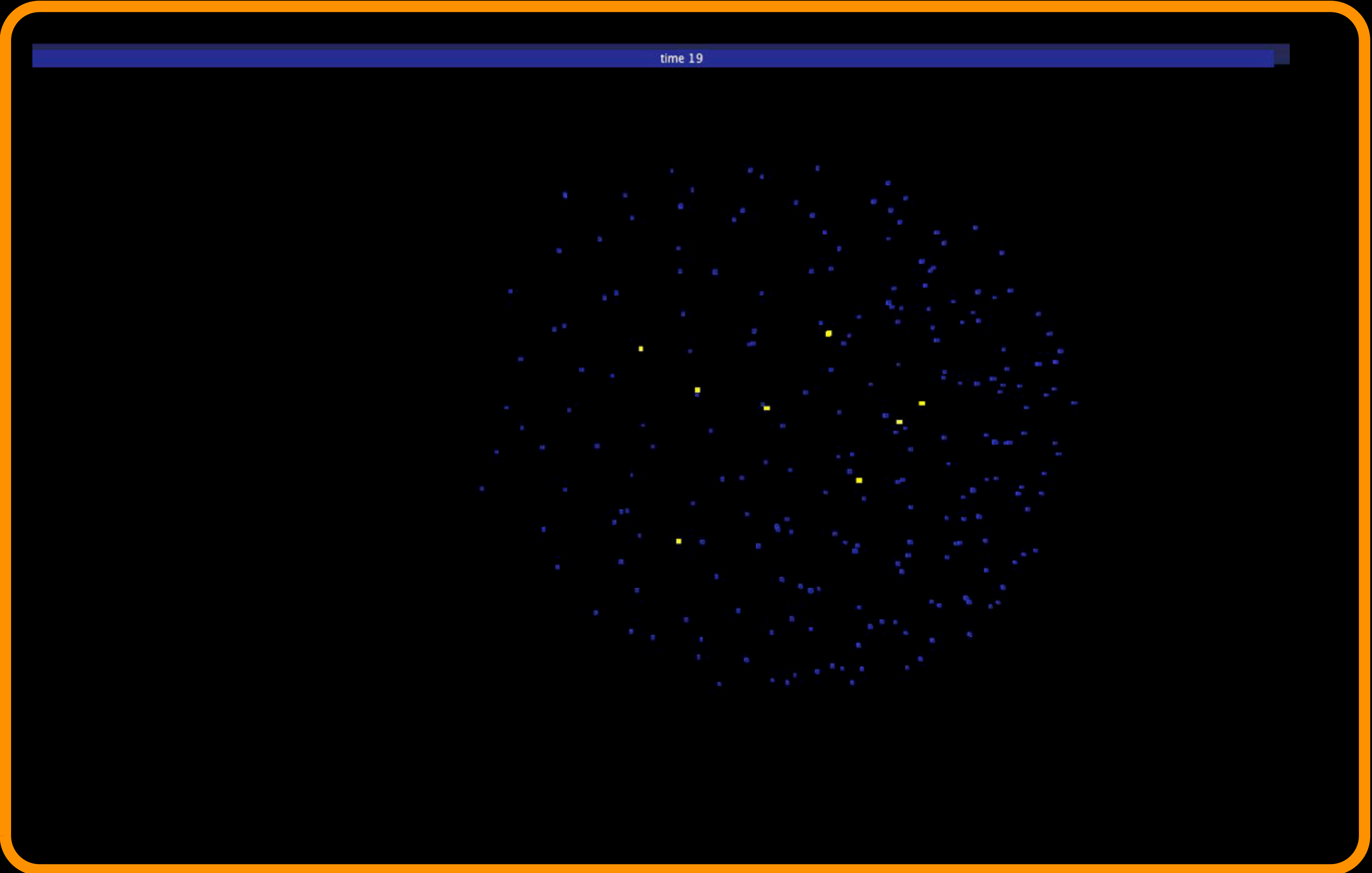
$$d = \mathcal{U}(d_0 - \frac{w_d}{2}, d_0 + \frac{w_d}{2})$$

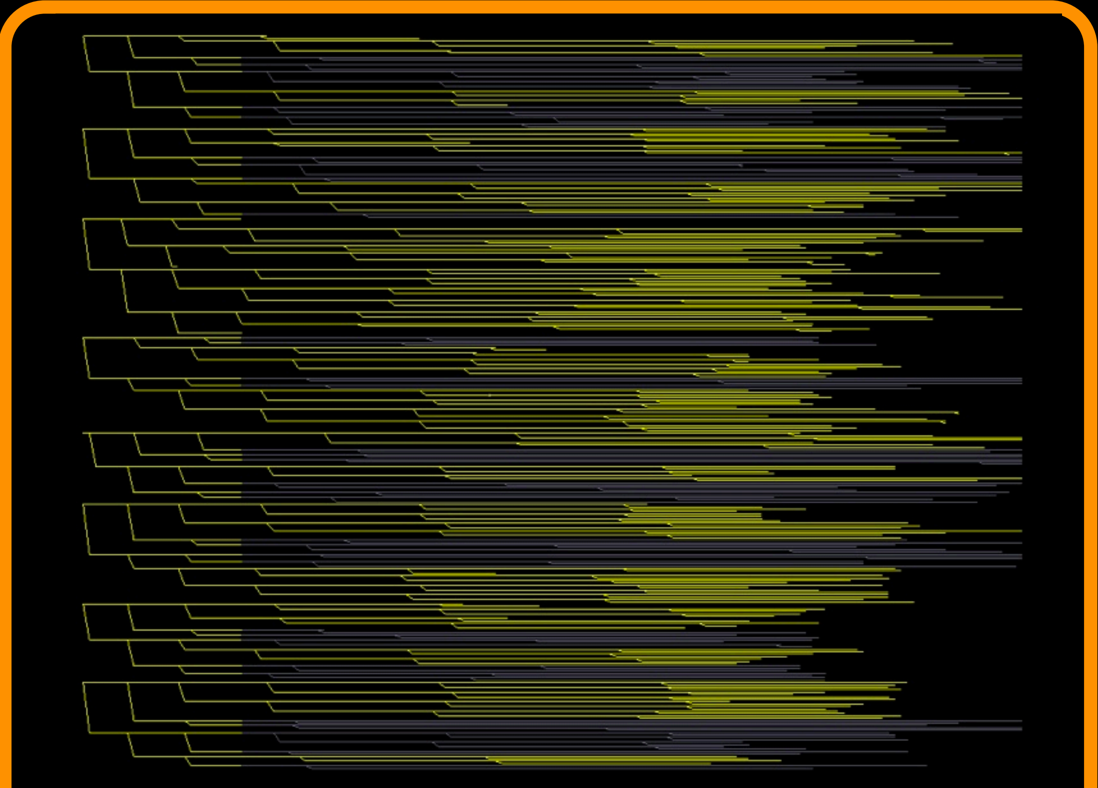
Live Specimen

Model

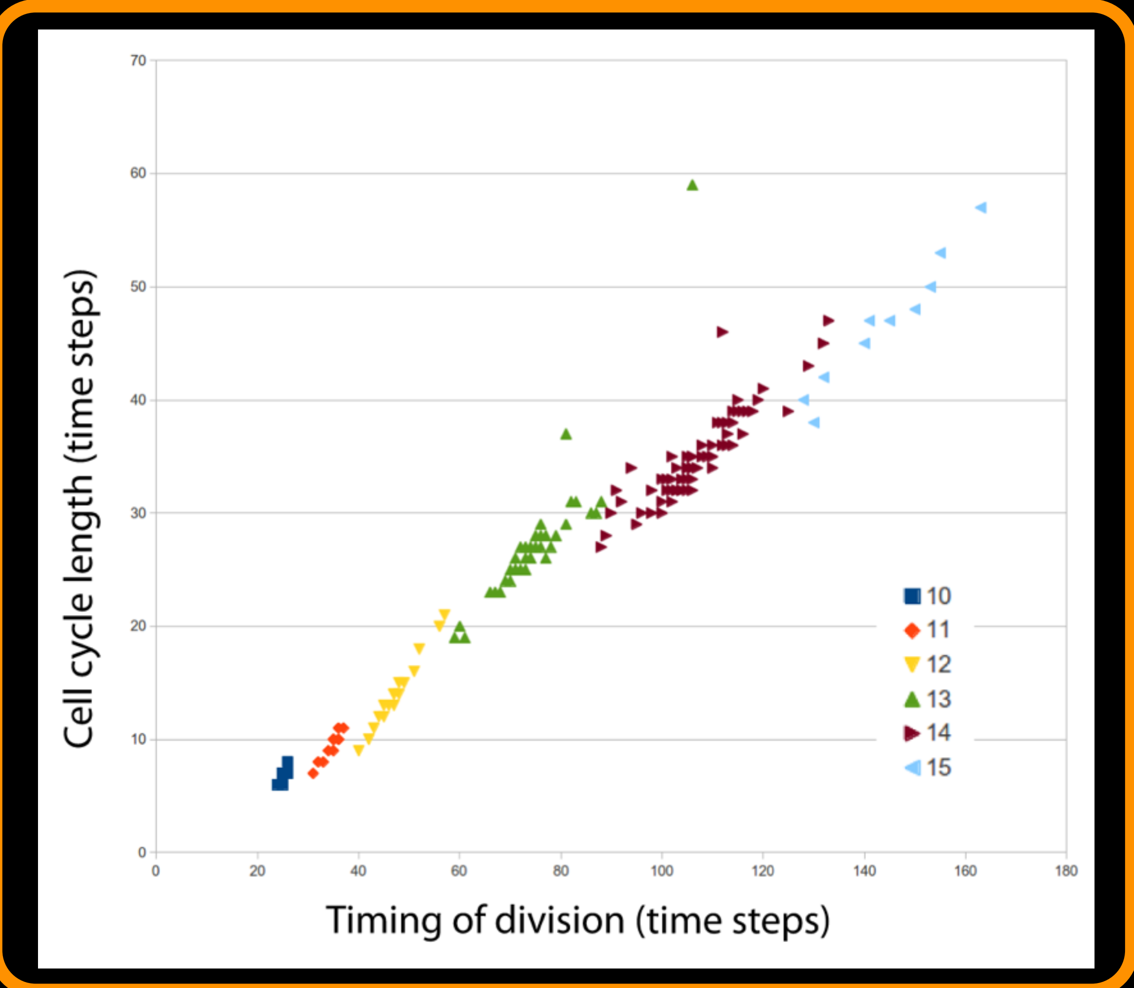
2. Cell Proliferation Rate Along the Cell Lineage

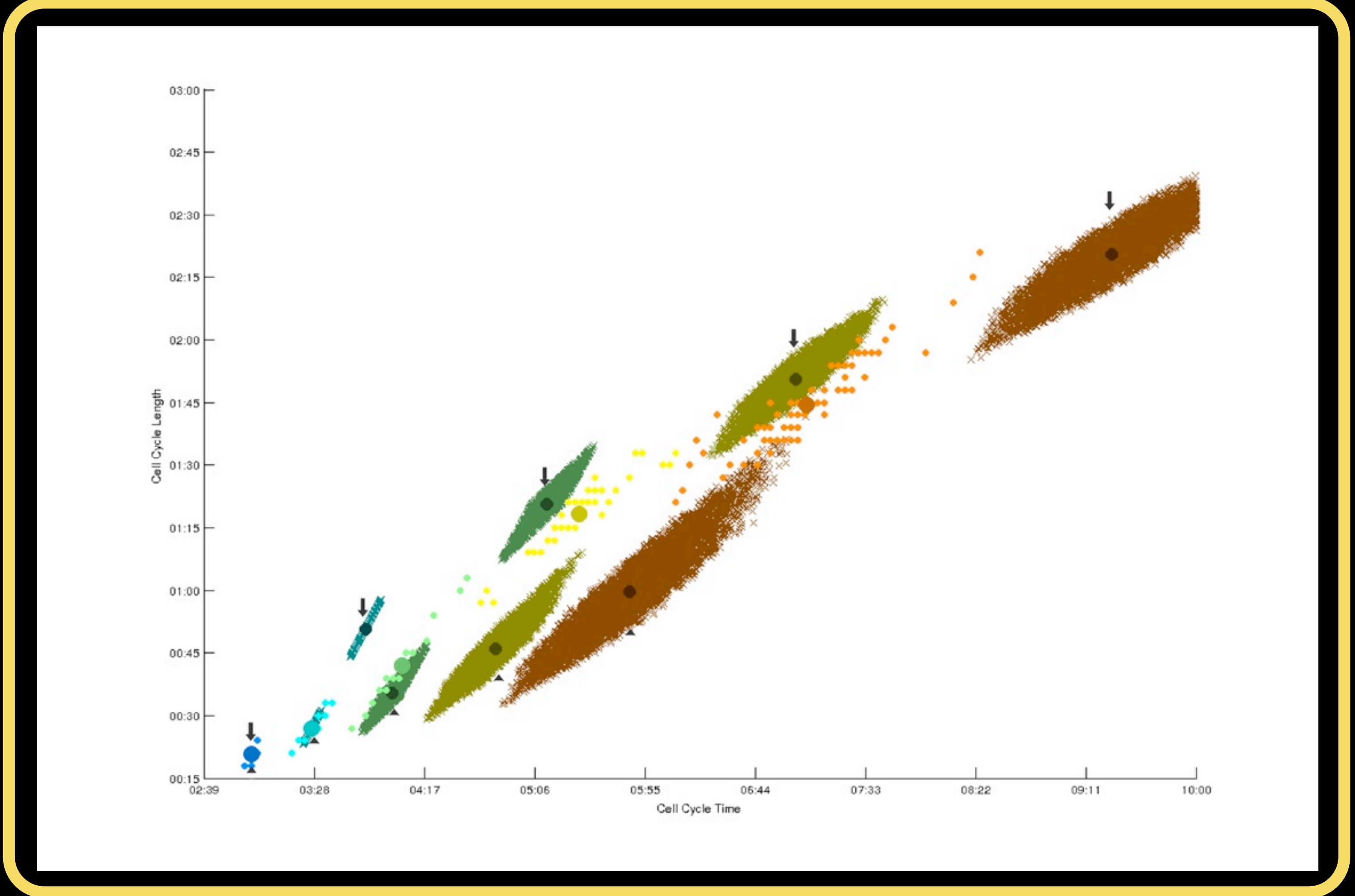
# Division times along the lineage from cycle 10 to 14



Manual correction by Ingrid Colin



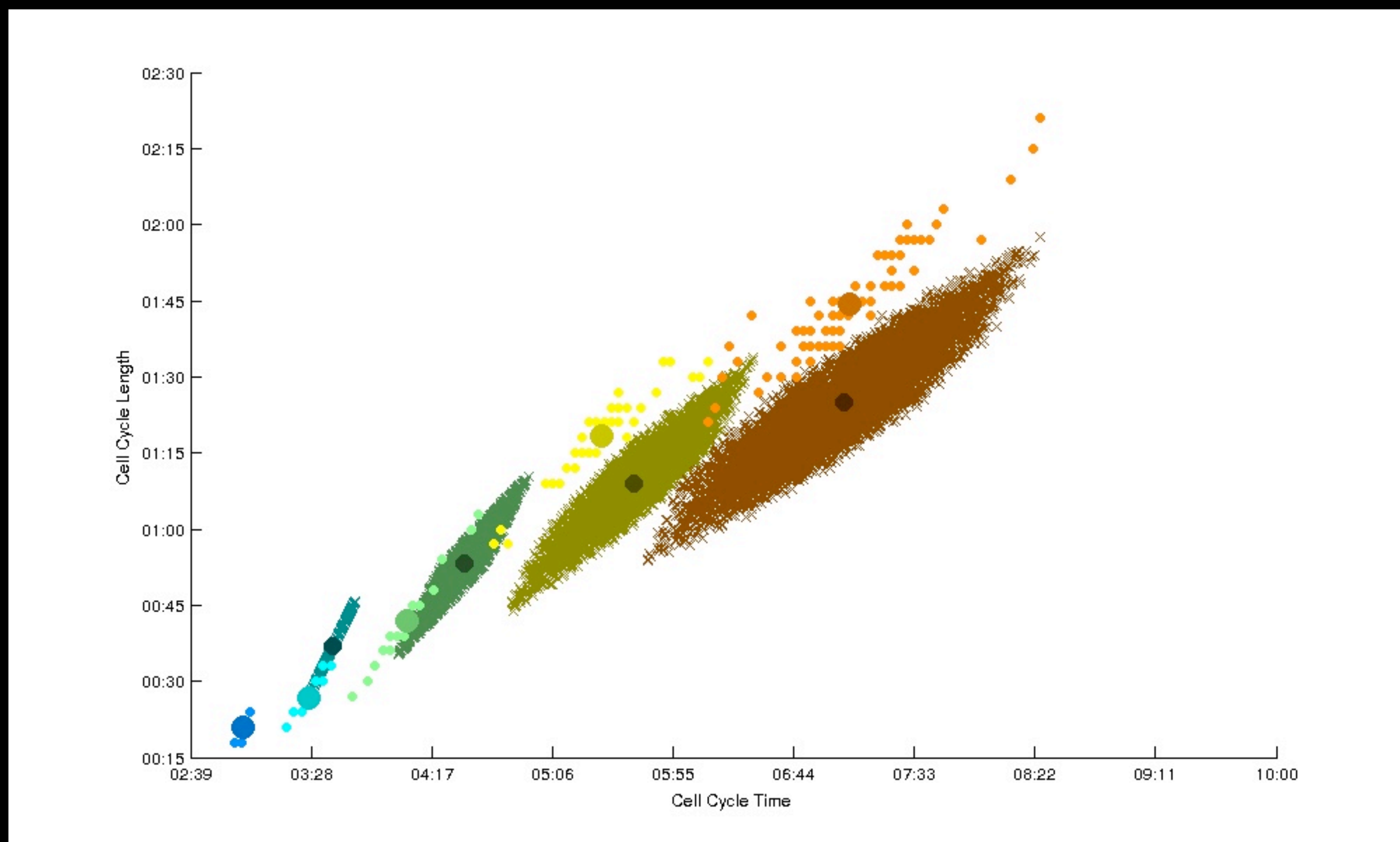




Live Measures

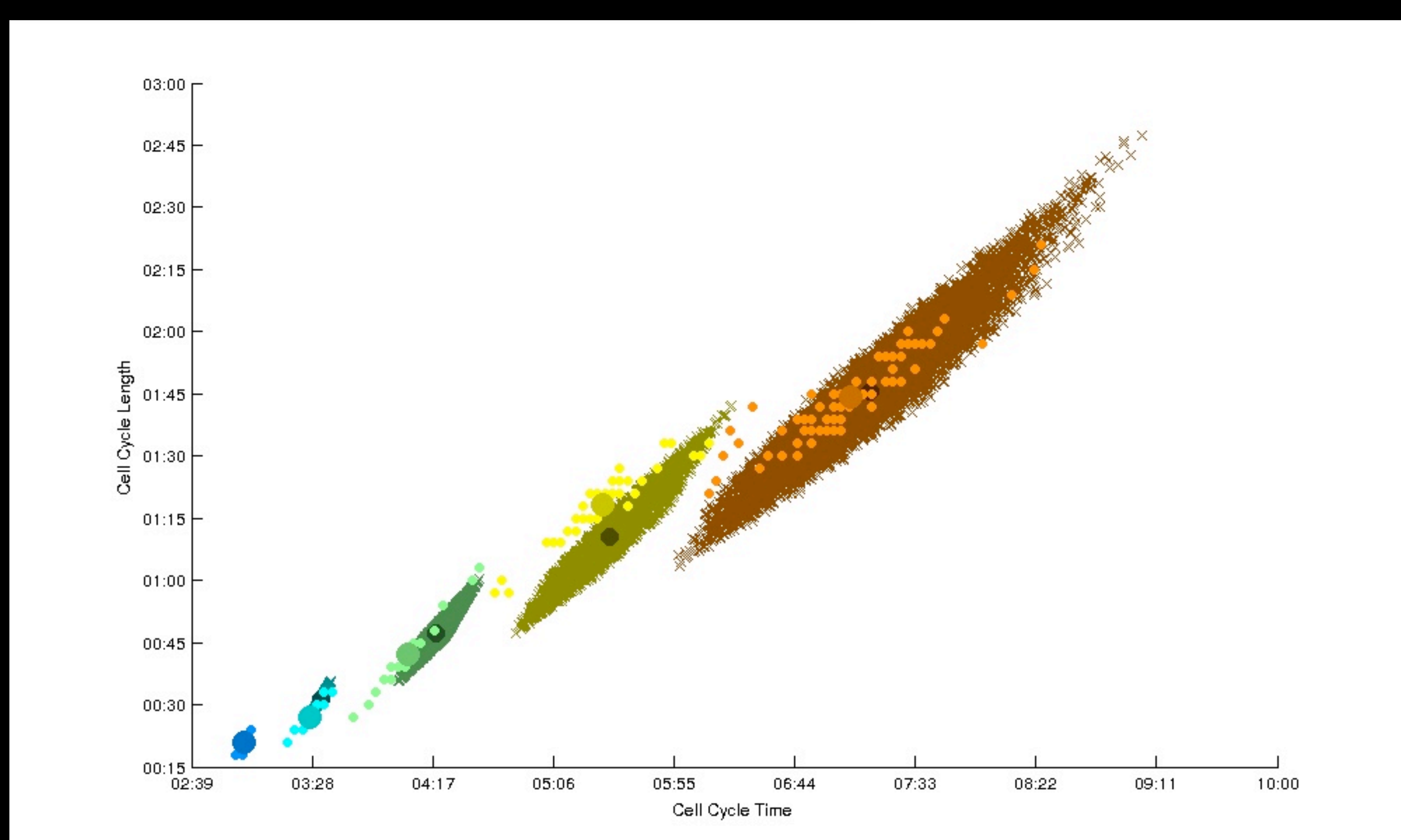
Simulated Measures

## 2. Cell Proliferation Rate Along the Cell Lineage

# The geometric progression provides the best fitness



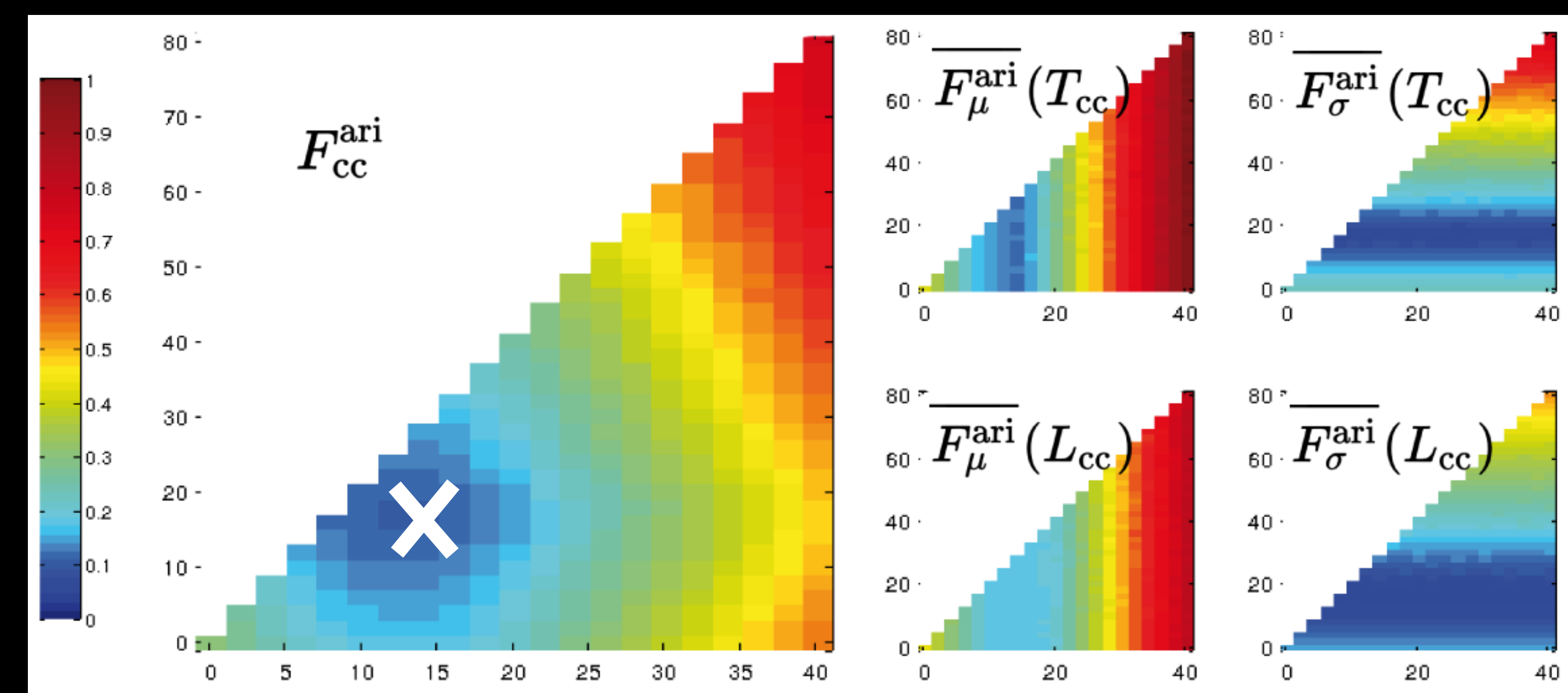

Best couple of parameters for the arithmetic progression rule. $d_0 = 14\,\text{min},\ w_d = 18\,\text{min}$

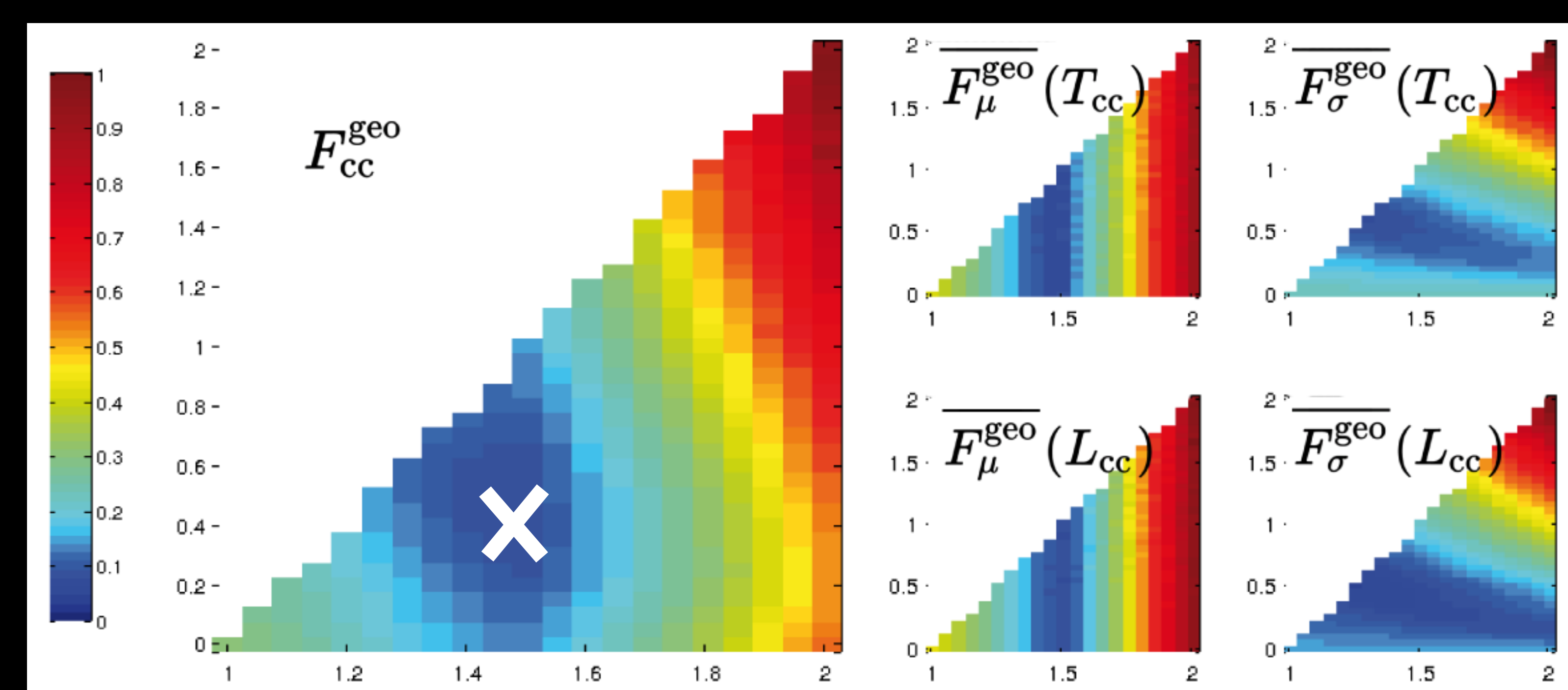



Best couple of parameters for the geometric progression rule. $r_0 = 1.5,\ w_r = 0.4$

$F_\mu(T_{cc})$ $F_\sigma(T_{cc})$ : difference of mean/std of the cell cycle times

$F_\mu(L_{cc})$ $F_\sigma(L_{cc})$ : difference of mean/std of the cell cycle lengths

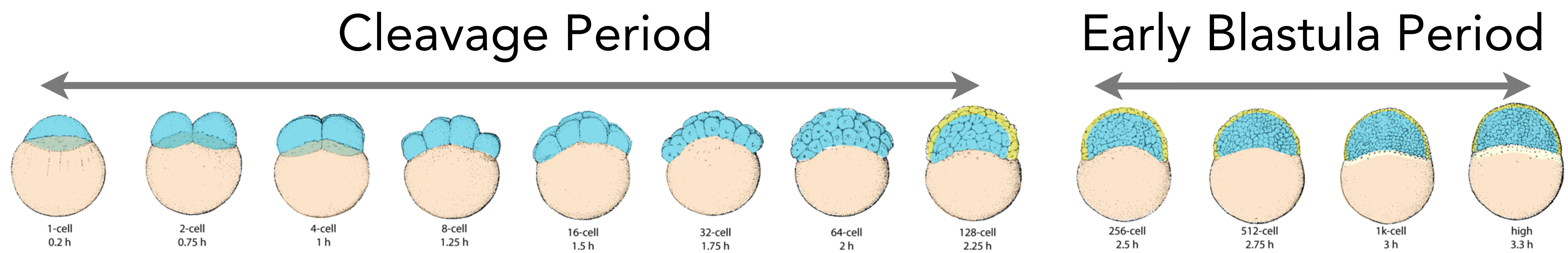


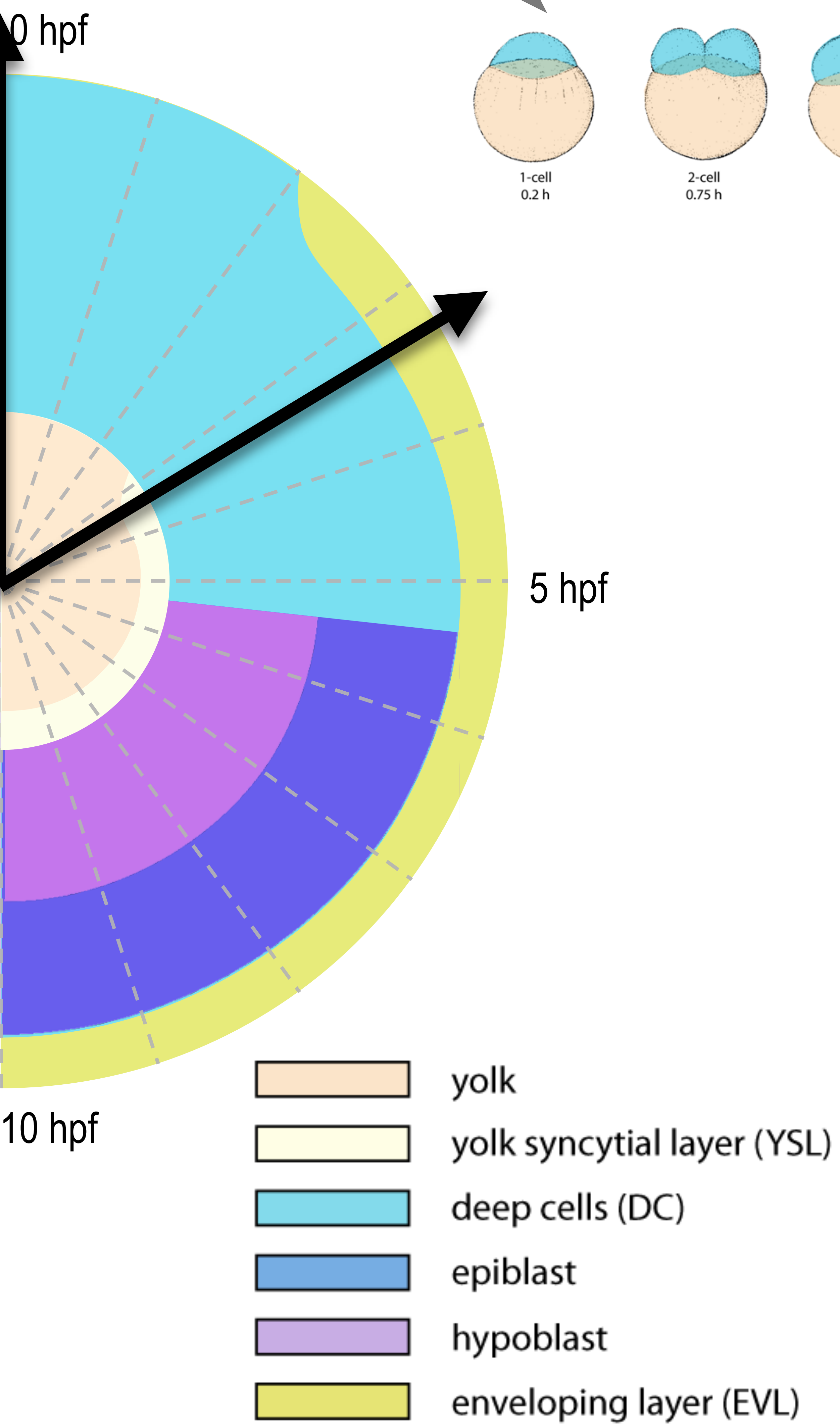



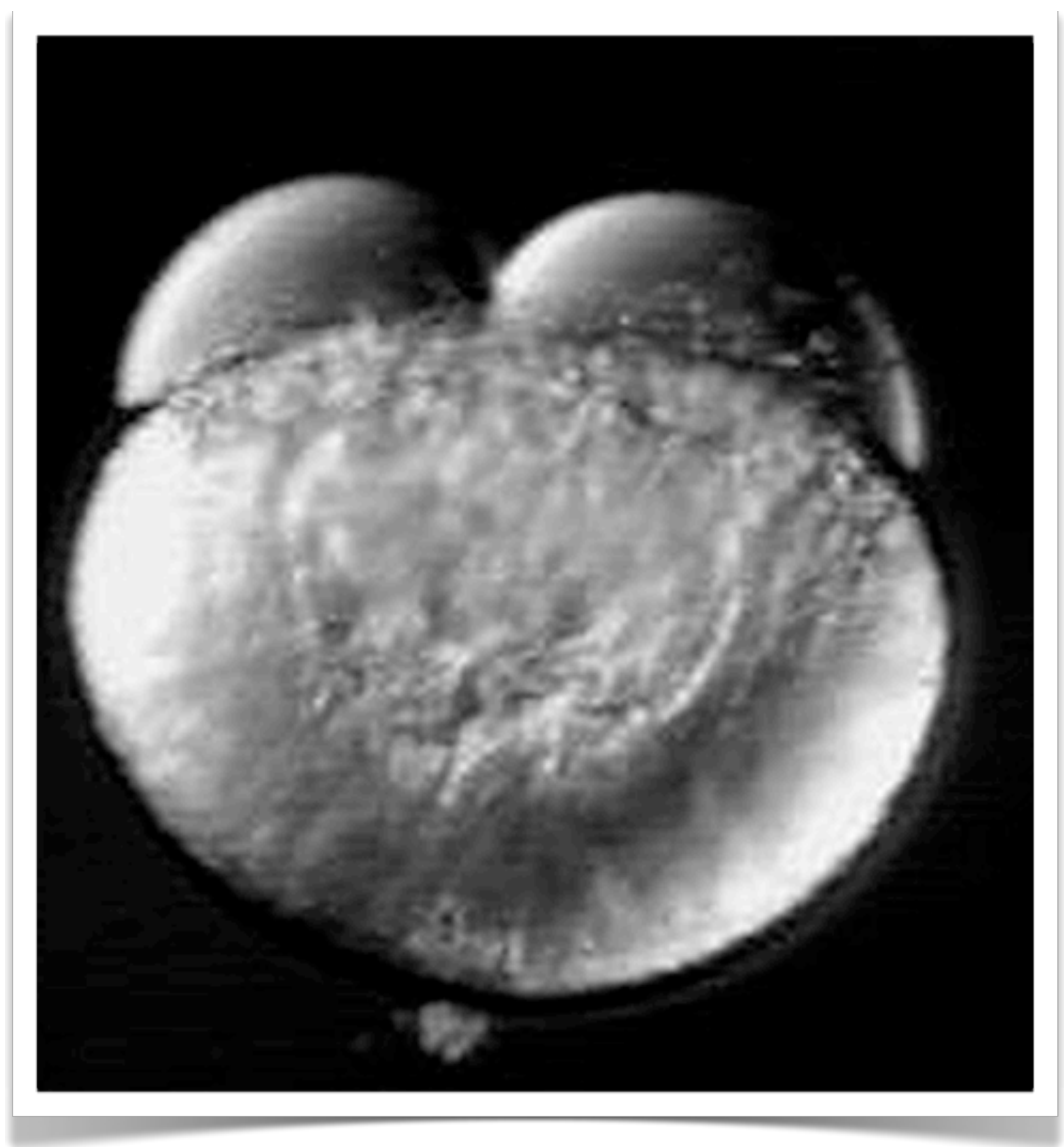

Validation

# 3. Shaping the Zebrafish Blastula

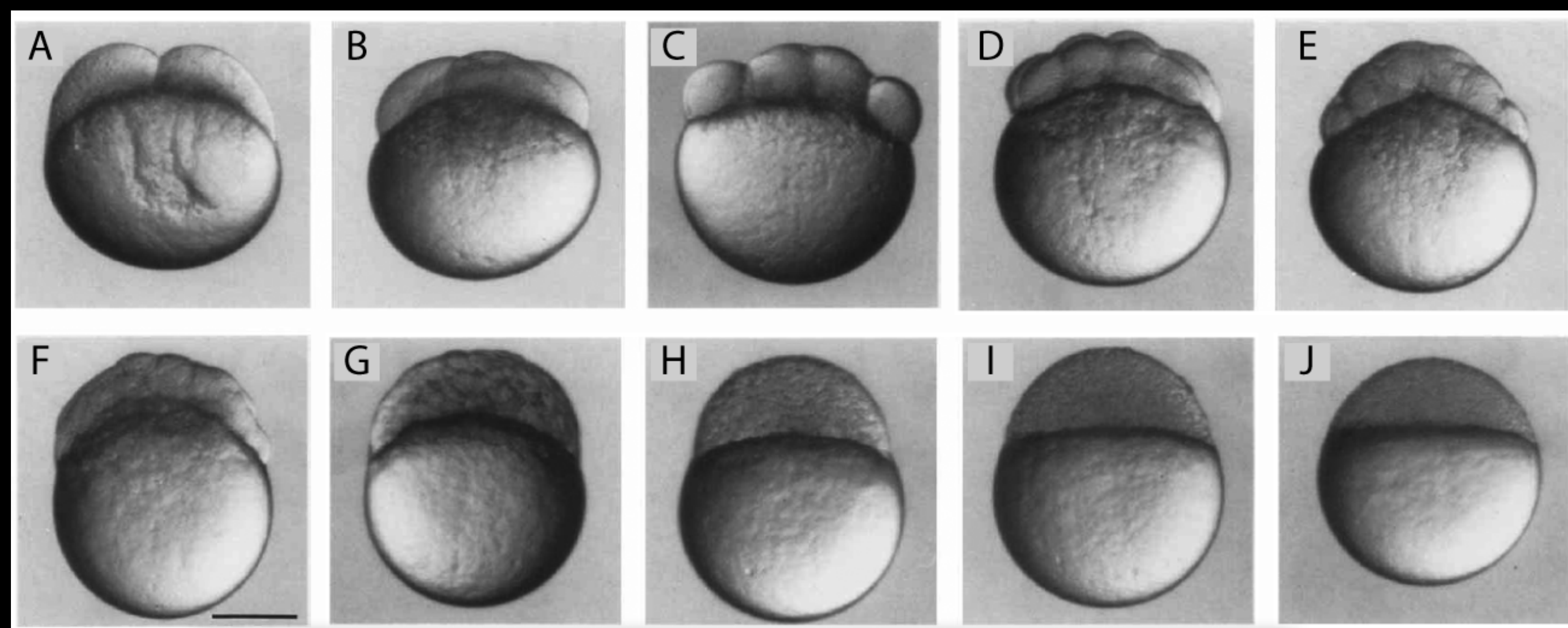
## *How is the blastula shape emerging from cell-cell interactions?*



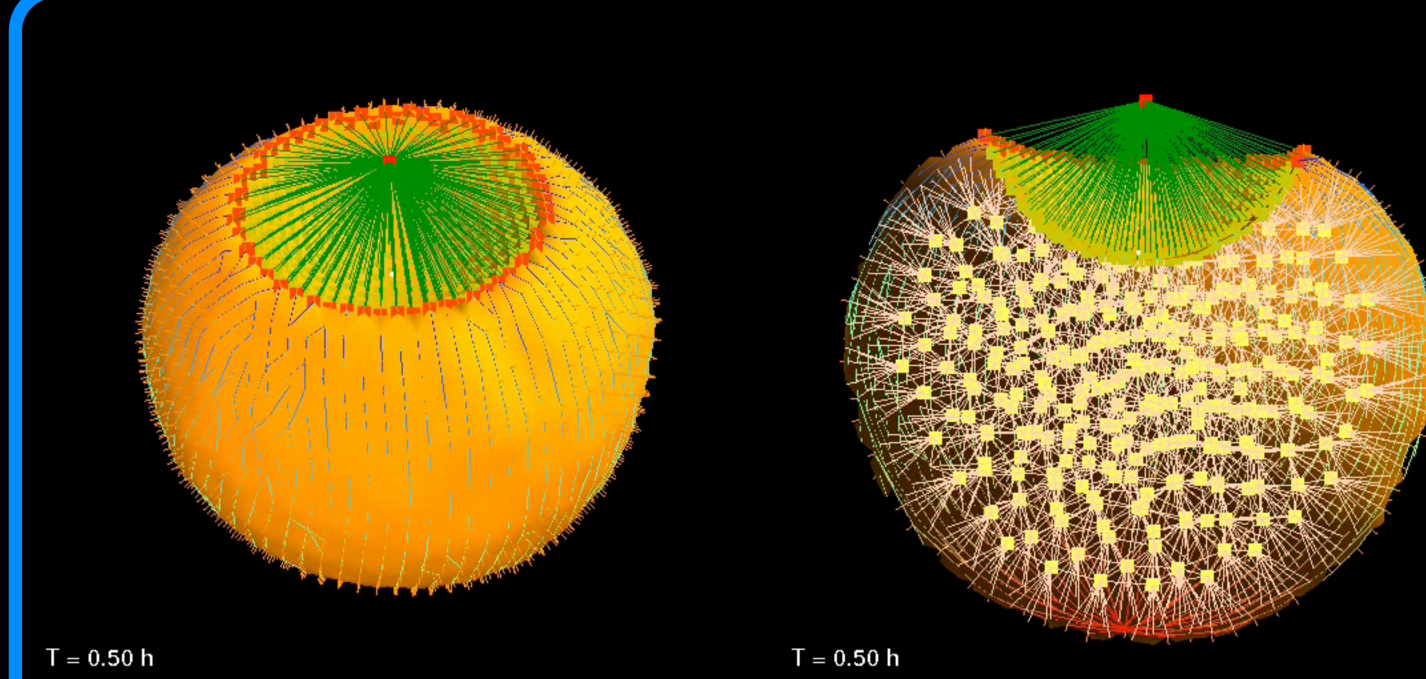



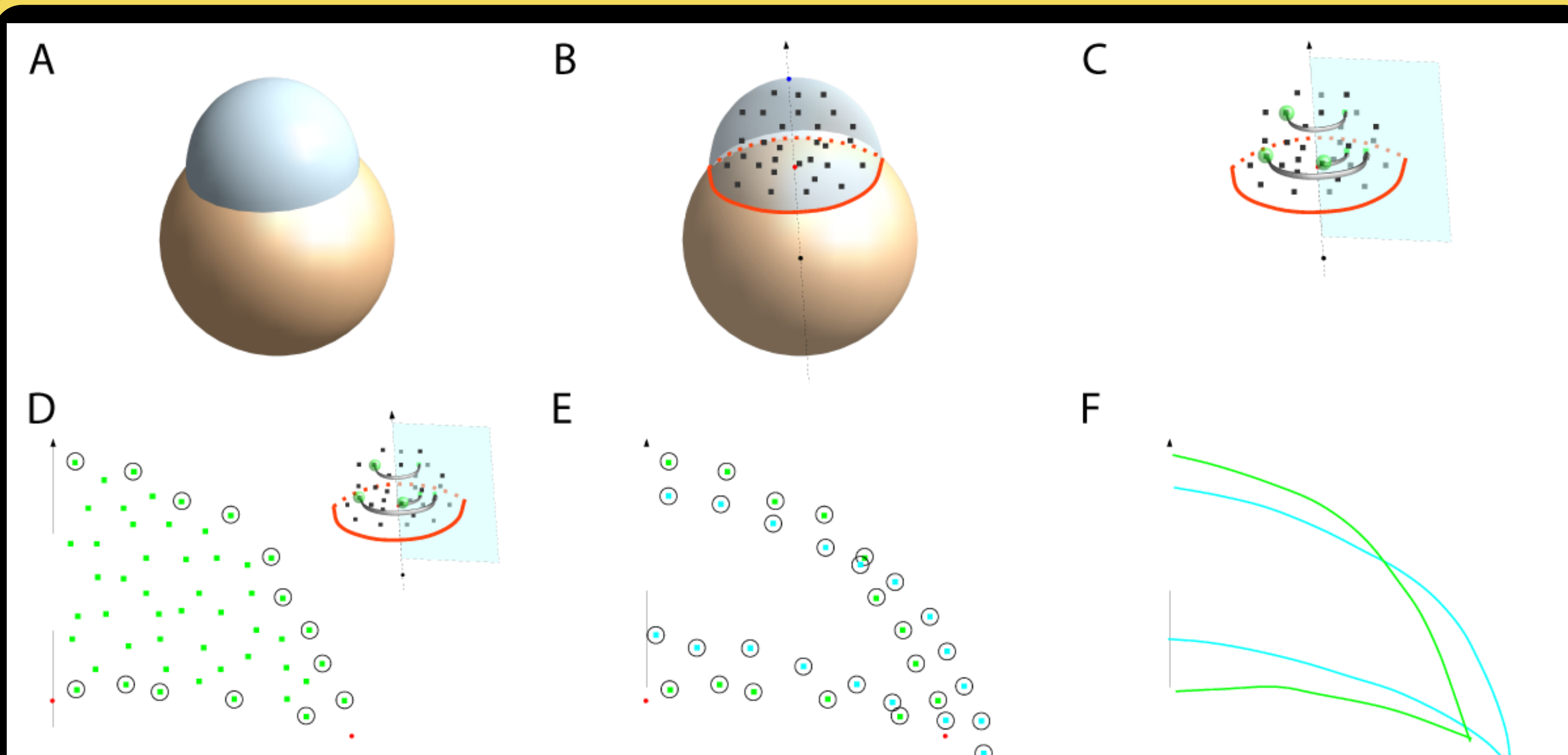
# Proposed fitness: volume/section superposition

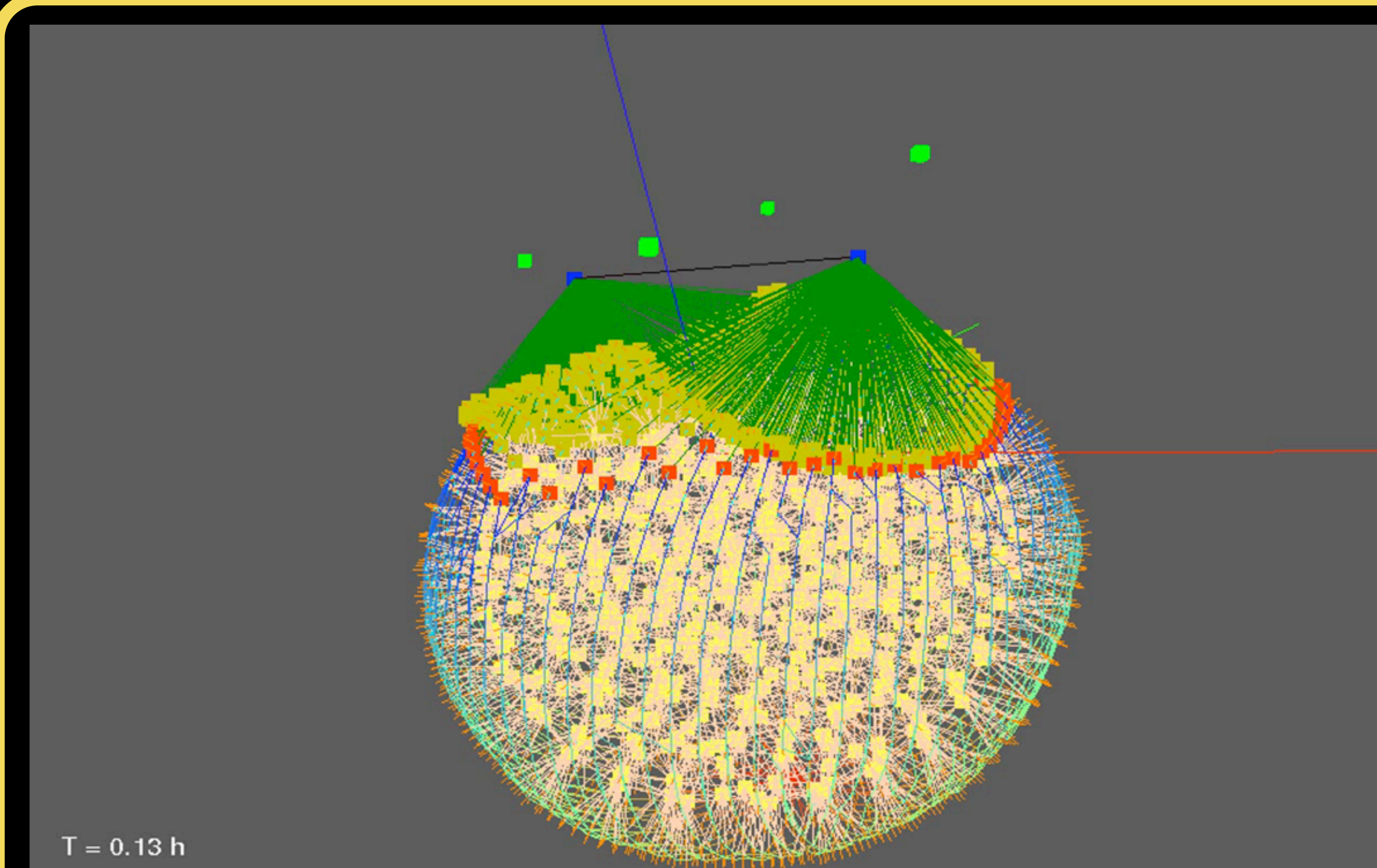



Kimmel, C.B. et al., 1995. Stages of embryonic development of the zebrafish. Developmental




Simulation of the cleavage period.



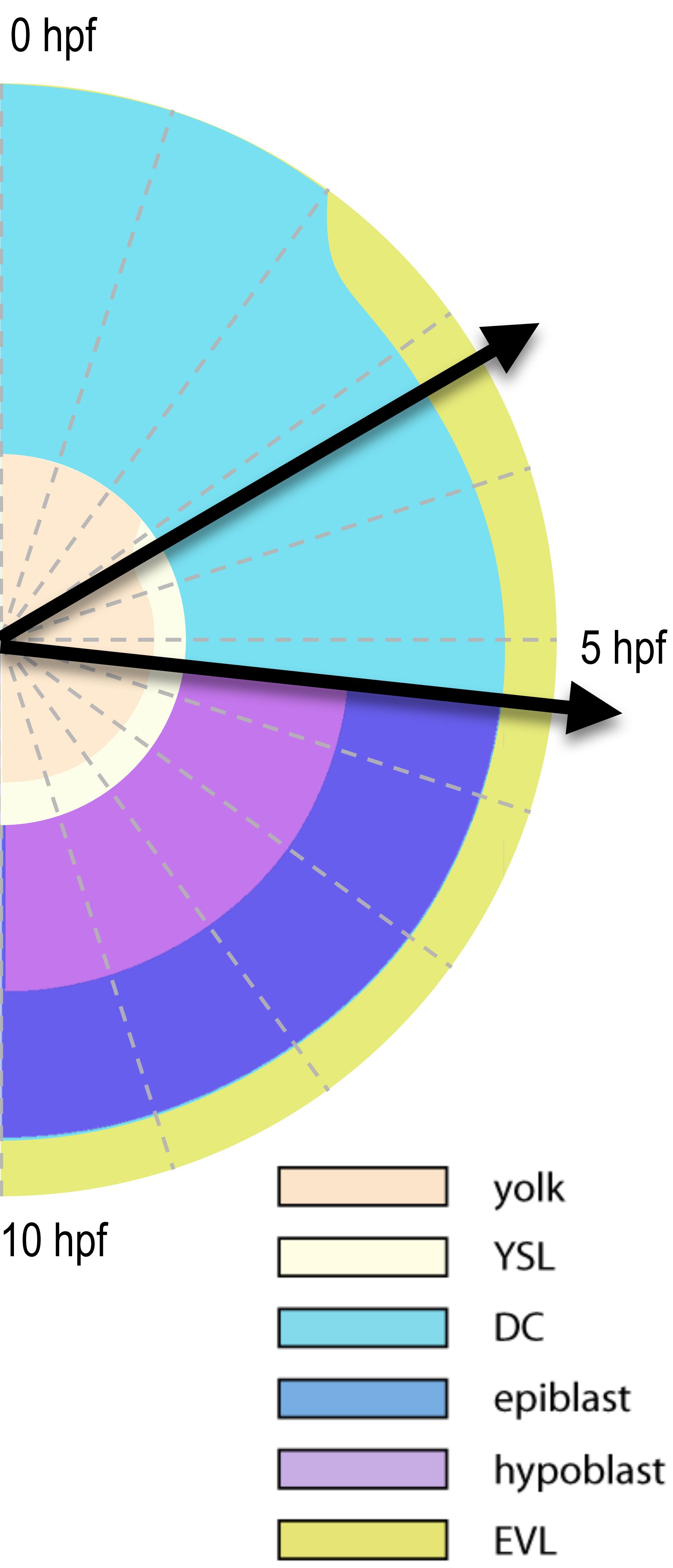

Live Specimen

Model

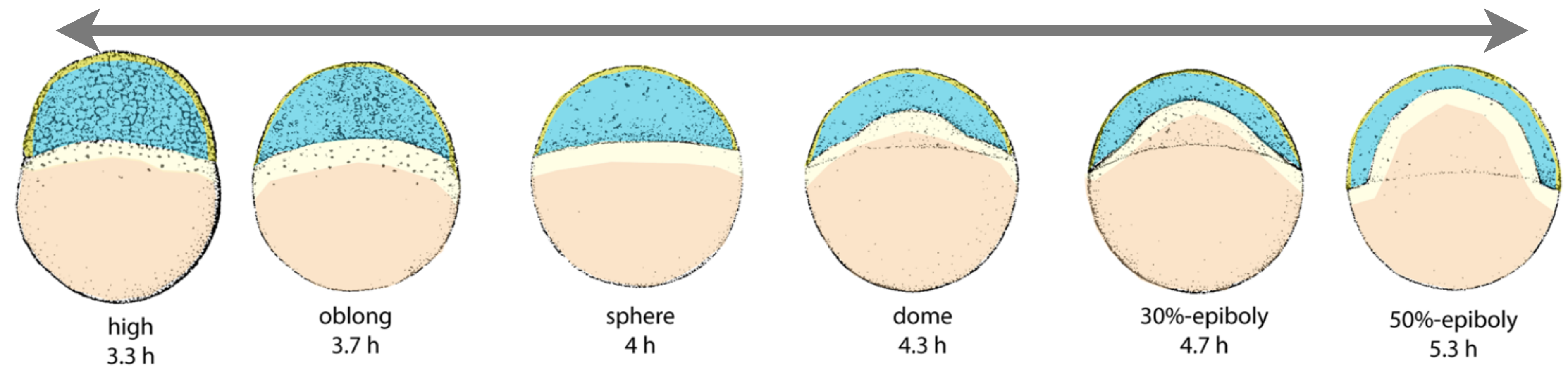
Simulated Measures

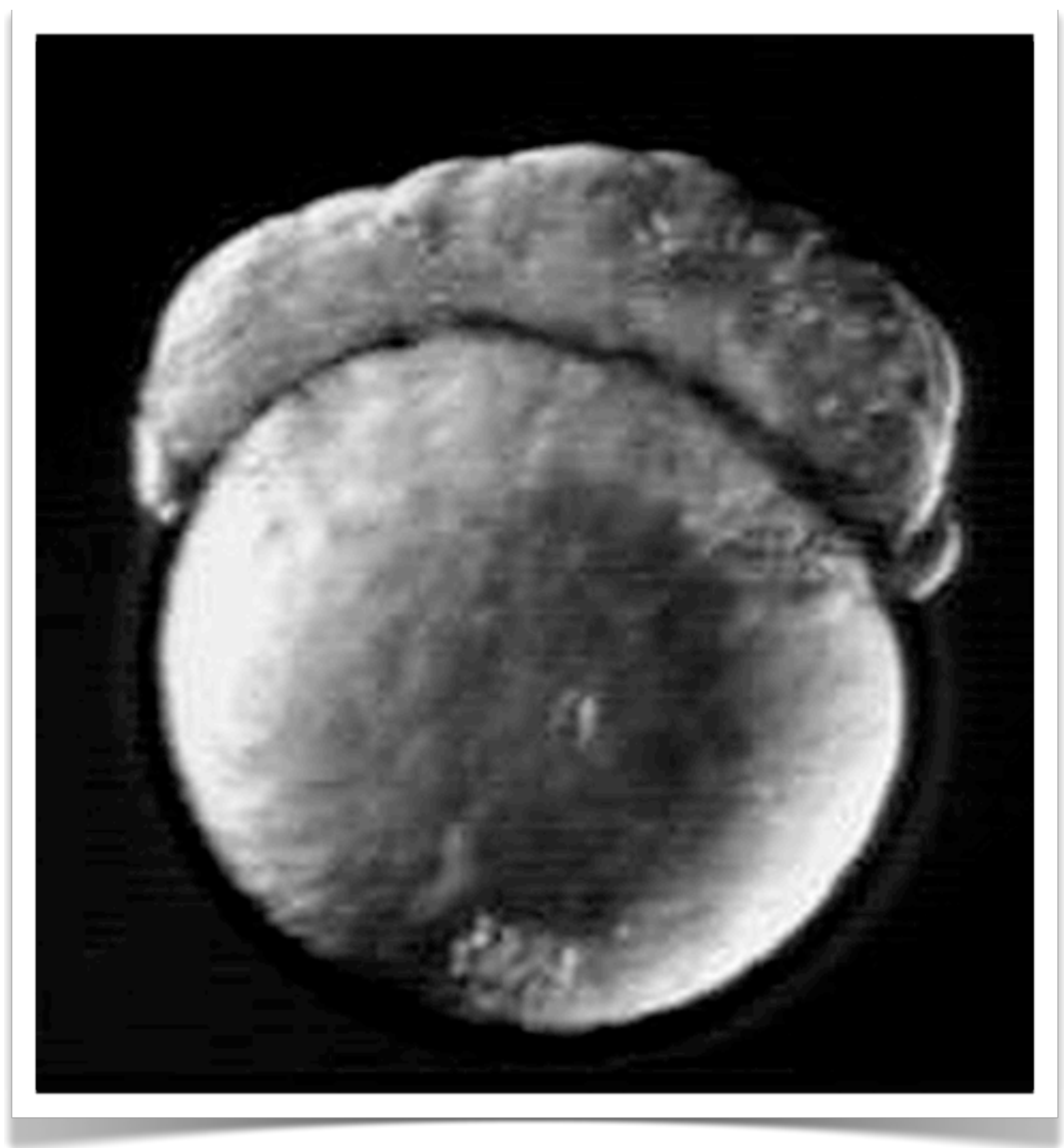
# 4. Cell Behaviors in the Enveloping Layer Compartment

## *What cell interactions lead to the EVL epithelial topology?*

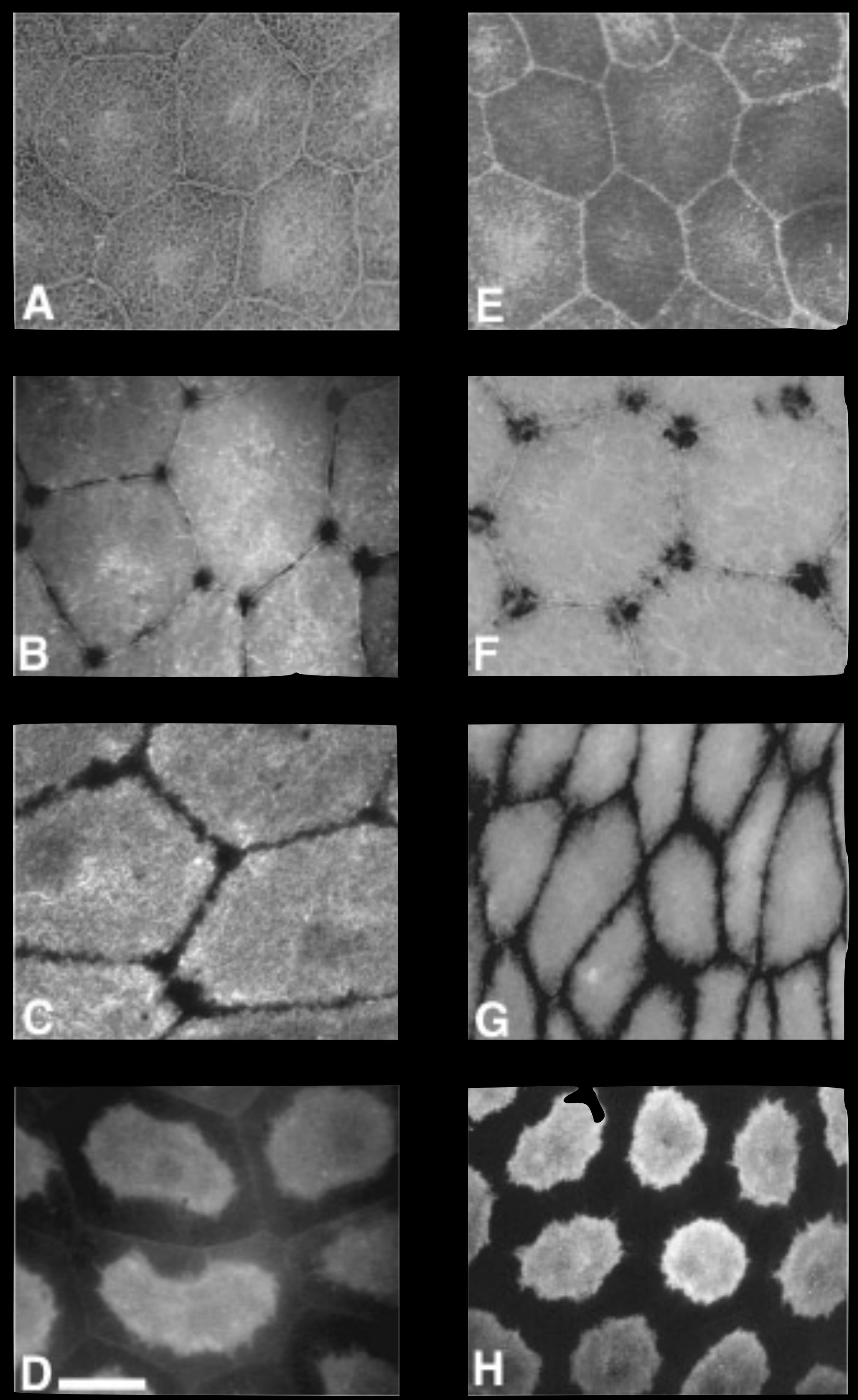



Late Blastula Period

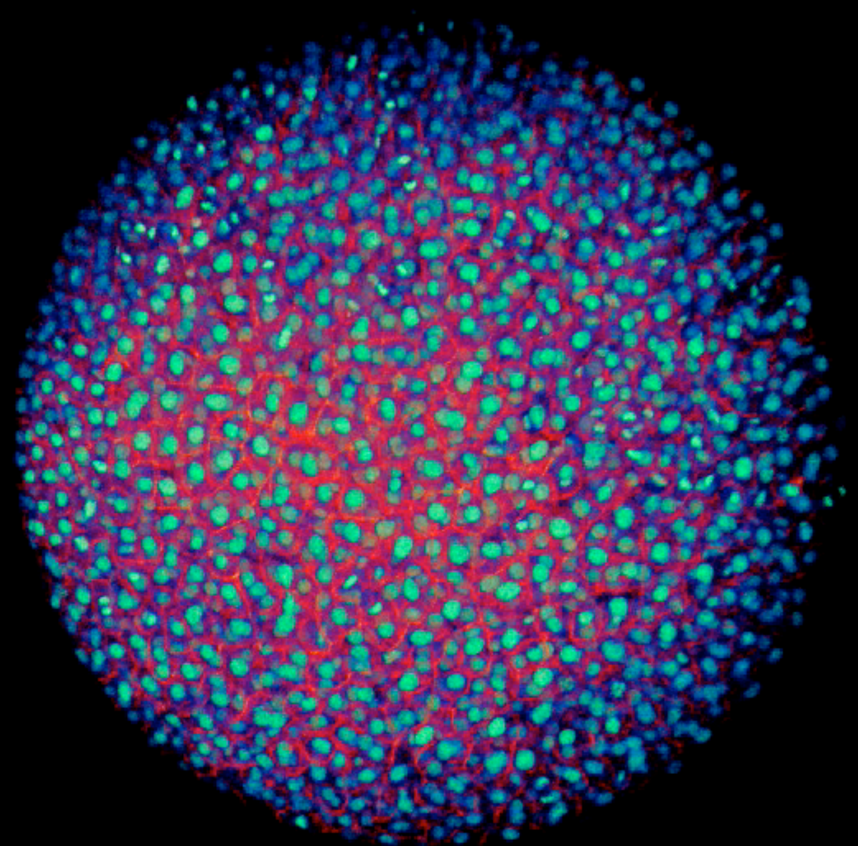


# Mechanical model for EVL cell proliferation




Regional membrane turnover within the apical membranes of Fundulus enveloping layer cells. Fink and Cooper, 1996.



Dataset ID 071222bF between 32-cell stage (1.75 hpf) and gastrulation.

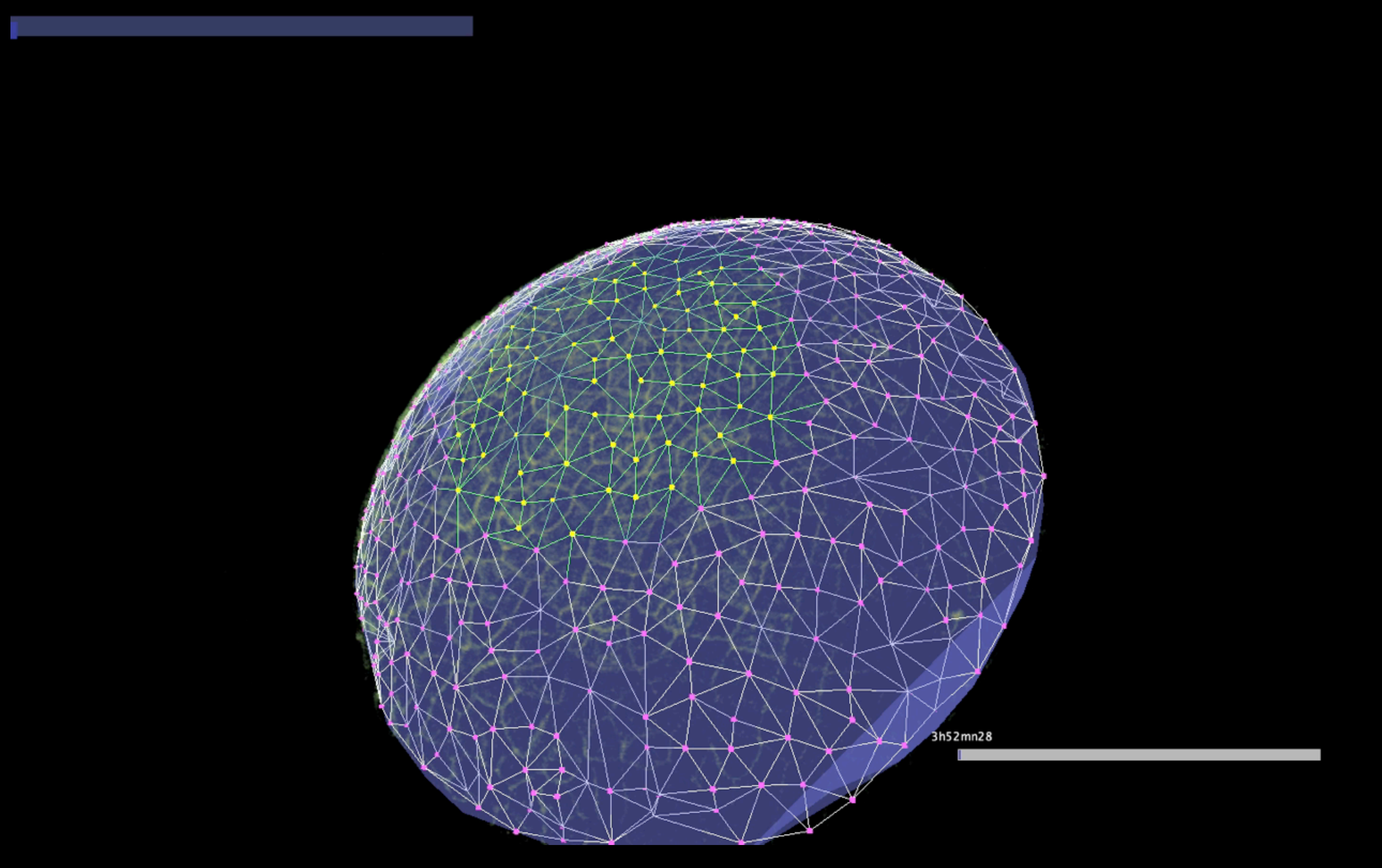
3 parameters :

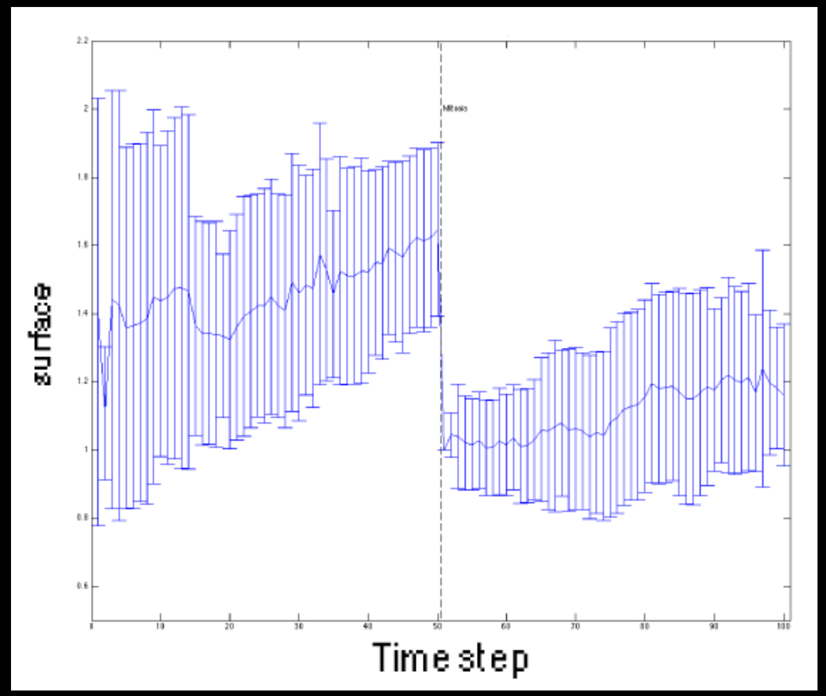
- limit radius coefficient $c_{\text{limit}}^{lat,\text{evl}}$
- EVL growth ratio $\gamma_{\text{evl}}$
- tension threshold $\theta_{\text{evl}}^{-}$

Hypotheses Live Specimen Model

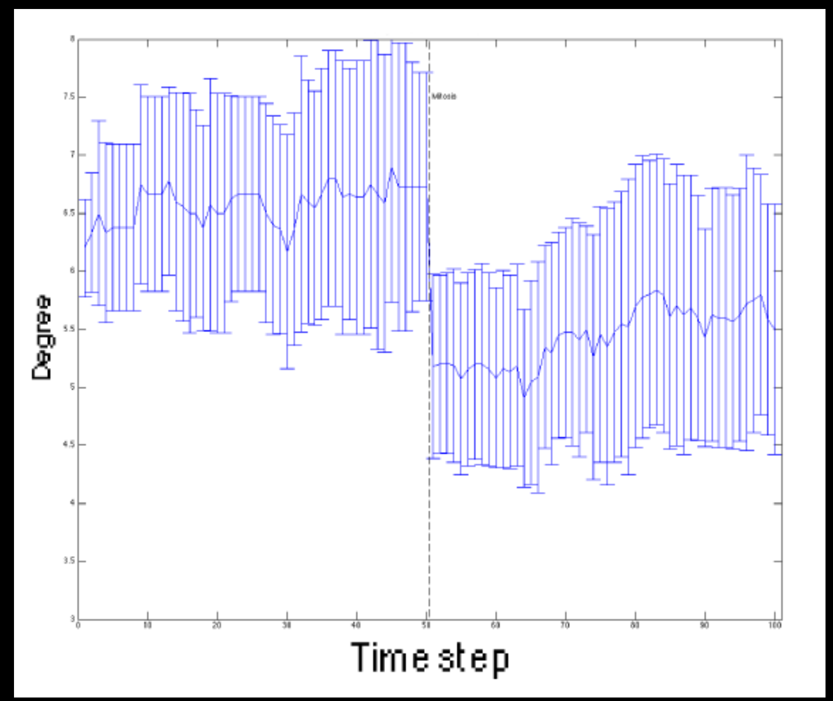
4. Cell Behaviors in the Enveloping Layer Compartment

# Cell degree and surface over time and around mitosis

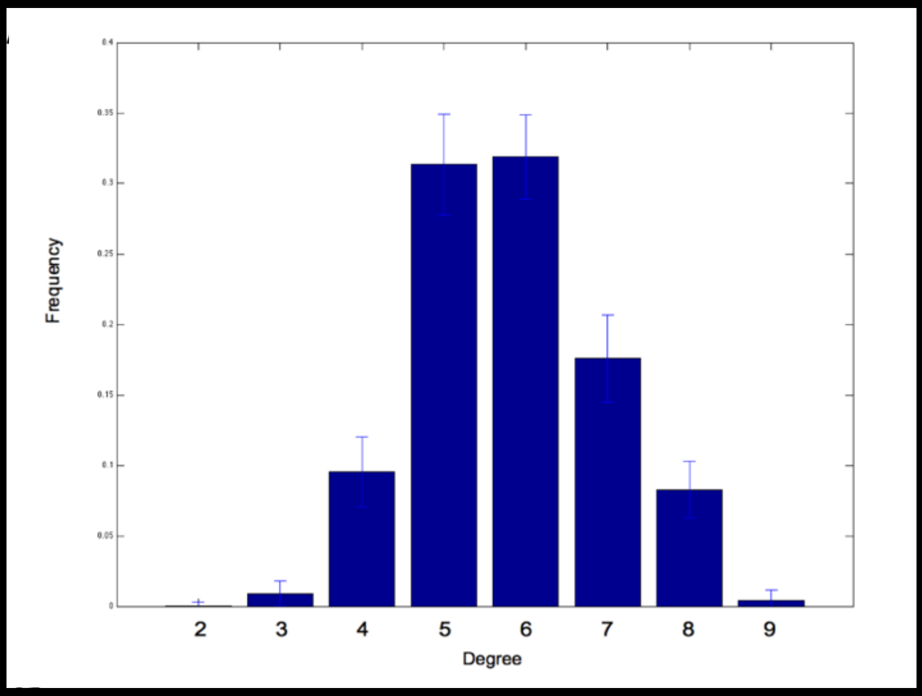




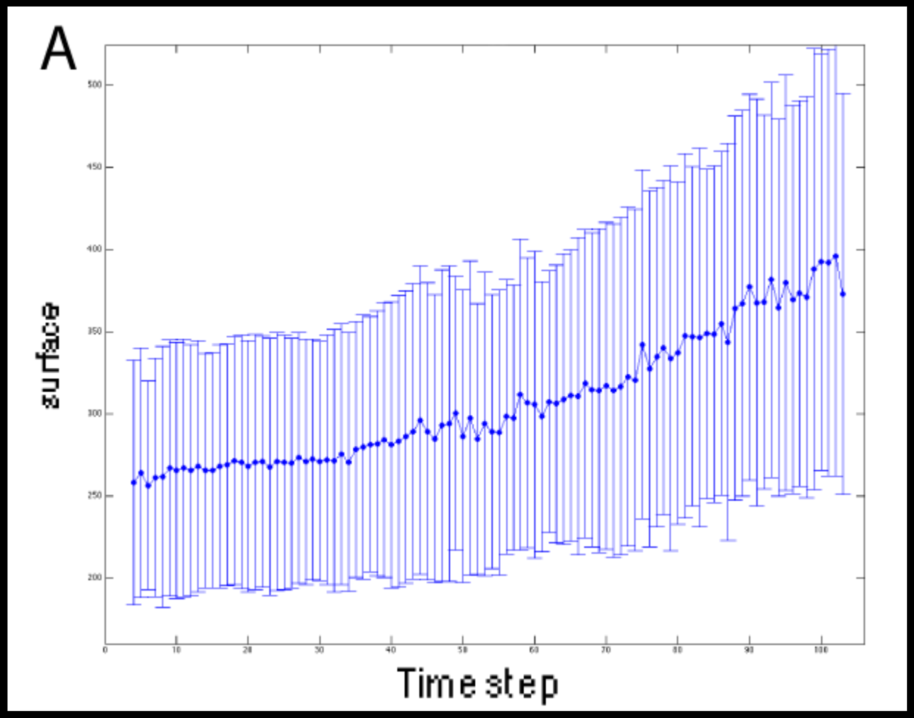

Evolution of the EVL cells' average surface around mitosis.



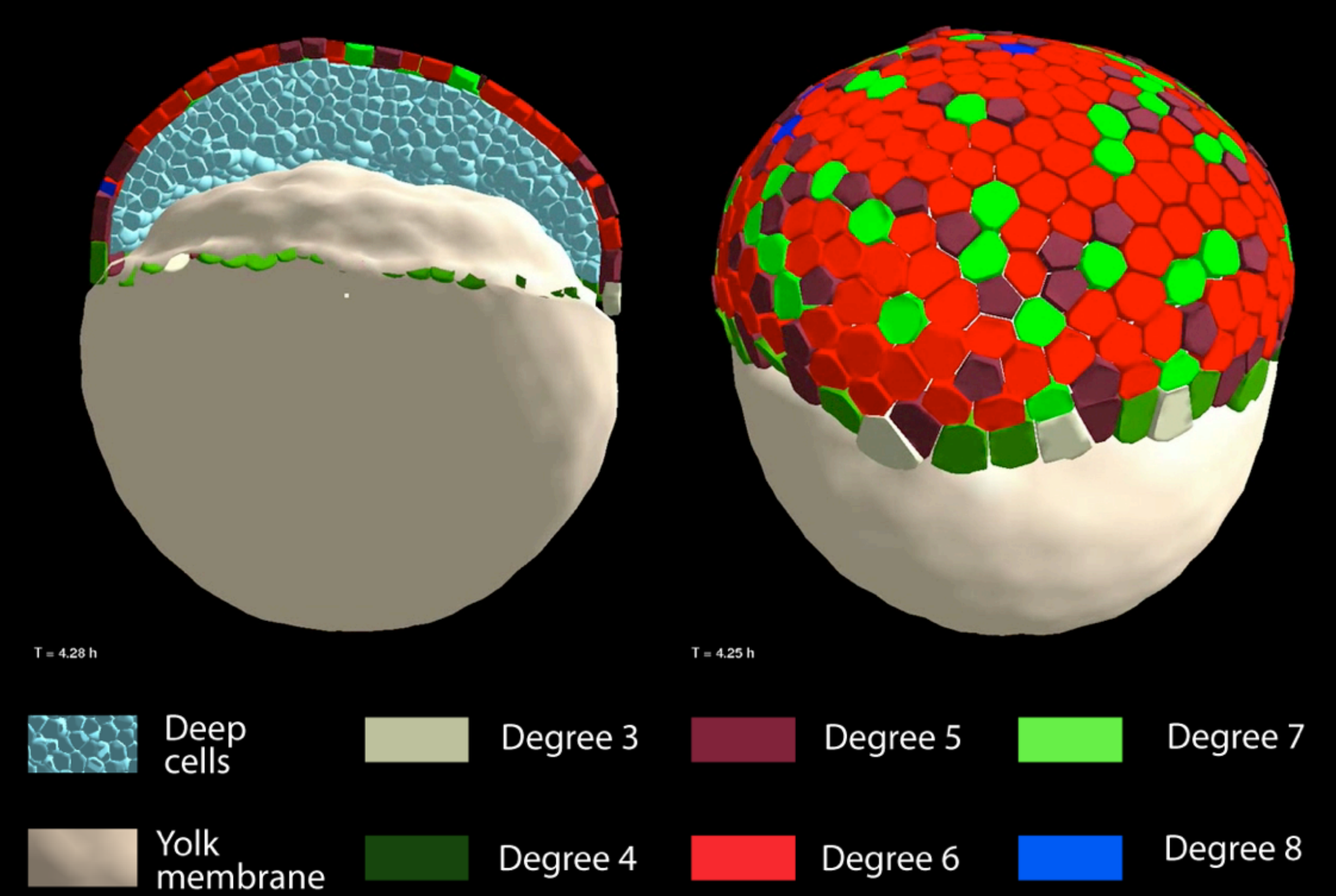

Evolution of the EVL cells' average degree around mitosis.




Histogram of the number of neighbors (degree) in the whole population.




Evolution of the EVL cells' average surface through time.




Manual correction by Adeline Boyreau

Live measures by Paul Villoutreix

Live Measures

Simulated Measures

# The simulation catches the geometrical features better than the topological ones

| | Min. | Max. | Cardinality | Unit |
|---|---|---|---|---|
| $c_{\text{limit}}^{lat,\text{evl}}$ | 1.1 | 3 | 15 | - |
| $\gamma_{\text{evl}}$ | 1.001 | 1.1 | 15 | - |
| $\theta_{\text{evl}}^{-}$ | 20 | 50 | 15 | N |

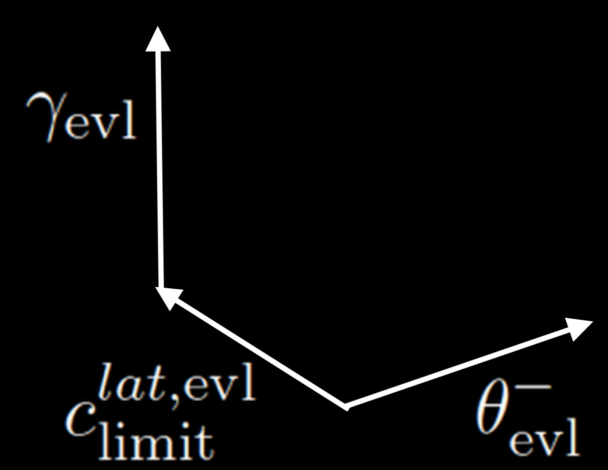
$$F_S = \sum_{t=1}^{100} \| < S^l(t) > - < S^s(t) > \|$$

$$F_n = \sum_{t=1}^{100} \| < n^l(t) > - < n^s(t) > \|$$

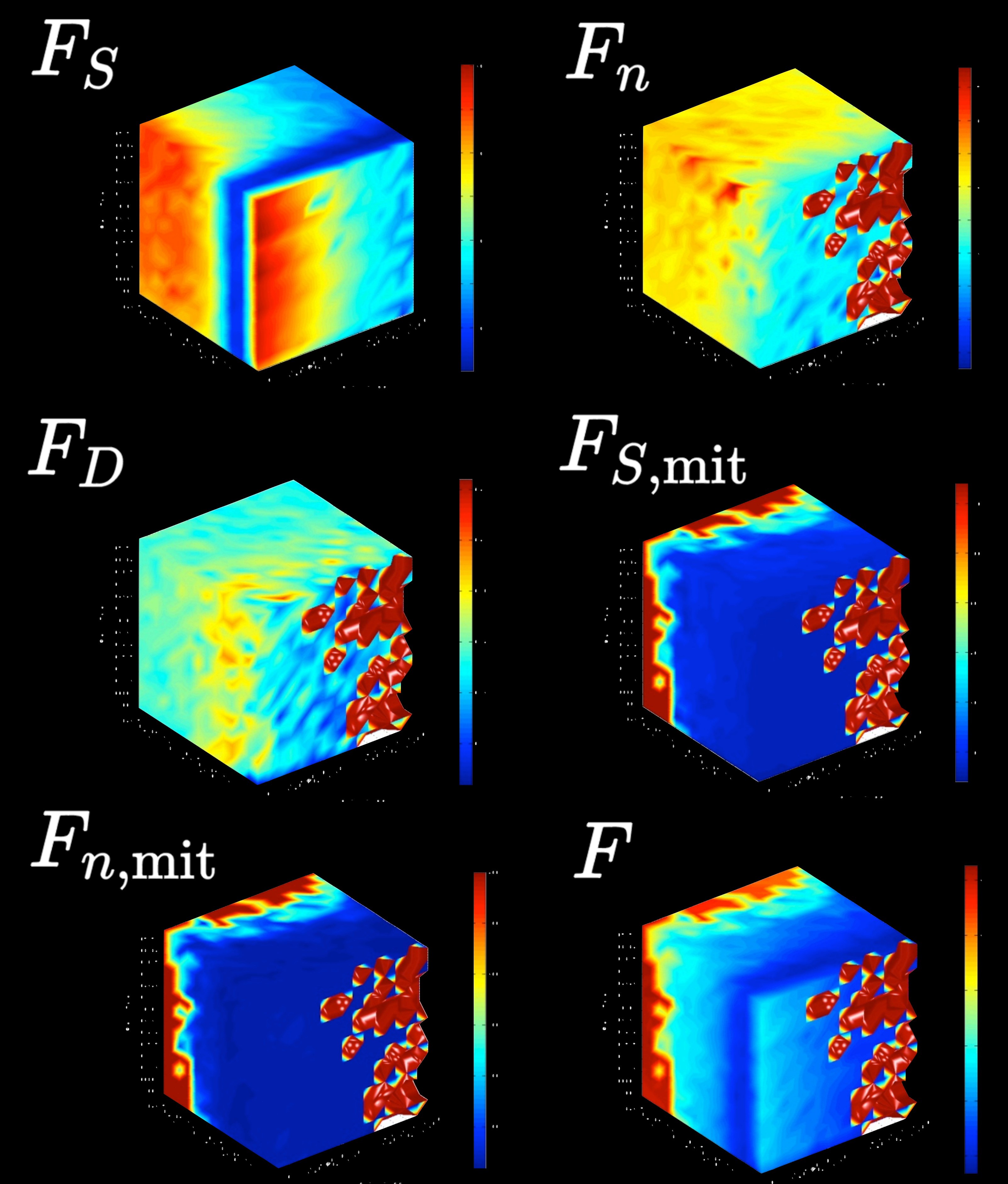
$$F_D = \sum_{k=3}^{9} \| D_k^l - D_k^s \|$$

$$F_{S,\text{mit}} = \sum_{t'=-50}^{50} \| S_{\text{mit}}^l(t') - S_{\text{mit}}^s(t') \|$$

$$F_{n,\text{mit}} = \sum_{t'=-50}^{50} \| n_{\text{mit}}^l(t') - n_{\text{mit}}^s(t') \|$$

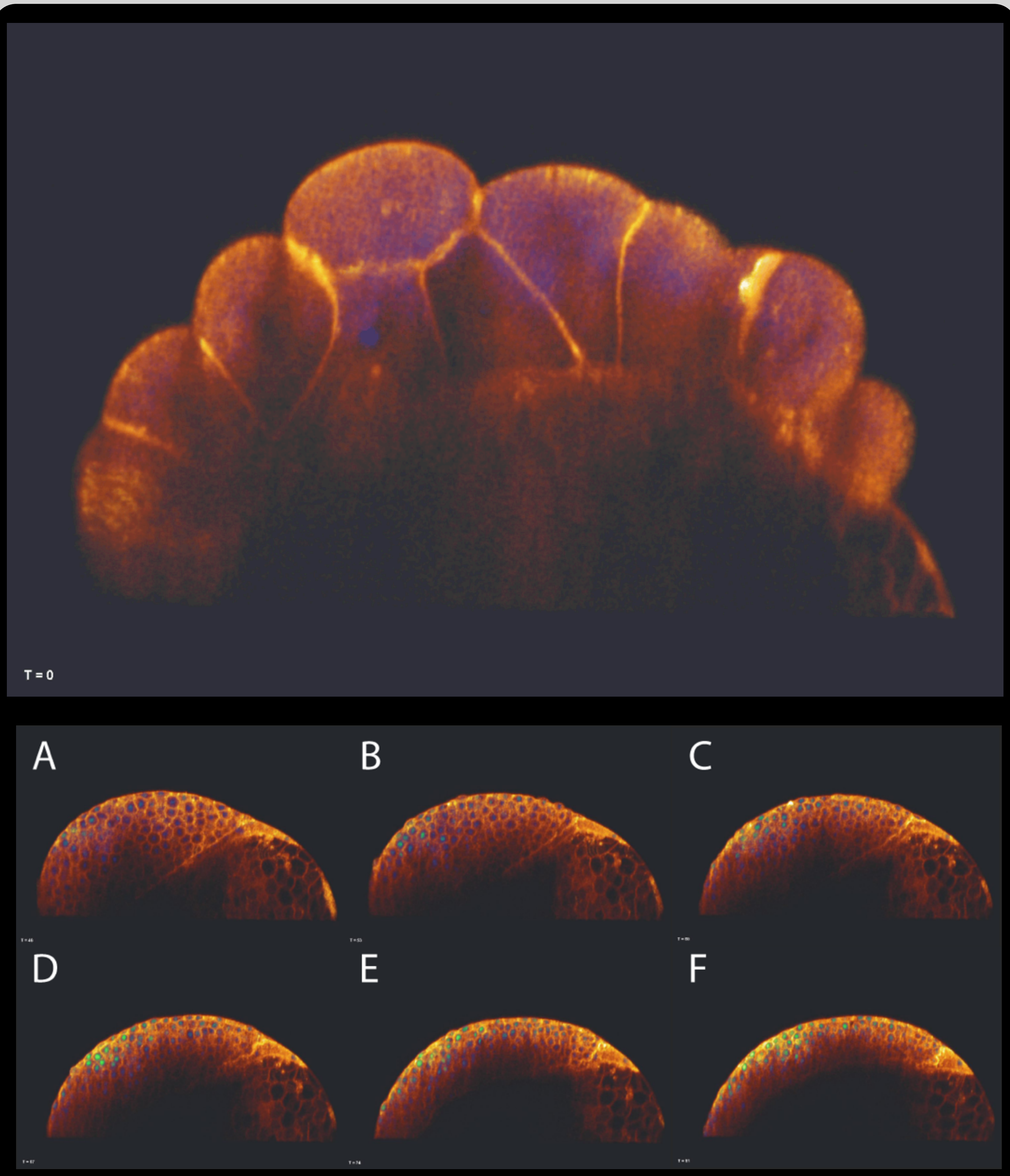



$$F = \frac{F_S}{\max F_S} + \frac{F_n}{\max F_n} + \frac{F_D}{\max F_D} + \frac{F_{S,\text{mit}}}{\max F_{S,\text{mit}}} + \frac{F_{n,\text{mit}}}{\max F_{n,\text{mit}}}$$

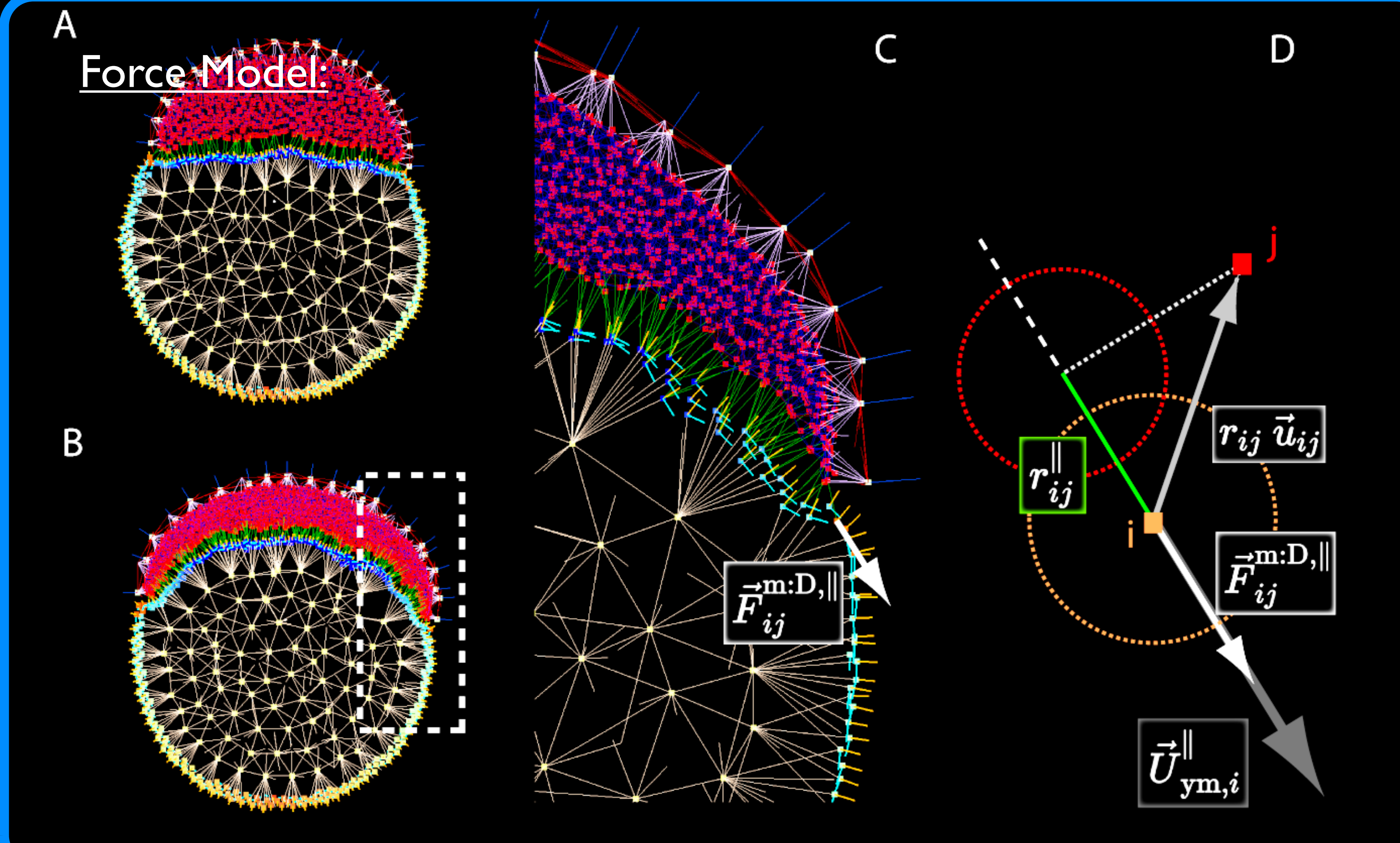


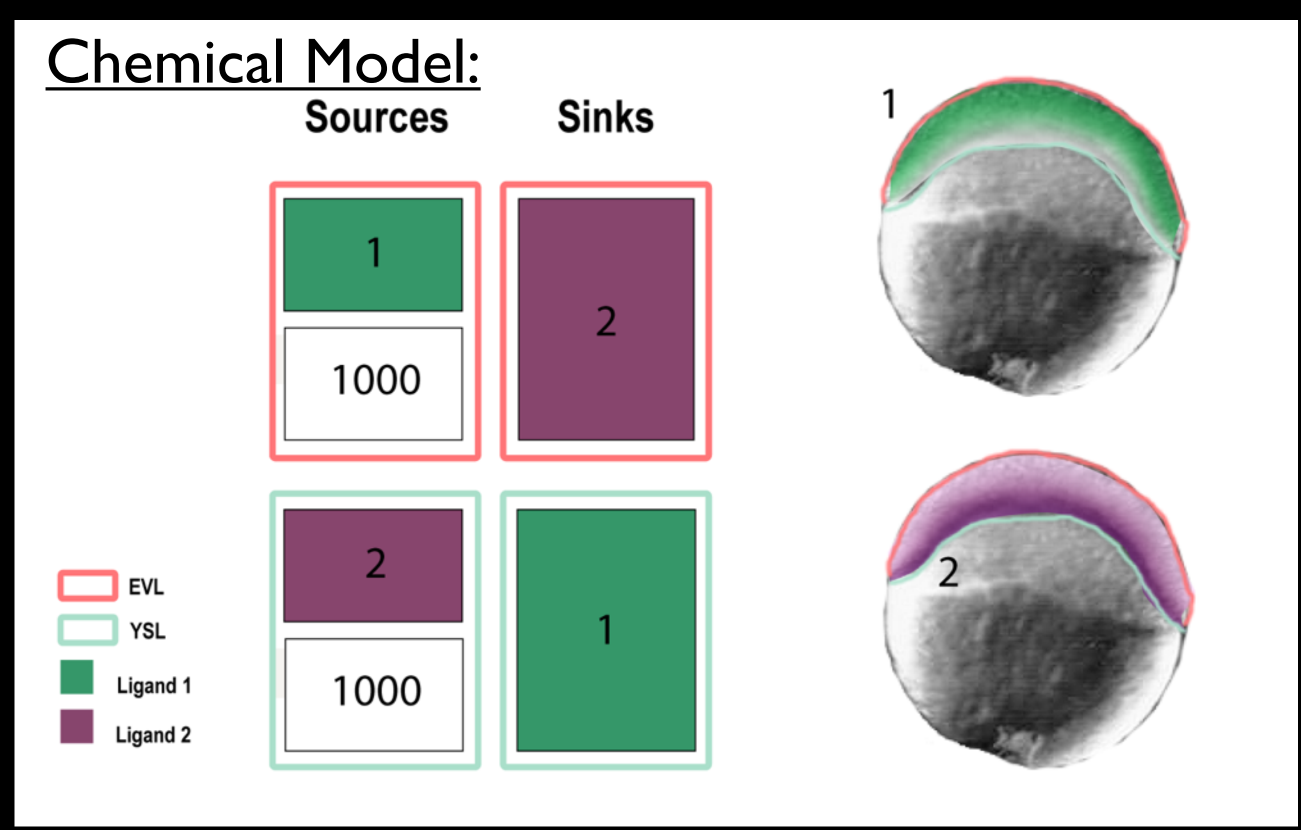

Validation

# Are protrusions sufficient to drive epiboly ?




Dataset ID 071222bF between 32-cell stage (1.75 hpf) and 75% epiboly stage.



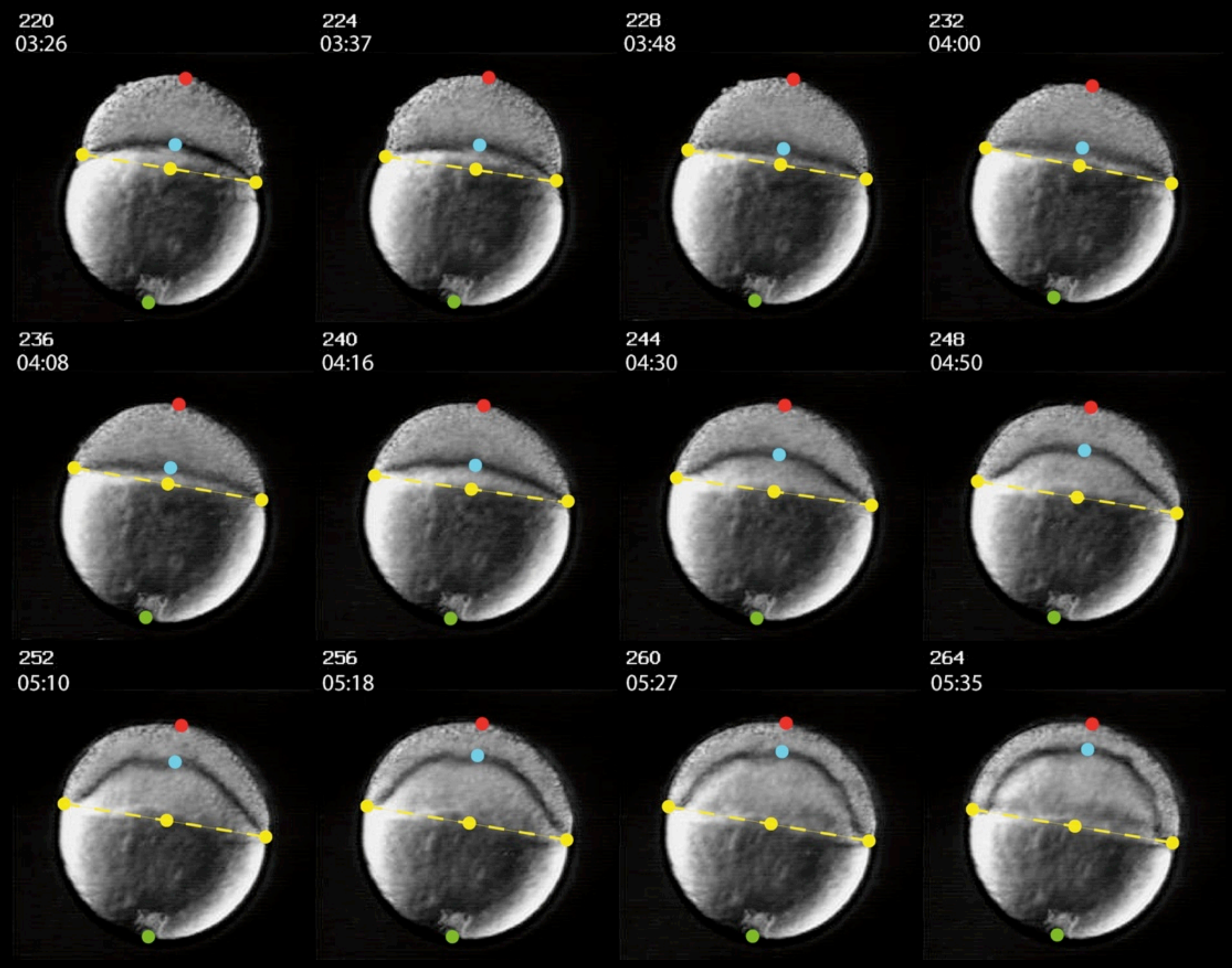




Hypotheses

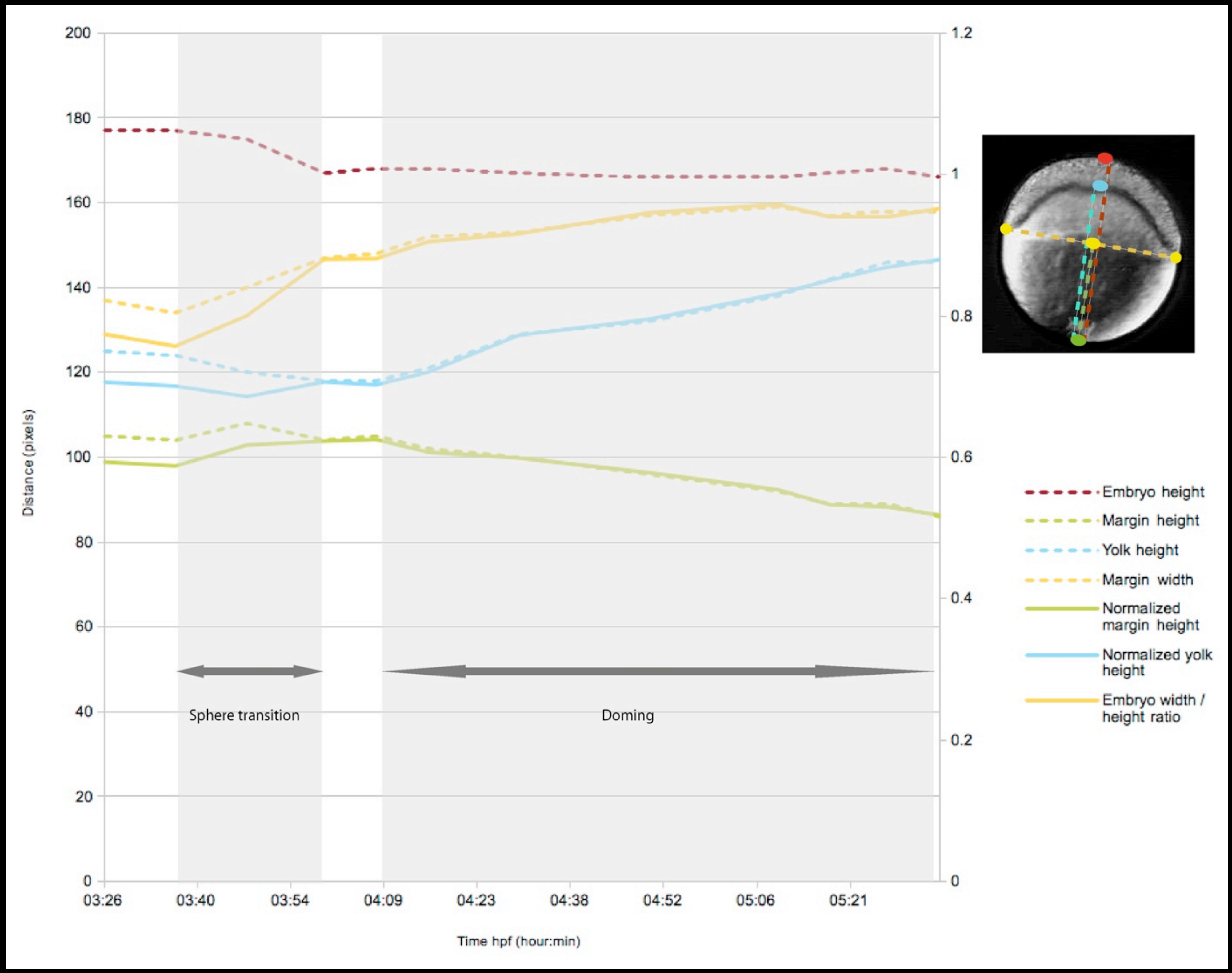
Model

5. Intercalation Patterns

# Macroscopic landmarks characterizing epiboly in the live specimen



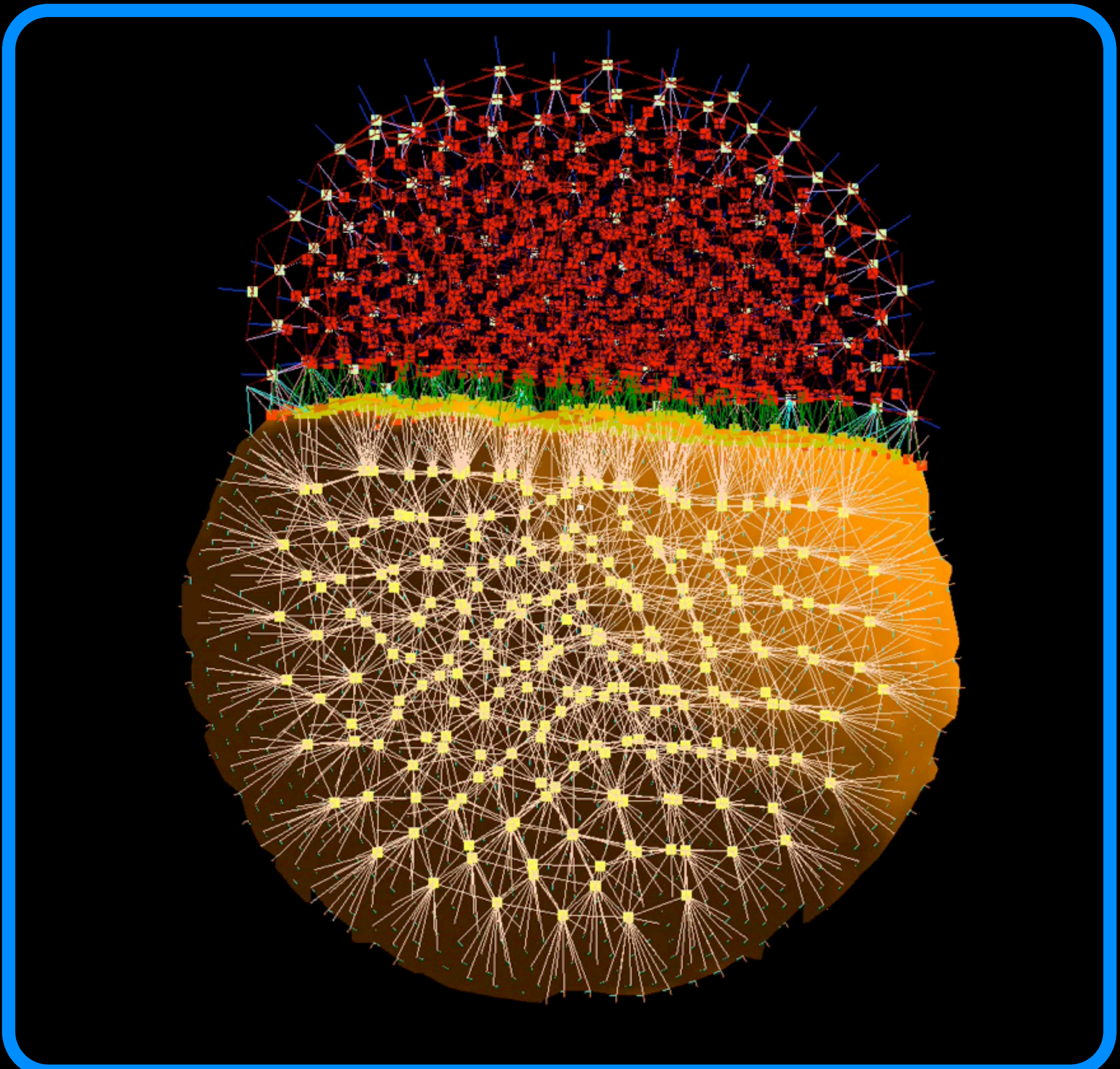

Macroscopic landmarks of the epibolic deformation from the “flipbook” specimen.

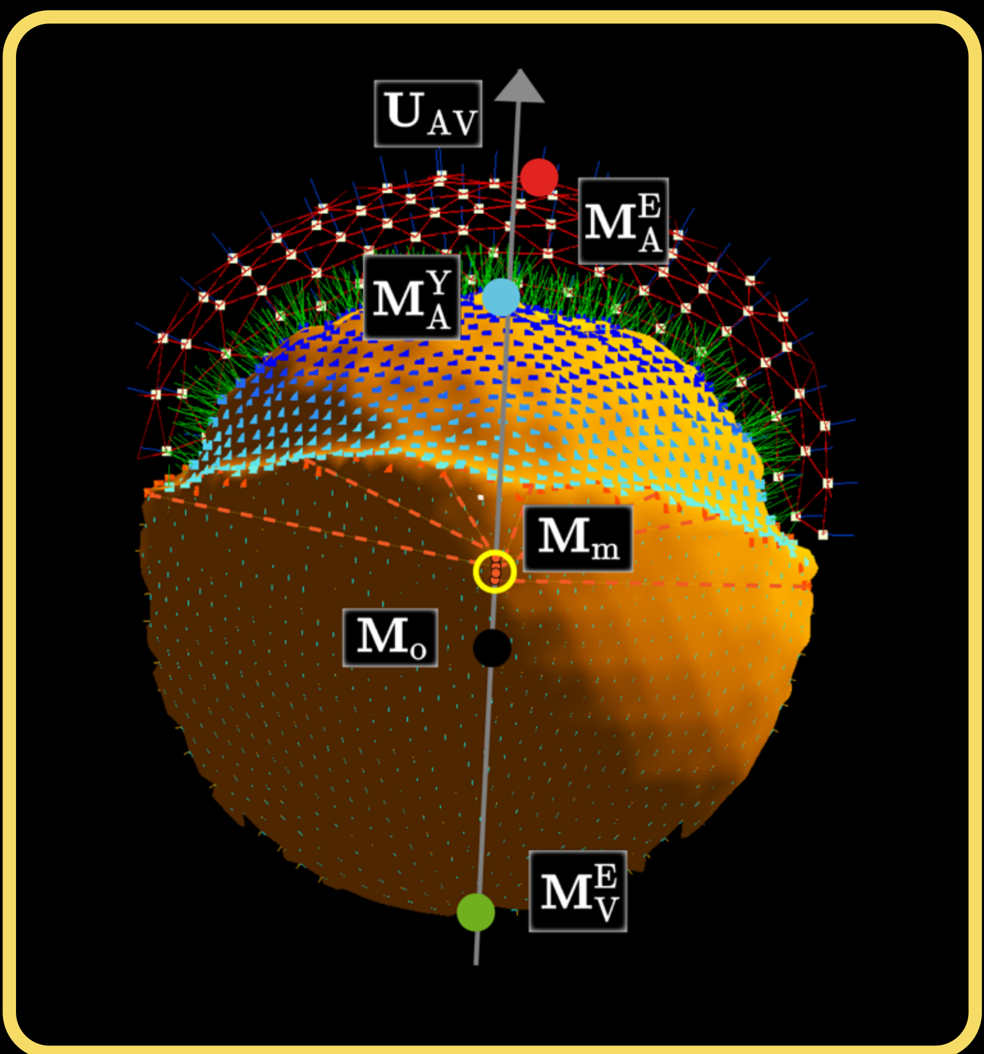


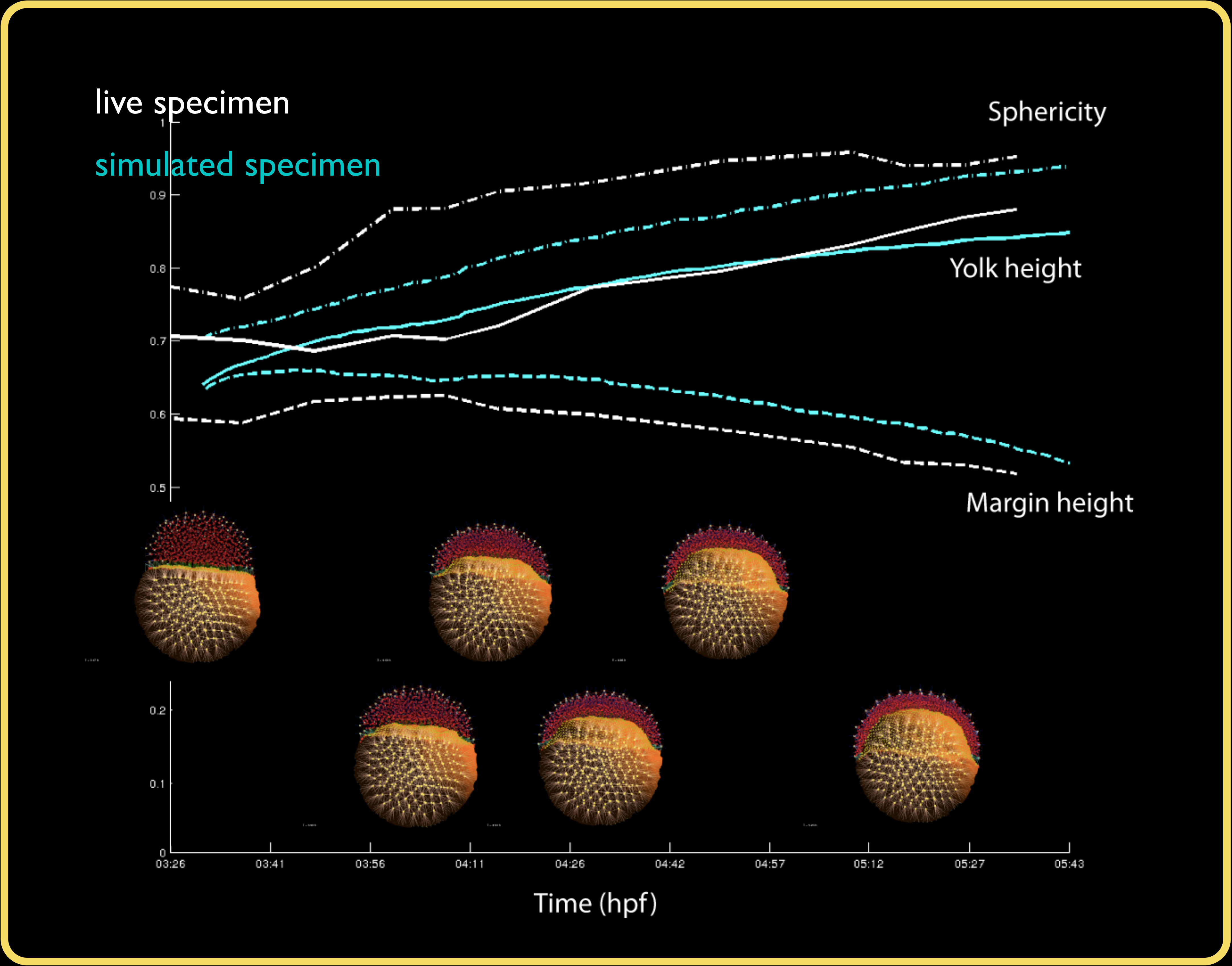

Live Measures

# 5. Intercalation Patterns

## Macroscopic landmarks characterizing epiboly in the simulated specimen



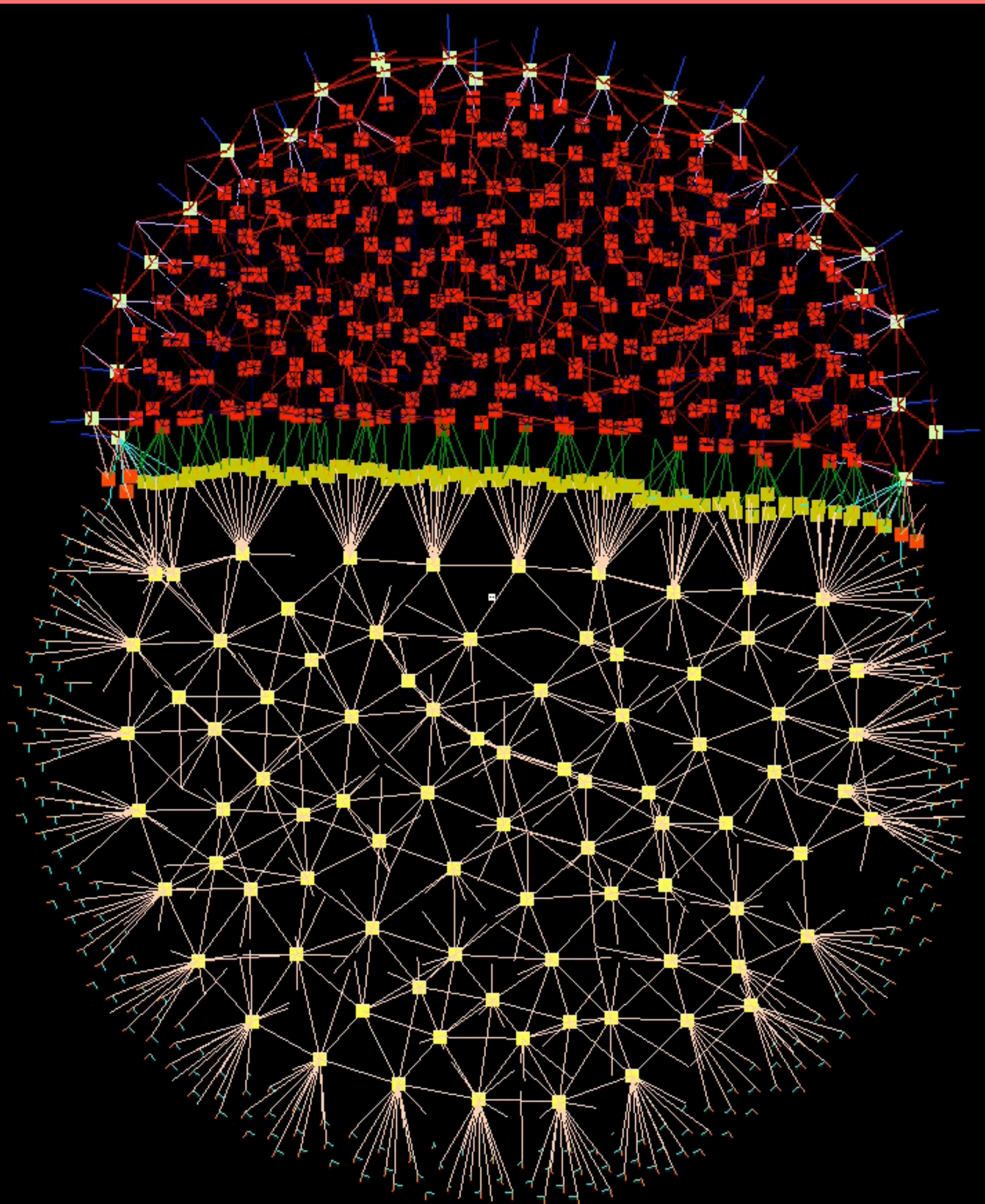


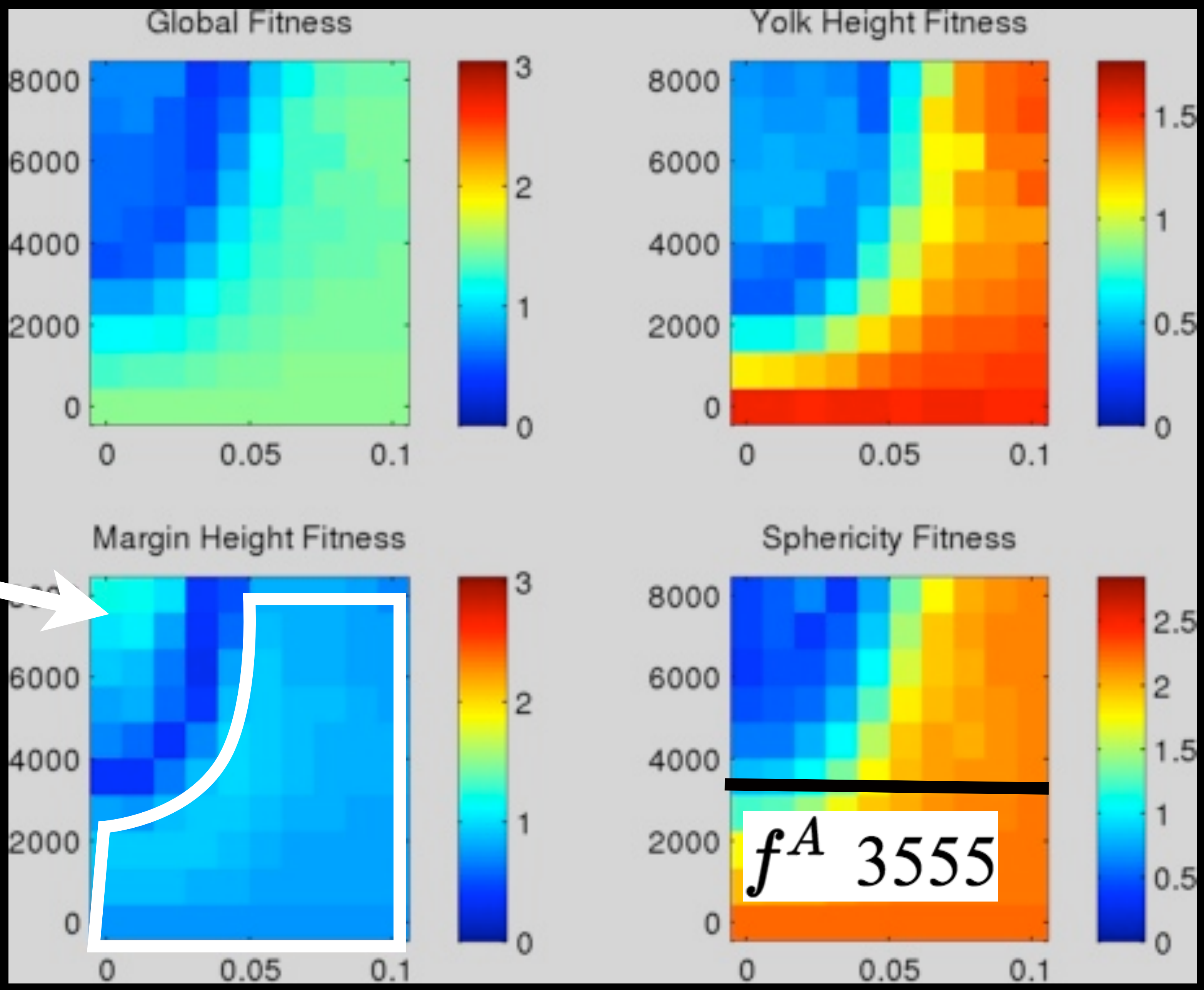

Simulated Measures

5. Intercalation Patterns

# Protrusion force intensity and polarization orientation randomness have counterbalancing effects



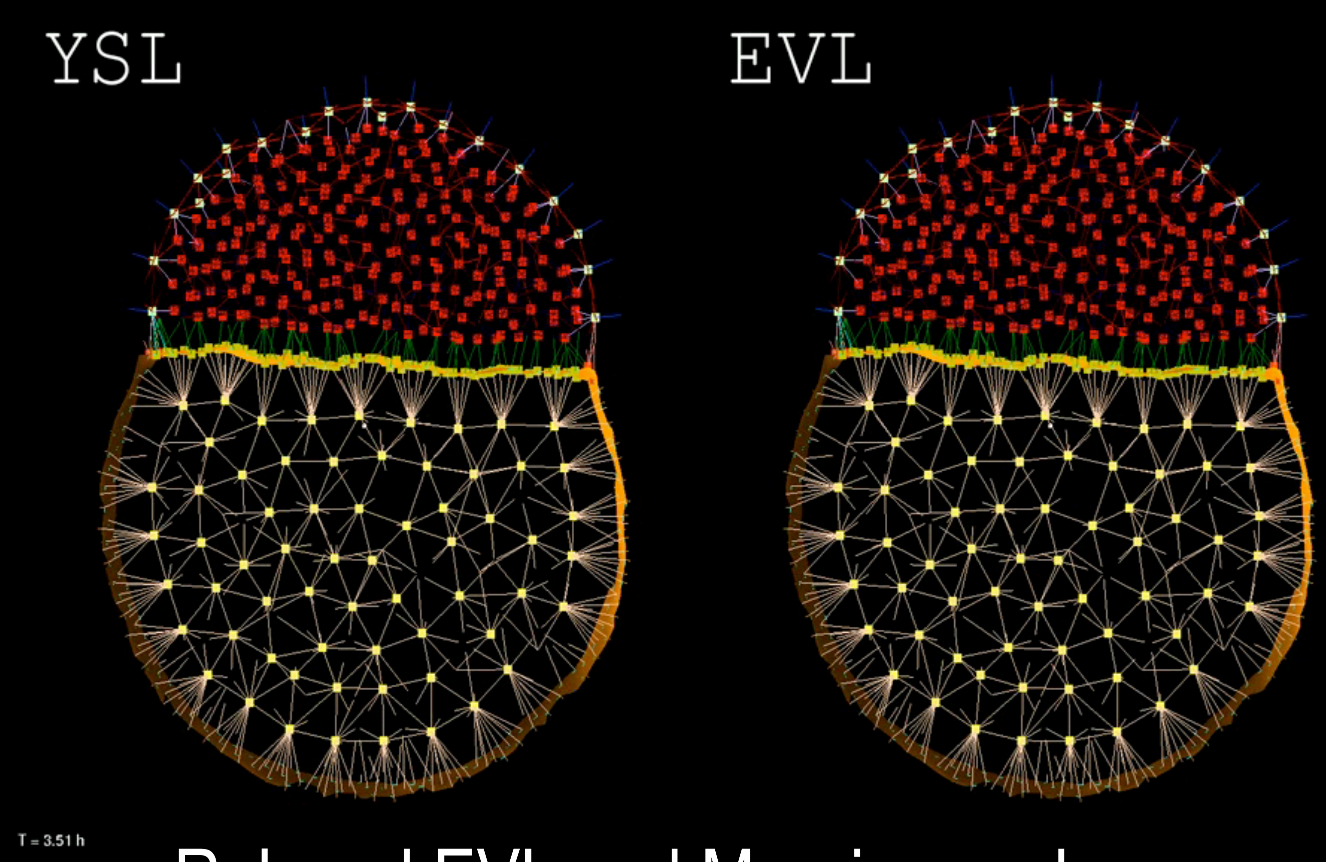
Fluid-like intercalation



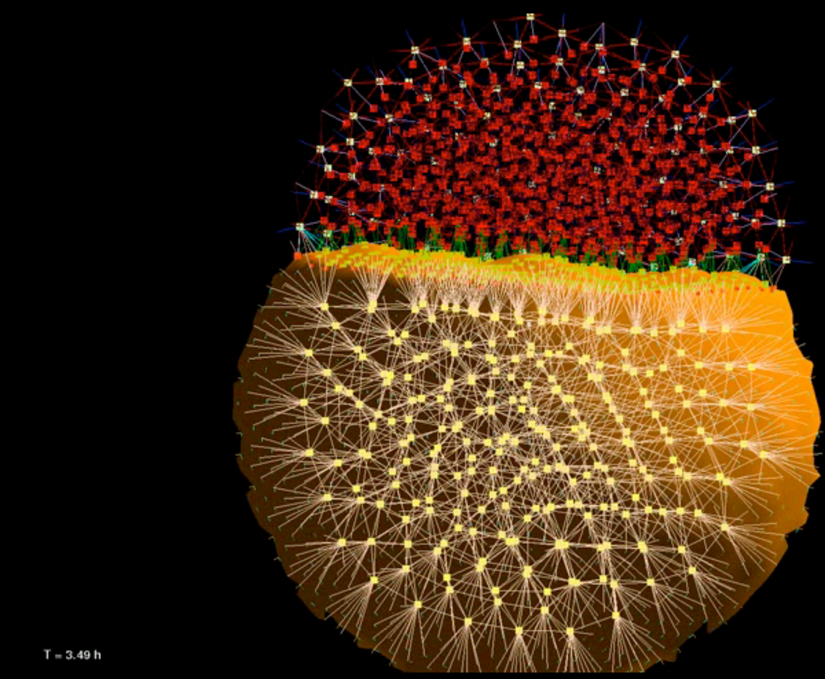

Validation

# 5. Intercalation Patterns

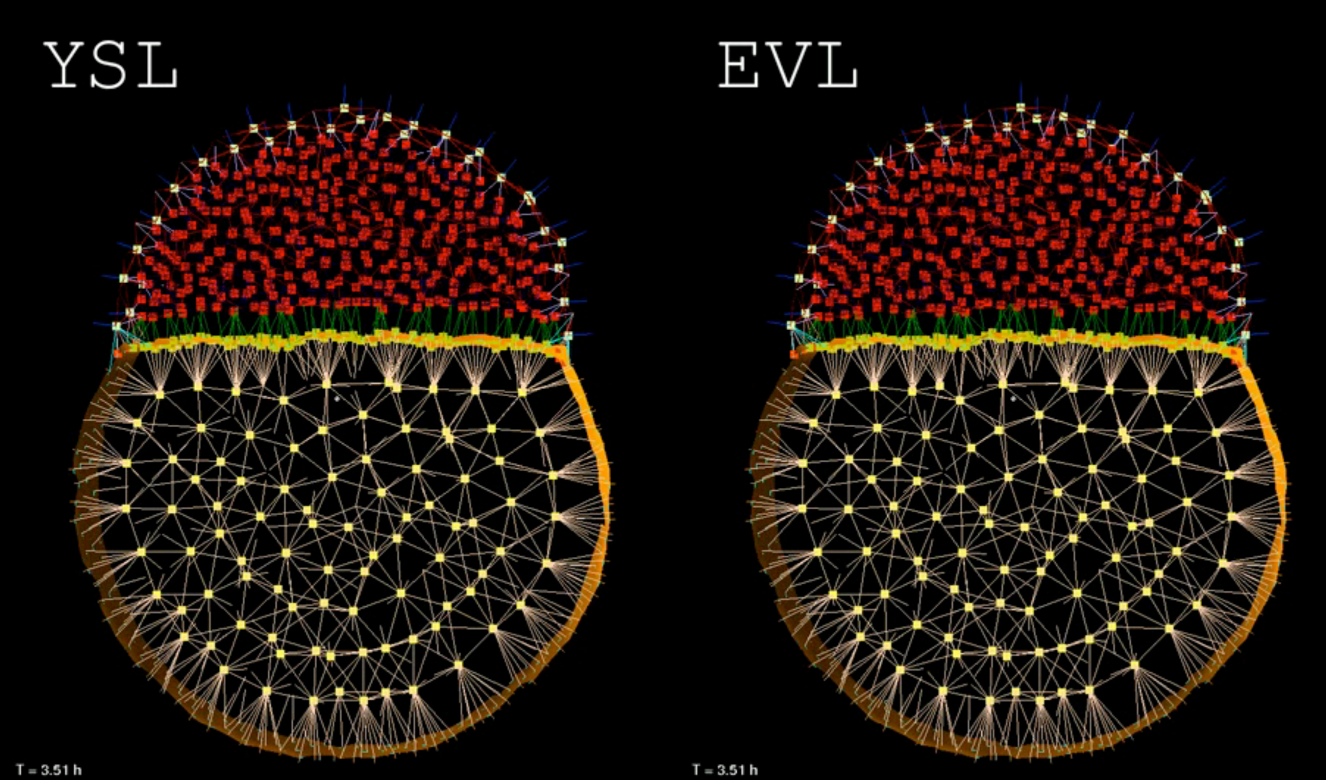
## Phase 1 epiboly and intercalation are very sensitive to mechanical parameters



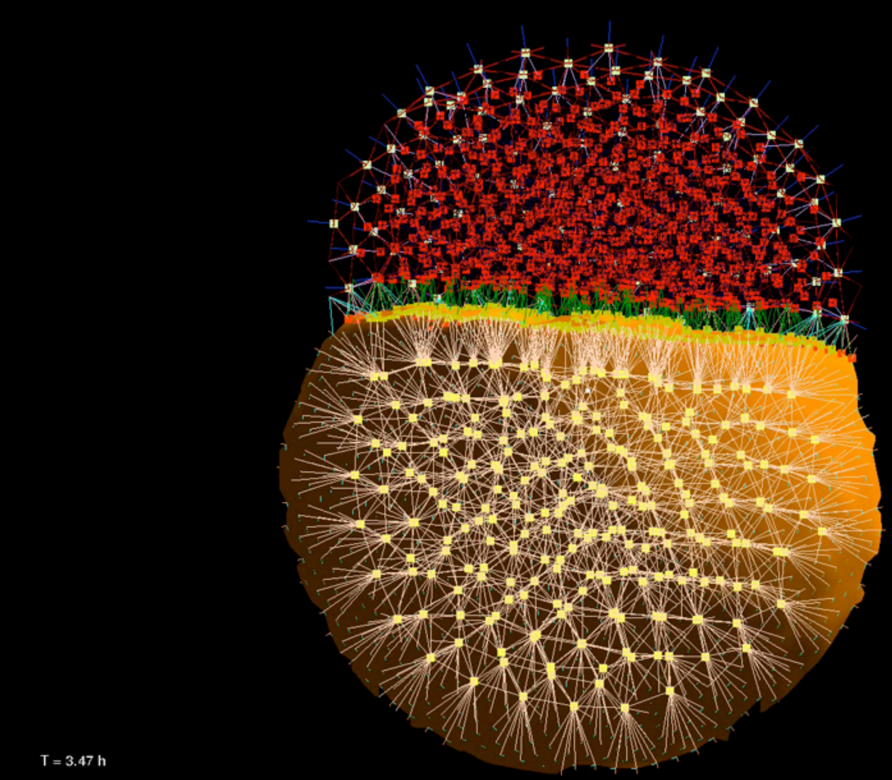

Relaxed EVL and Margin, random polarization axes




Rolling yolk membrane, DC perforating the yolk membrane, strong EVL resistance and weak margin resistance




Constraining EVL and margin, regular polarization axes

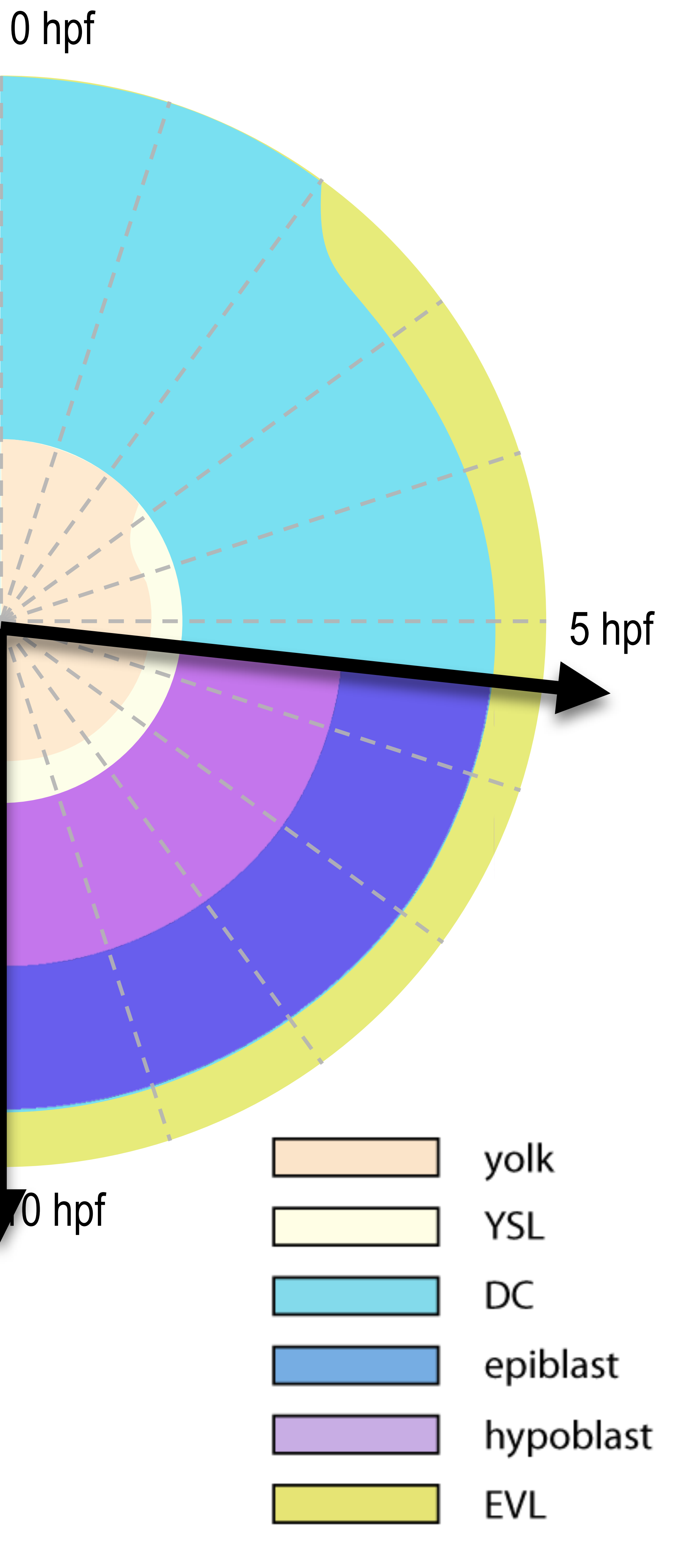


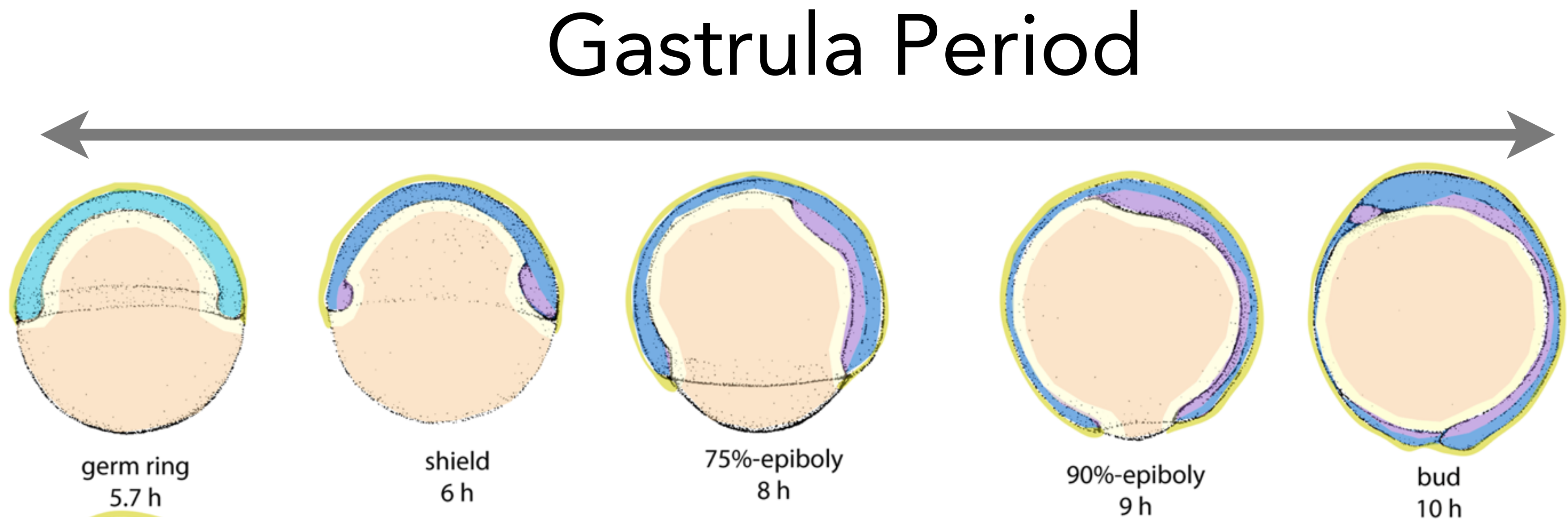

Good fitness simulated phenotype, Yolk as a source, low margin resistance resistance, intermediate EVL resistance

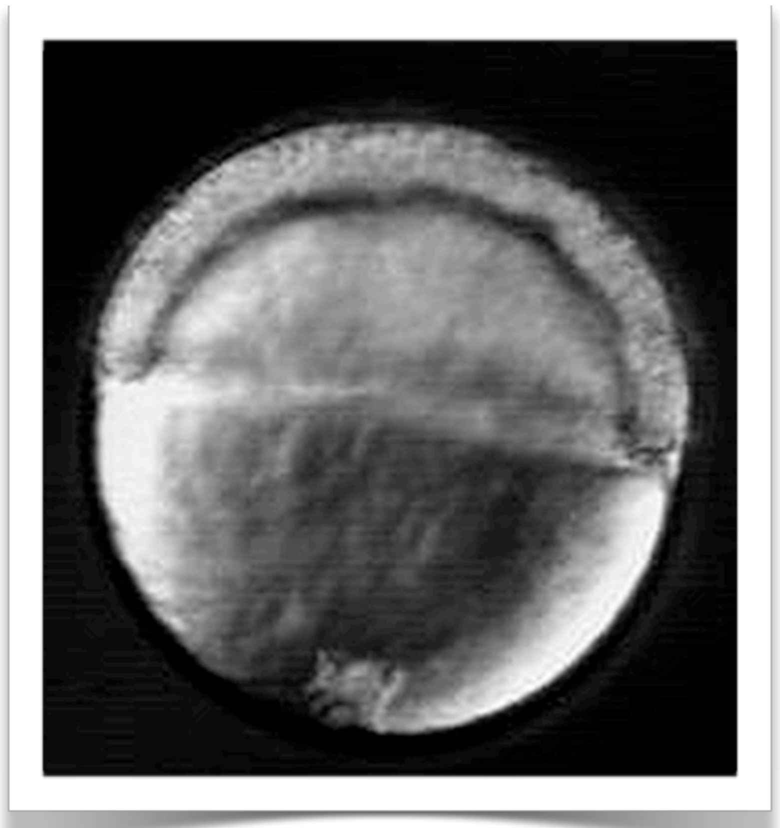
Validation

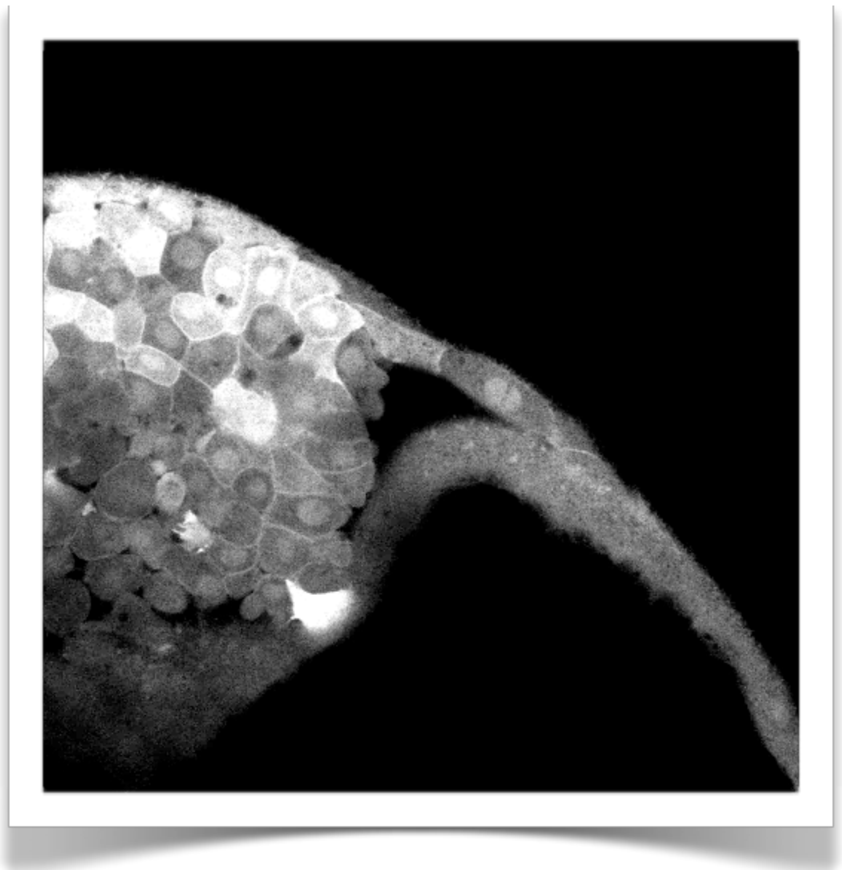
# 6. Gastrulation

*Cell division orientation and CE? Epiblast and hypoblast movement interdependence? Polarization field in CE? Internalization and phase 2 epiboly?*

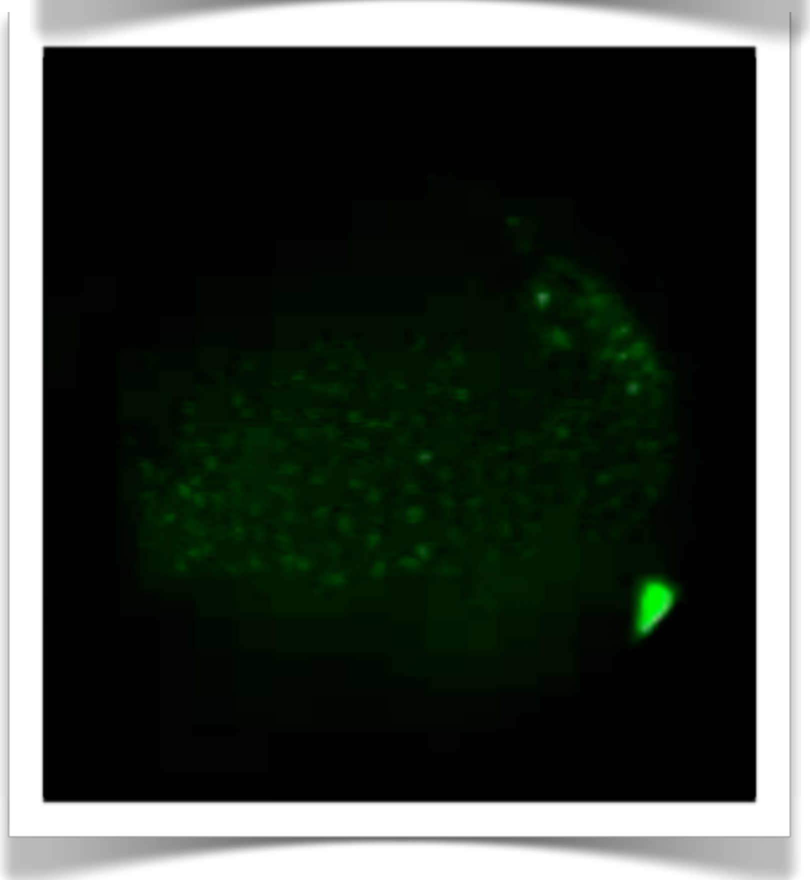


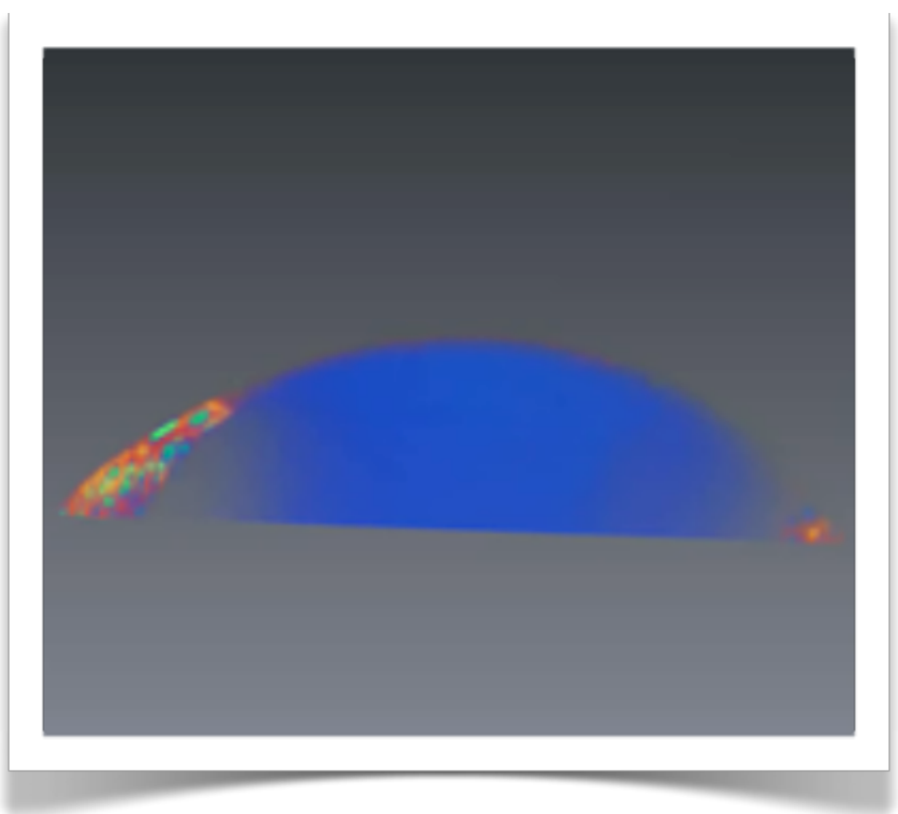





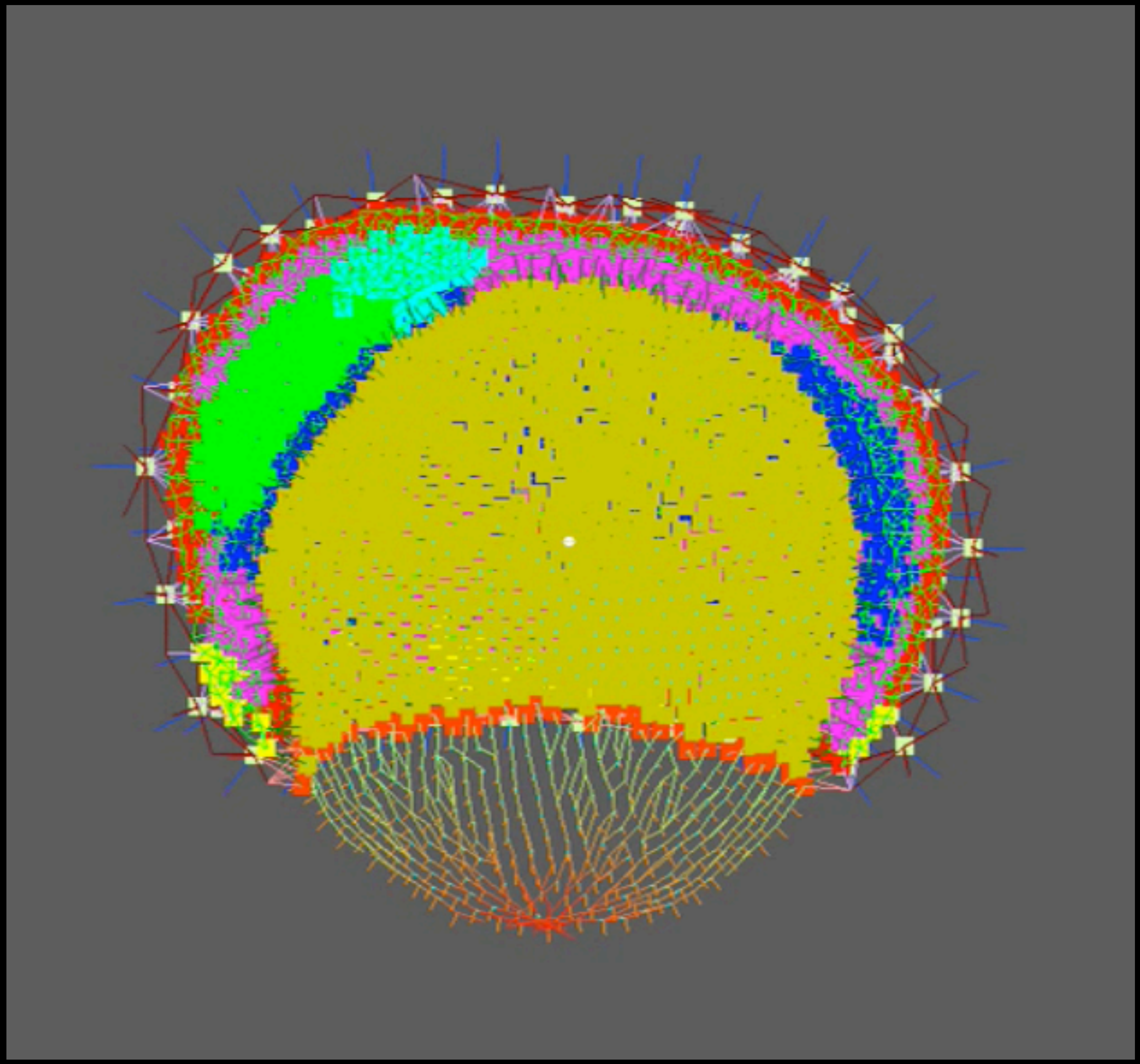
Montero & Heisenberg, 2004.



Mizoguchi et al., 2008



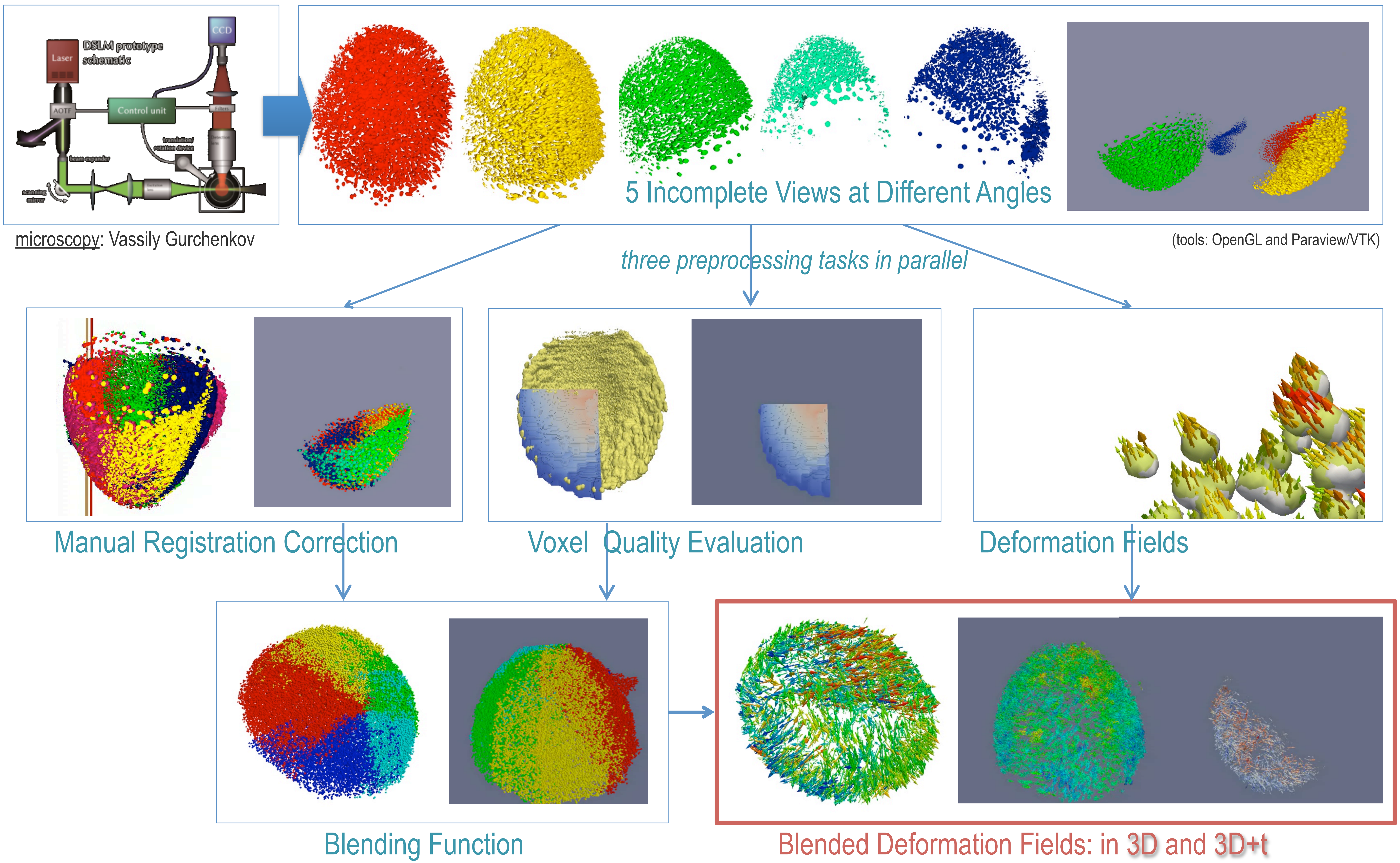
# Back to the exploration of the parameter space to perform gastrulation steps



Model

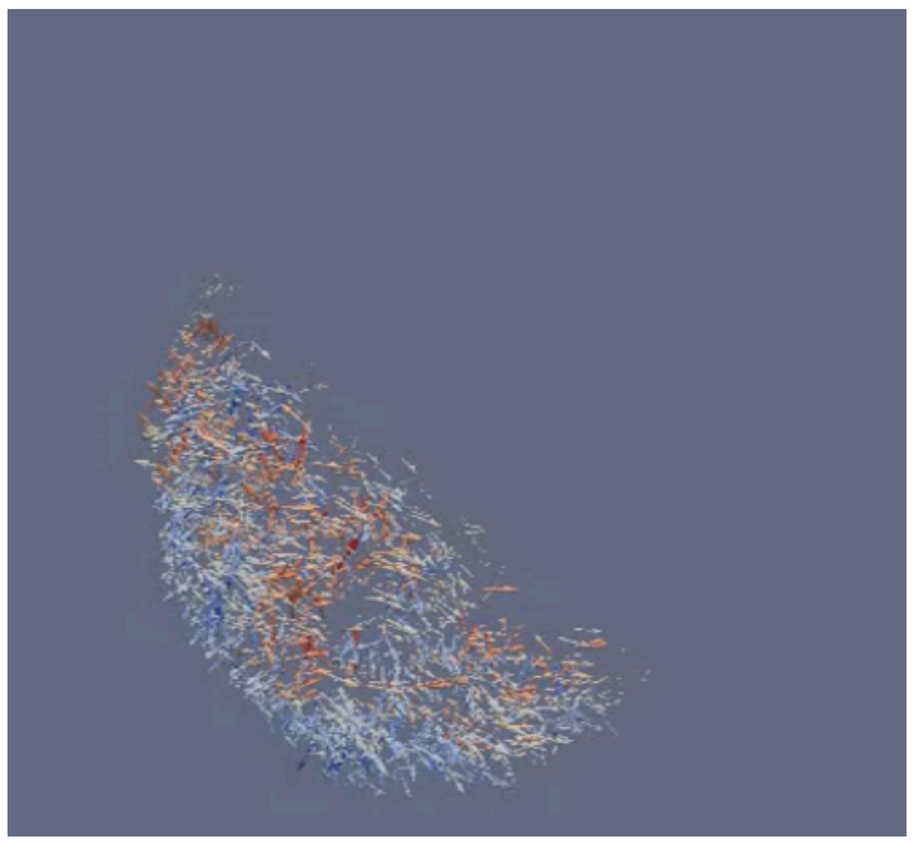
Future goal: toward a fitness based on extensive local measurements

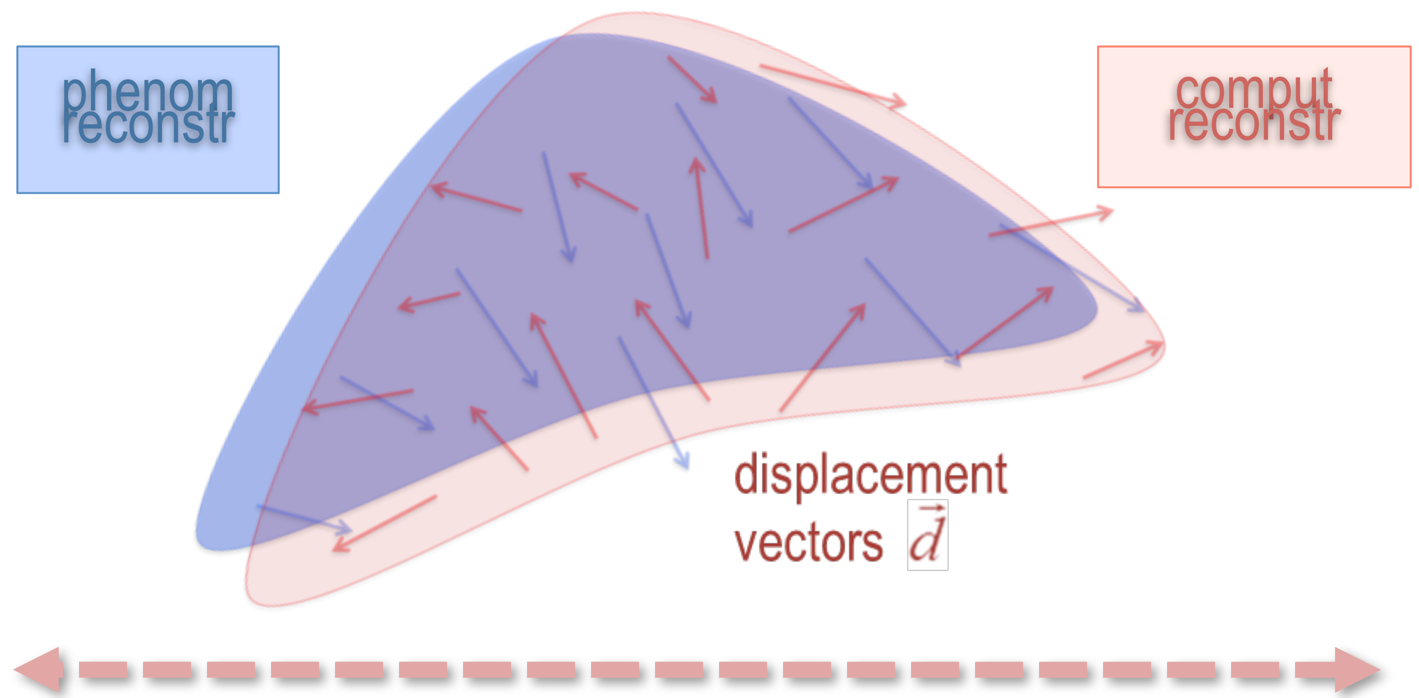
# *in toto* blended deformation fields

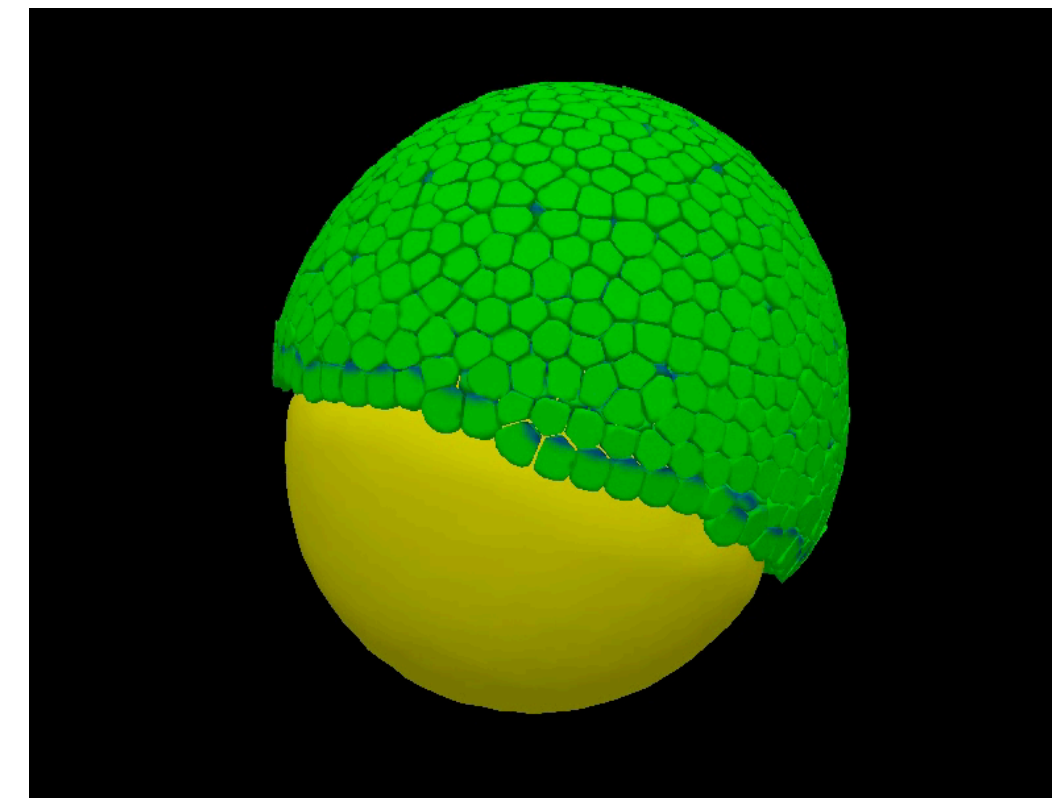


Future goal: toward a fitness based on extensive local measurements

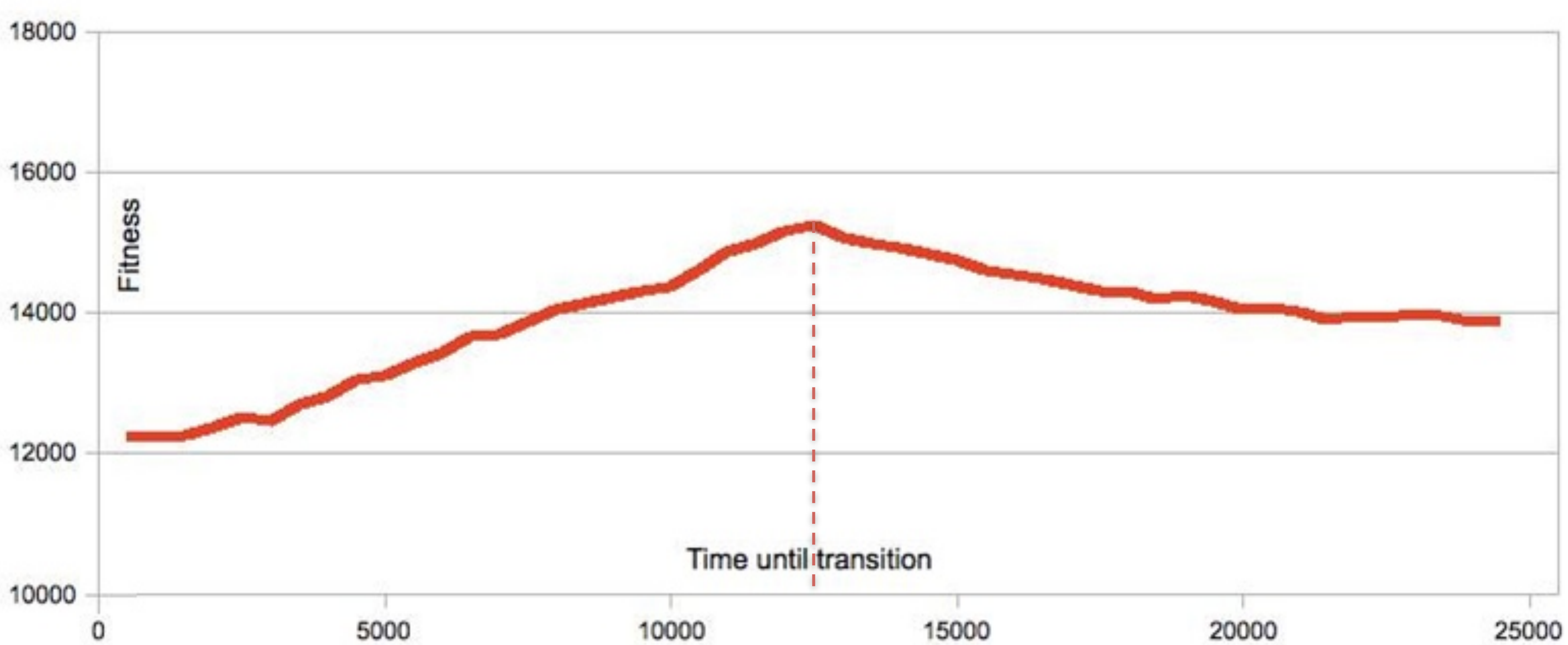
# *The fitness is the extensive sum of local differences*



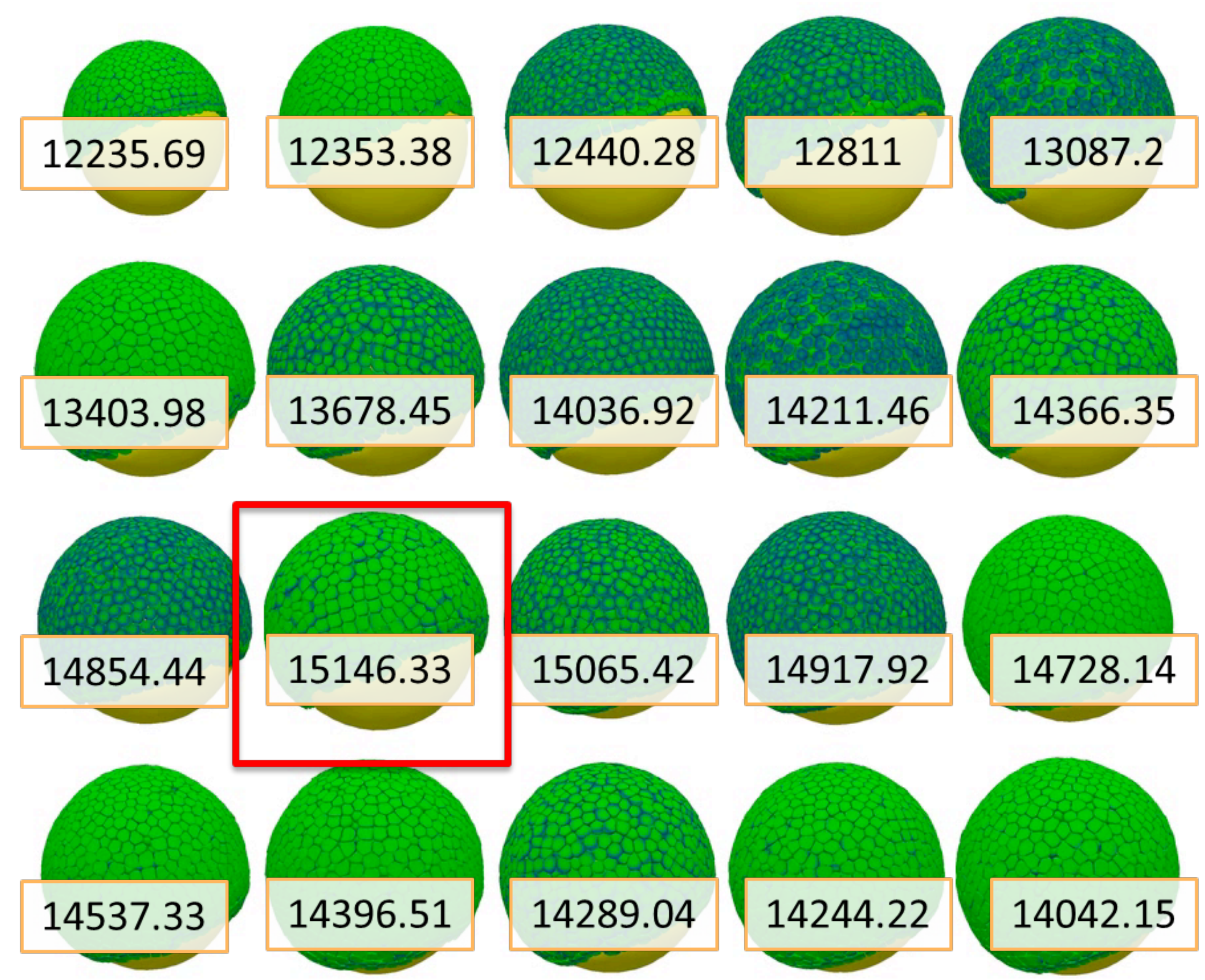


$$Fitness = \sum_{time\,t} \sum_{cells\,i} \max\left(0, \vec{d}_{simul}(i,t) \cdot \vec{d}_{reconstr}(i,t)\right)$$




<u>*ex. of parameter*</u>*: time_until_transition_to_epiboly*



# Conclusion

- MECAGEN, a generic model of development
- Strategy of hypothesis validation through confrontation of reconstructed measures from live and simulated specimens
- Theoretical choices driven by computational feasibility
- Toward a GRN specification of the model
- Toward an “Evo-Devo” Perspective