

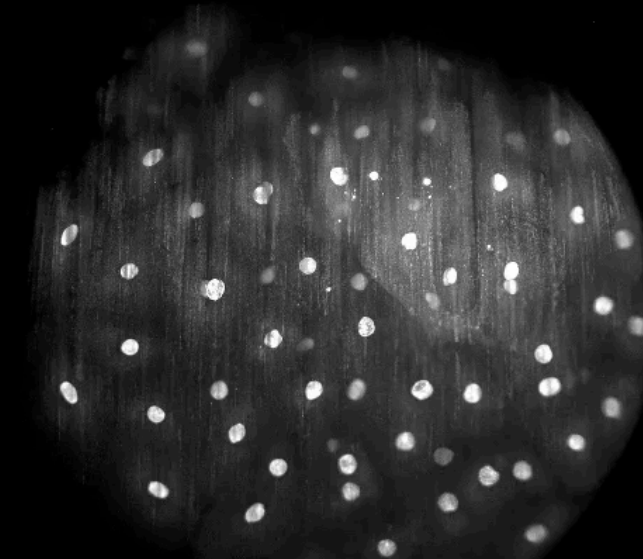
Diagnostic Imaging Revolution

Keller et al. (2008) *Science*

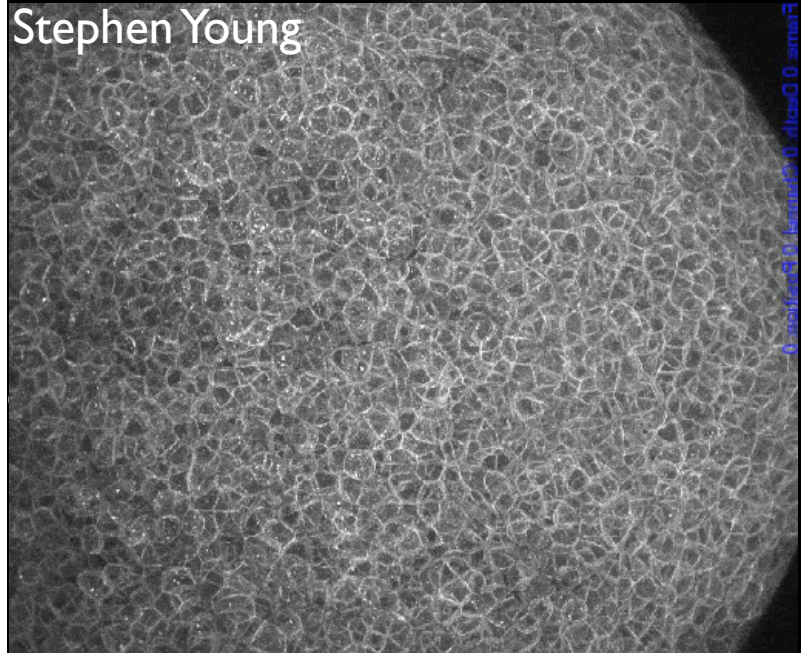
100 min

future dorsal side

animal view



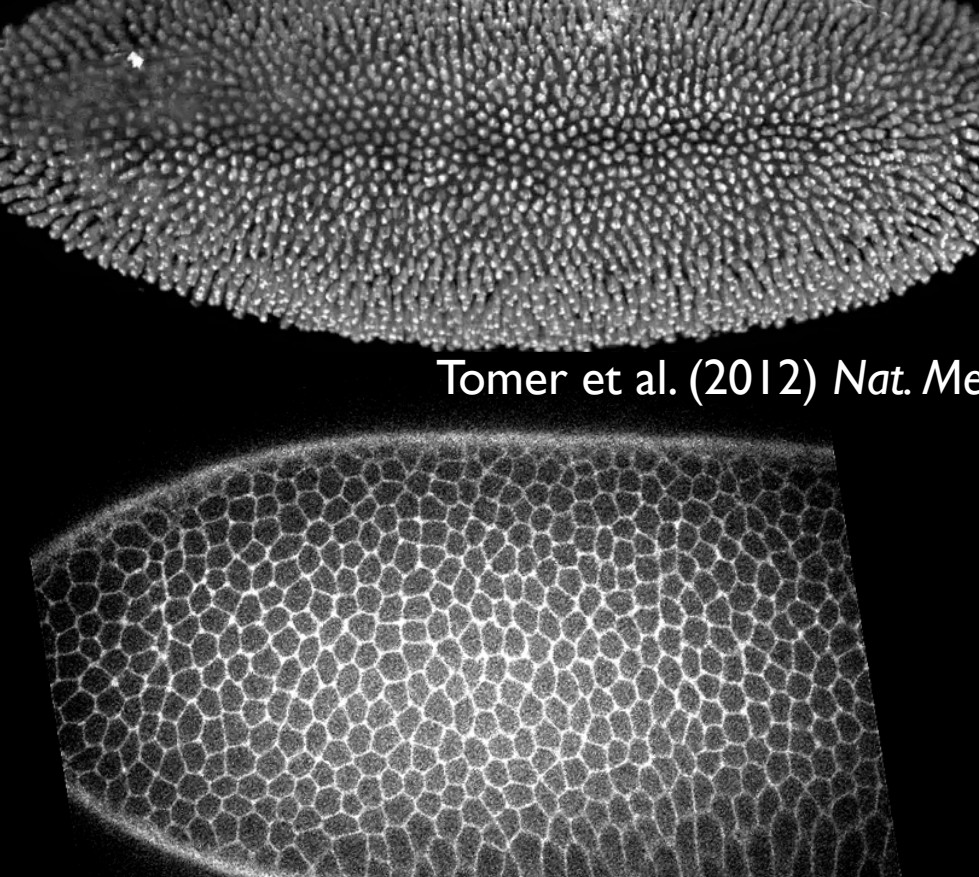
Stephen Young



Frame 0 Deck 0 (3/20/2014 11:57:10 AM)

ventrolateral view

02:52:30

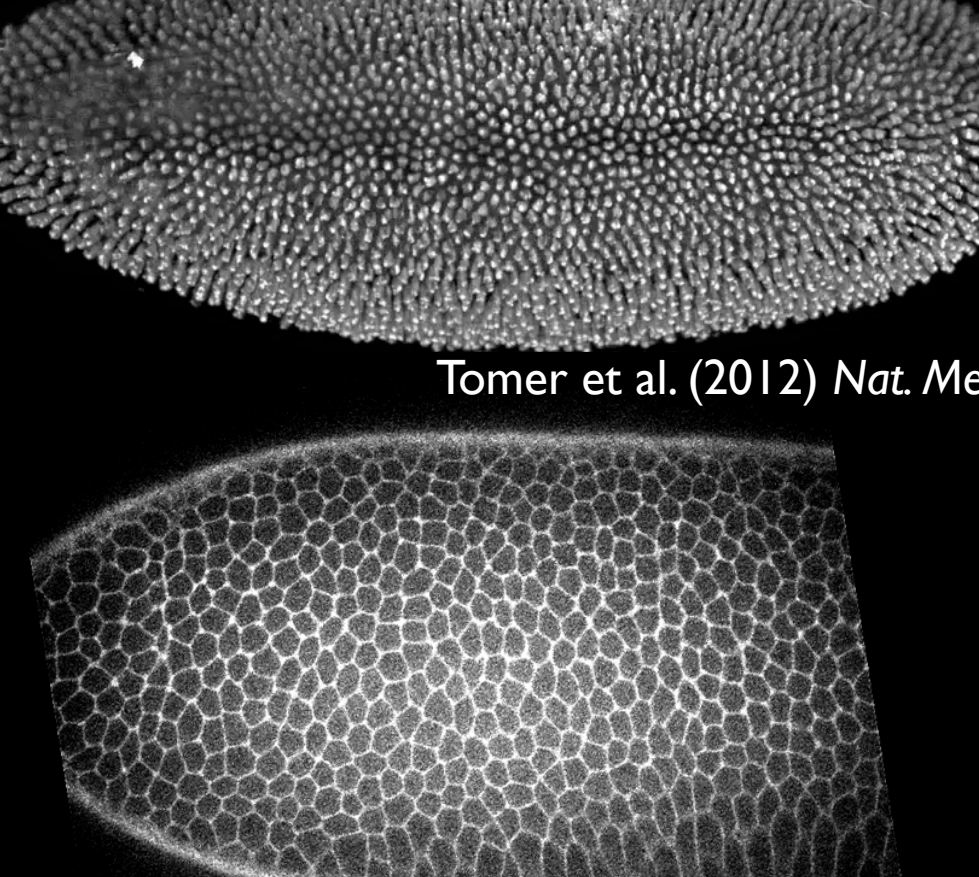


Tomer et al. (2012) *Nat. Methods*

Zallen lab

ventrolateral view

02:52:30

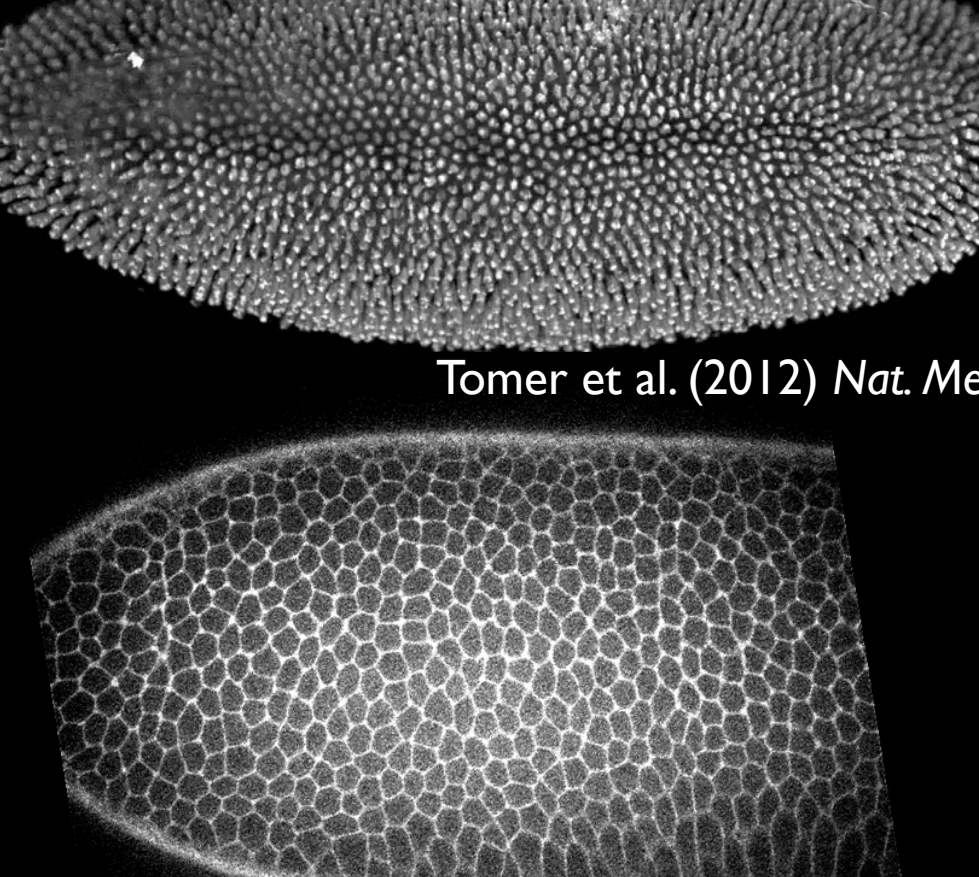


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Zallen lab

ventrolateral view

02:52:30

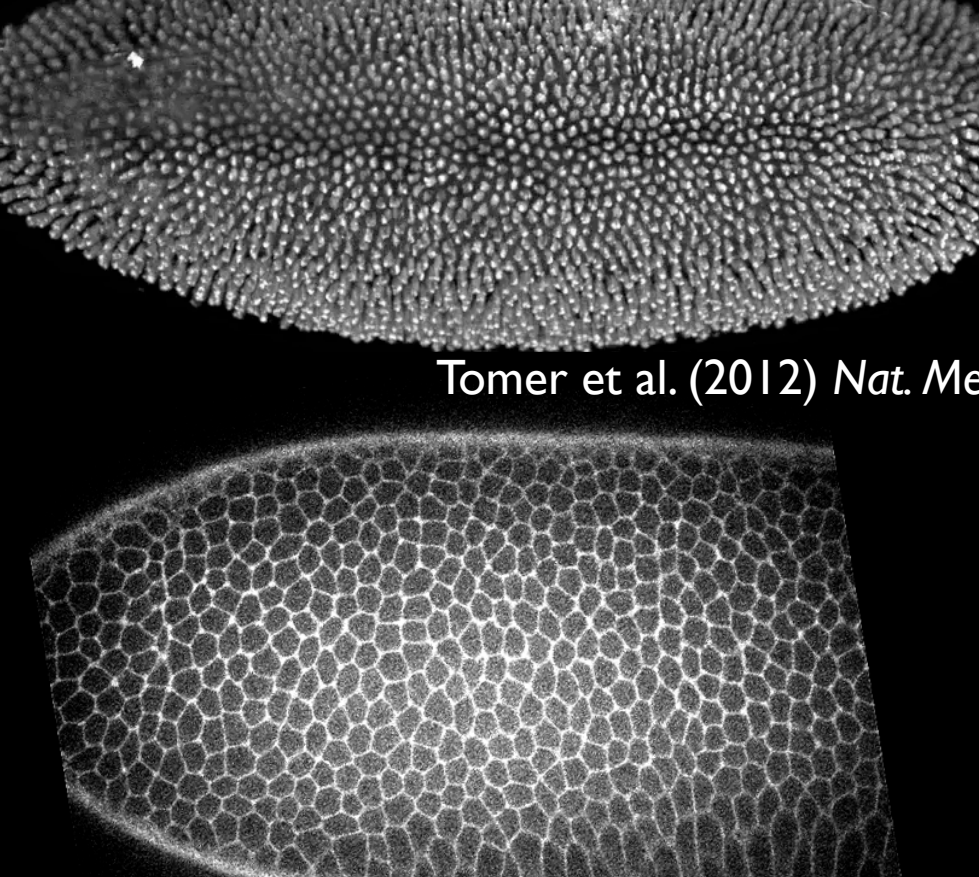


Tomer et al. (2012) *Nat. Methods*

Zallen lab

ventrolateral view

02:52:30



Tomer et al. (2012) *Nat. Methods*

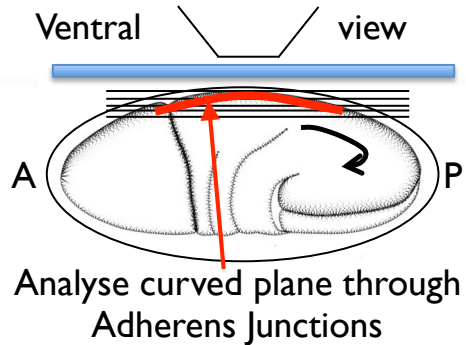
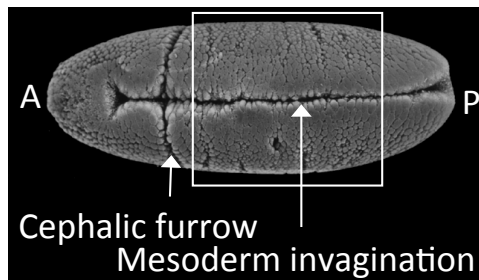
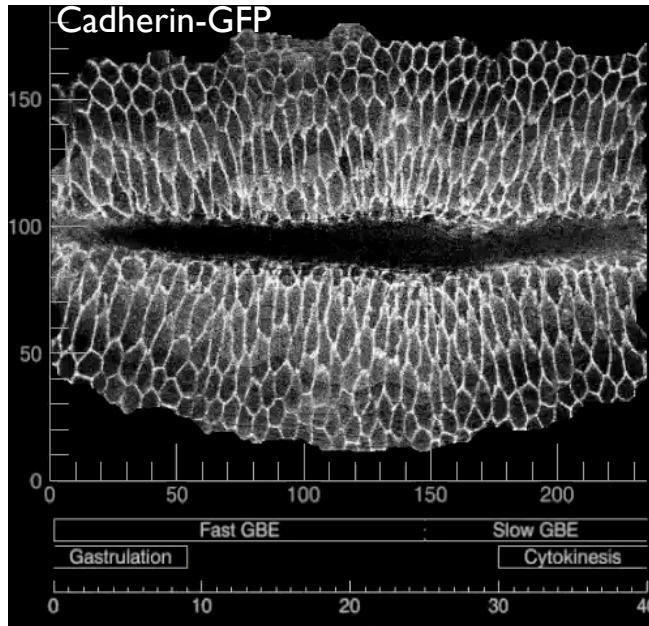
Zallen lab



Cell tracking & analysis

Raw movies

(ventral view of *Drosophila* germ-band,
with Lucy Butler & Benedicte Sanson)



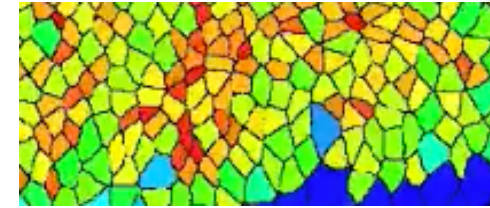
Blanchard et al. (2009) *Nat. Methods*



Analysis of Epithelial Morphogenesis

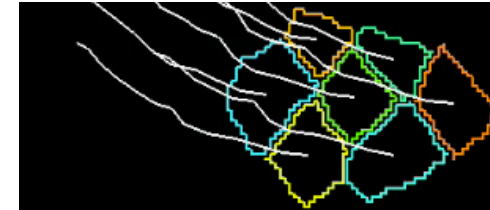
1 Static measures

- Cell shapes
- Expected cell shapes
- Cell-cell interface shapes
- Number of neighbours



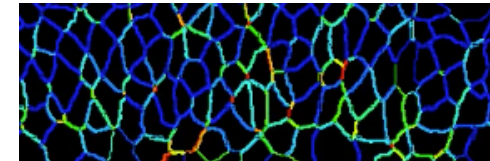
2 Dynamic measures

- Cell-cell interface dynamics
- Small domain deformations
- Impact of cell division
- Measuring fluctuations



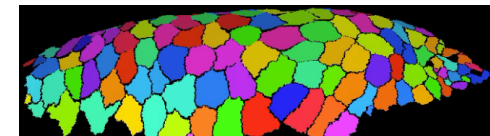
3 Fluorescence intensity

- Quantification of sub-cellular fluorescence intensity
- Medial dynamics
- Polarity of junctional proteins



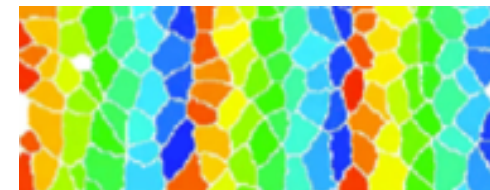
4 Towards 3D

- Epithelial tilt & curvature
- Combining tracking of apical & basal layers

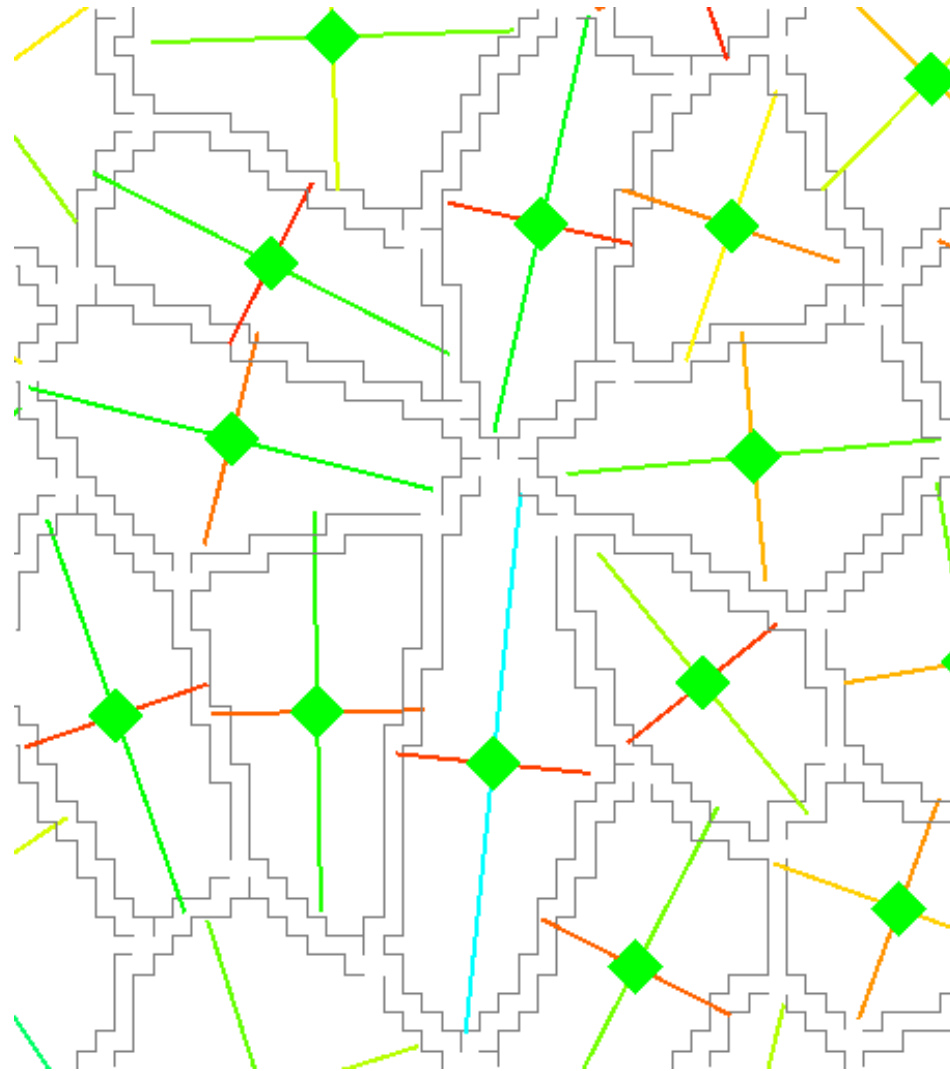


5 Time & space coordinate systems

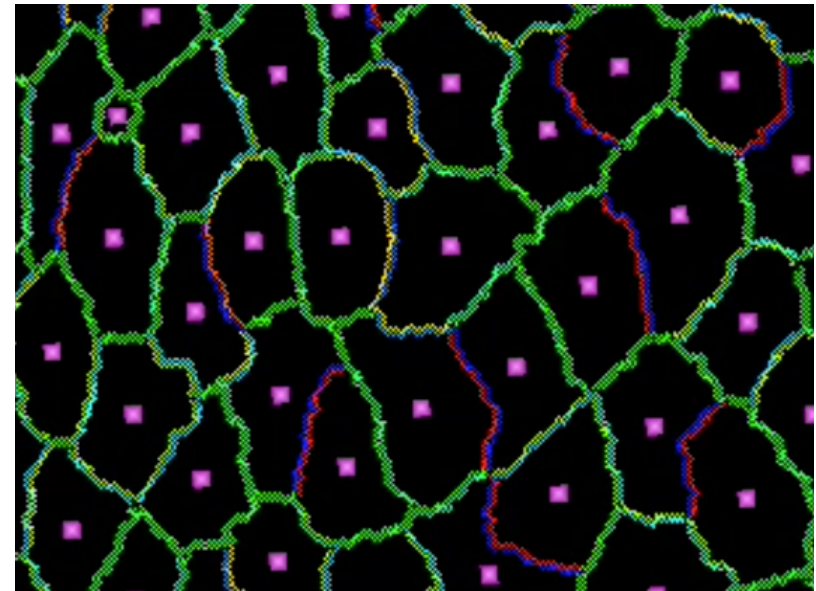
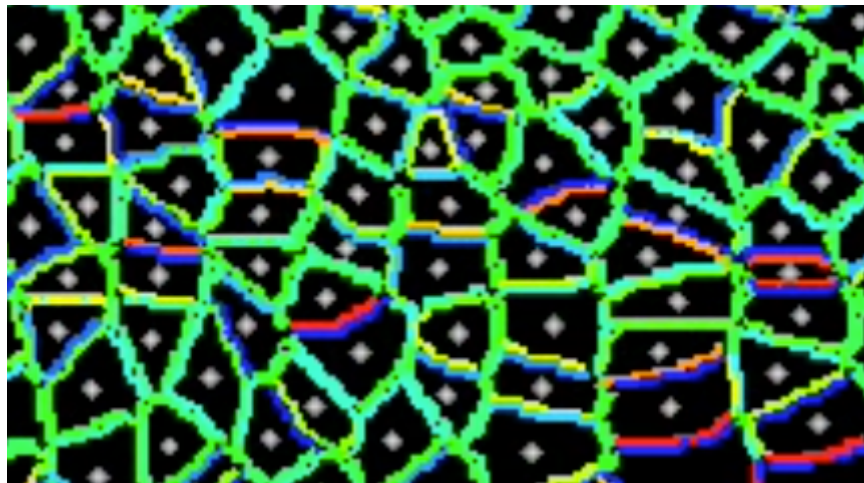
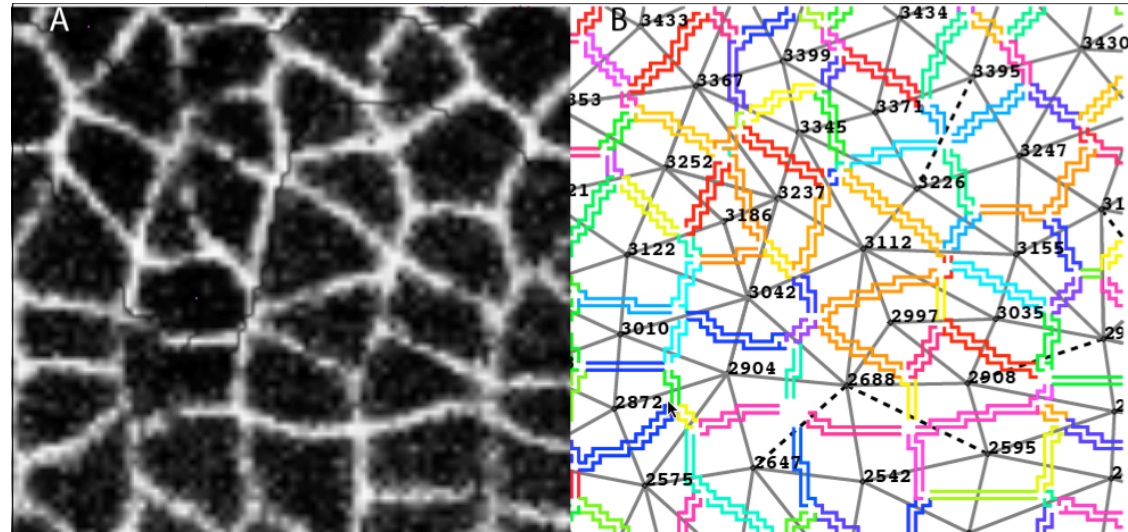
- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries



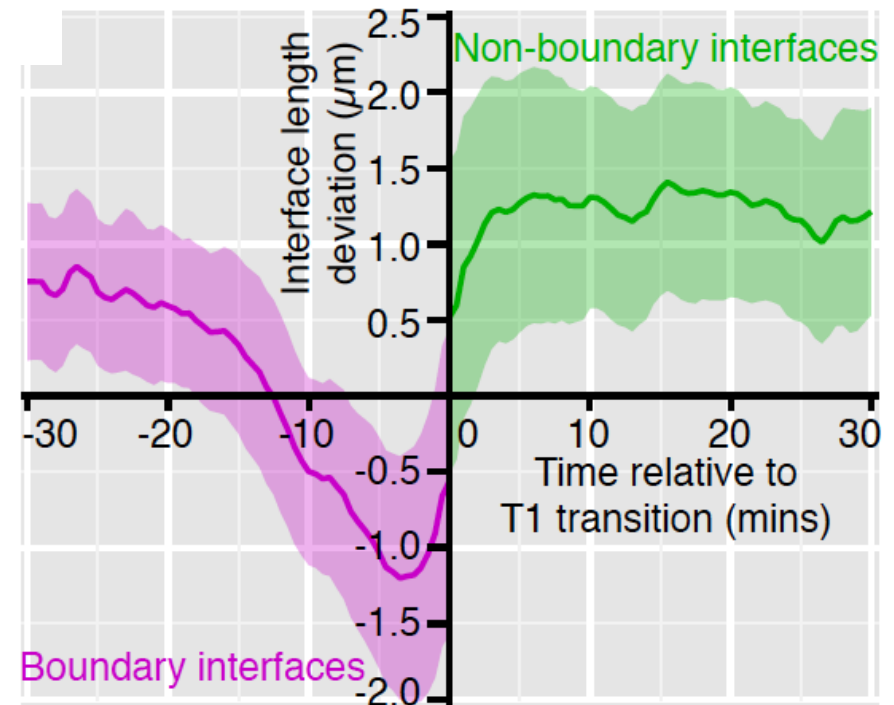
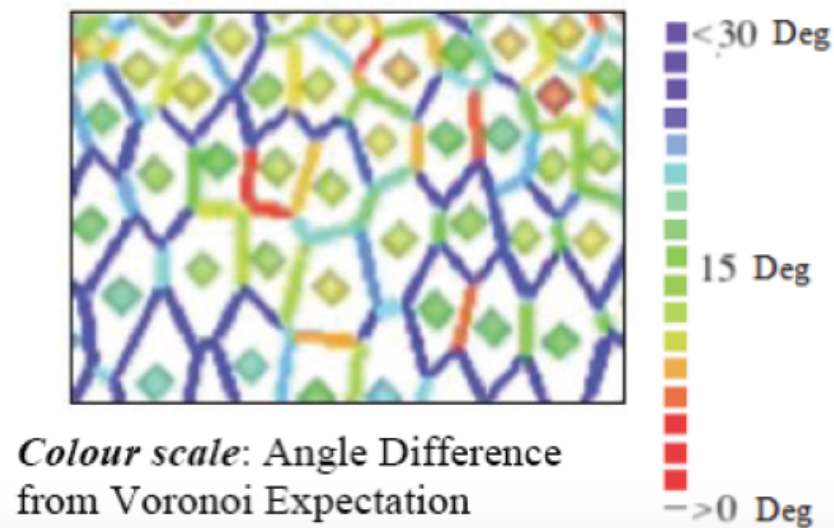
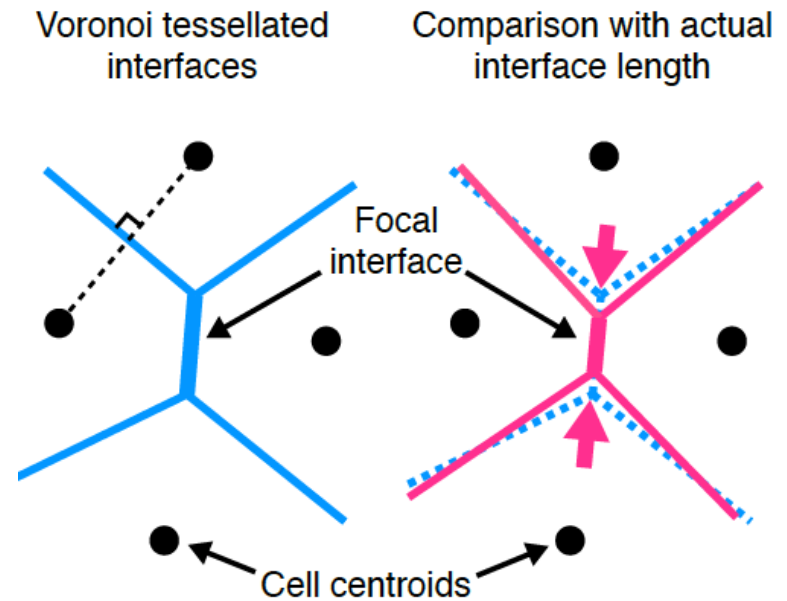
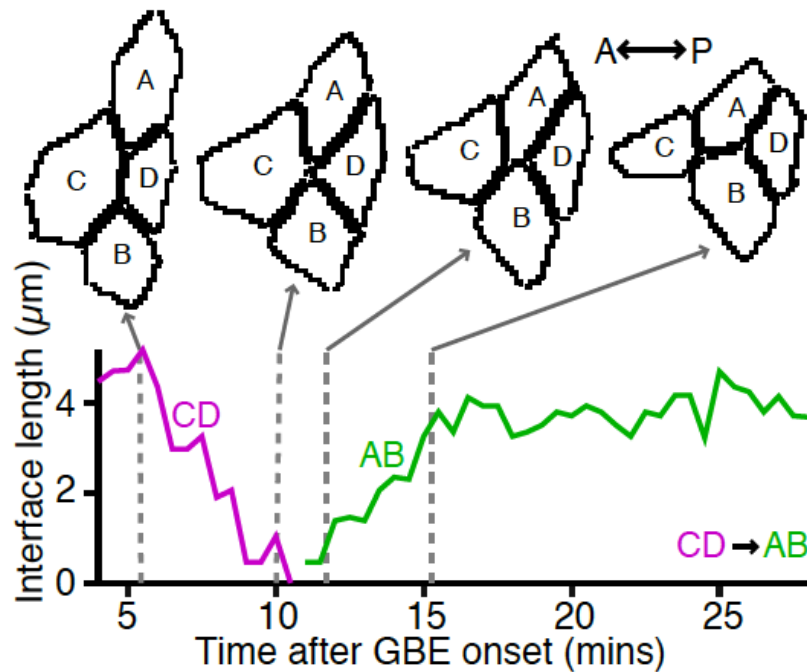
Cell shapes



Cell-cell interfaces



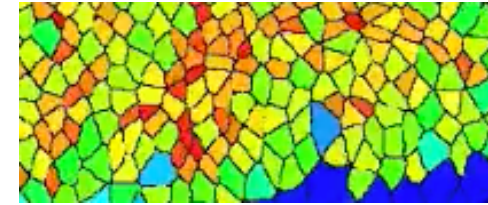
Cell-cell interface geometric stress



Analysis of Epithelial Morphogenesis

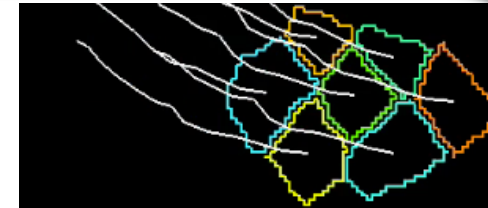
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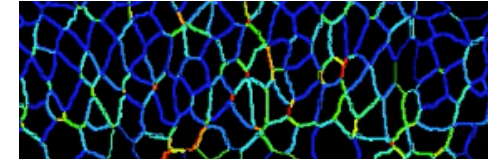
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- Measuring fluctuations



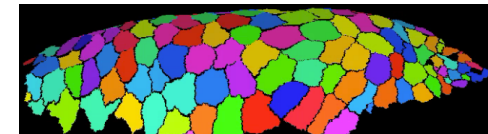
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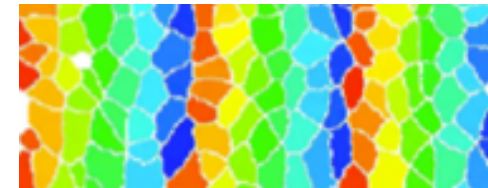
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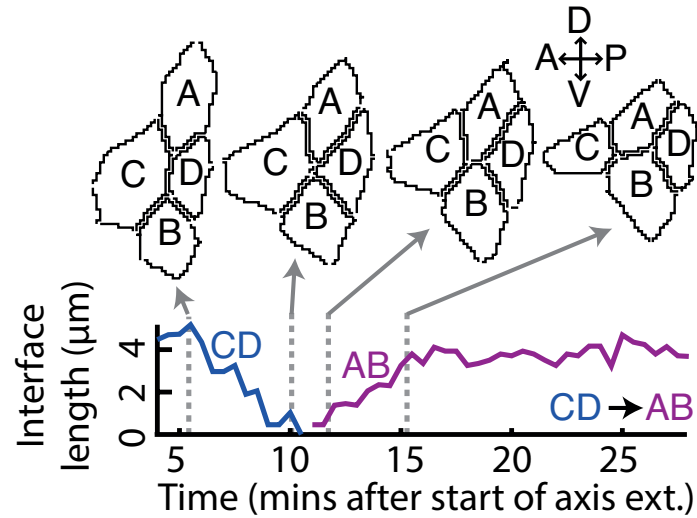
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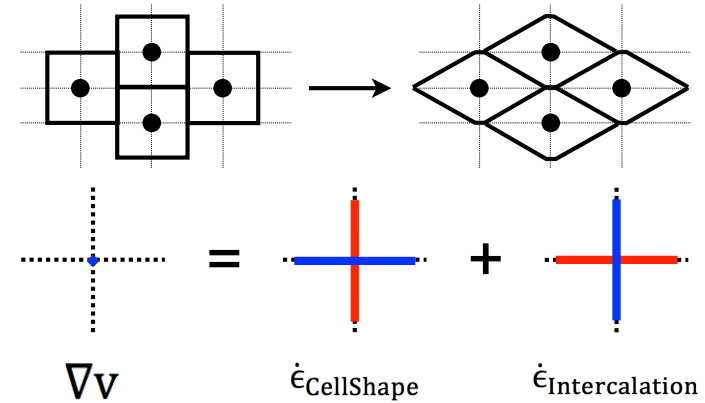


Cell-cell interface dynamics

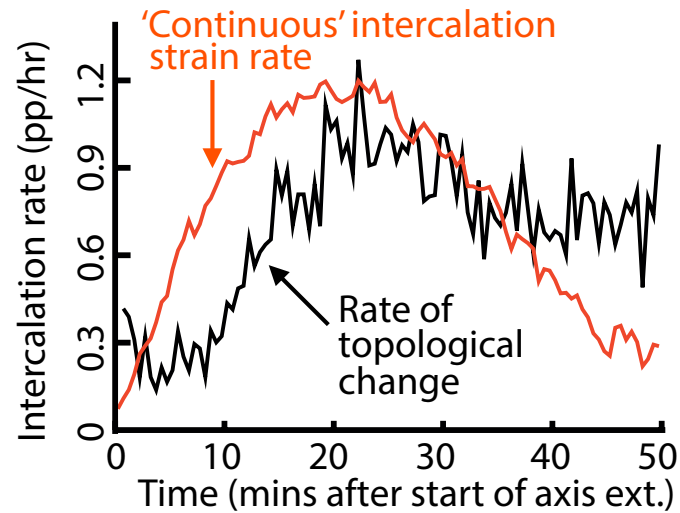
Context of T1 process



Continuous sliding behaviour



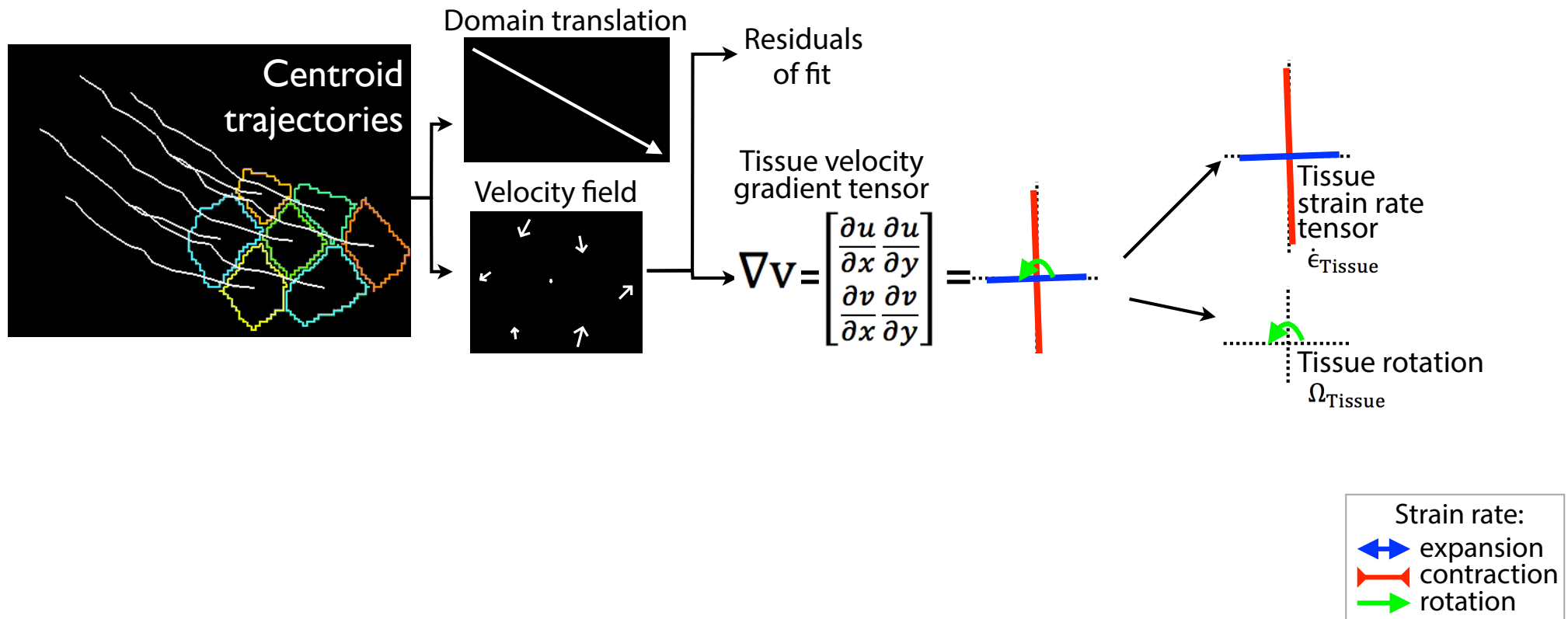
'Continuous' vs 'discrete' intercalation



Blanchard (2017) *Phil. Trans. R. Soc. B*

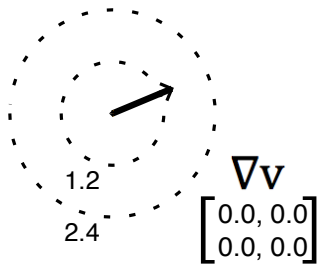
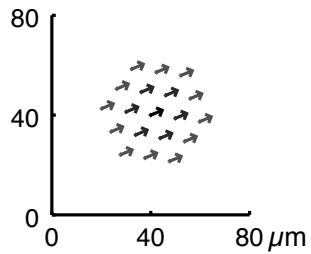


Small domain deformation rates

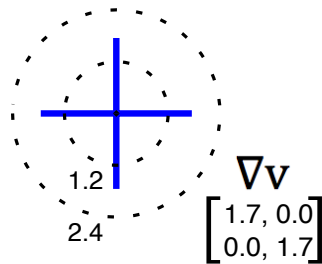
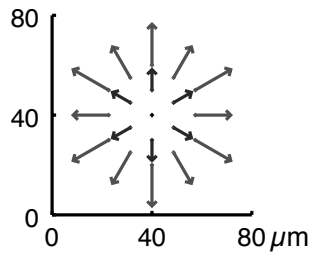


Tissue tectonics

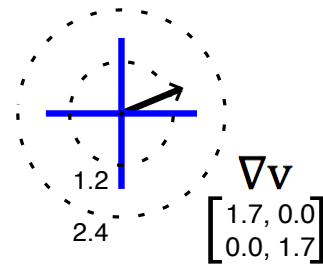
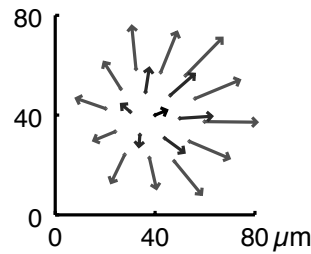
Translation



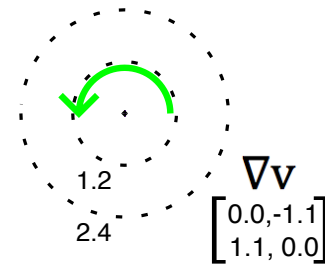
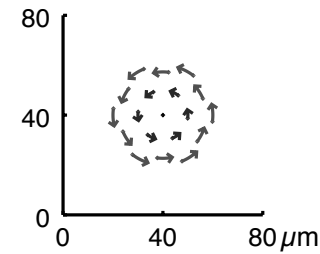
Dilation



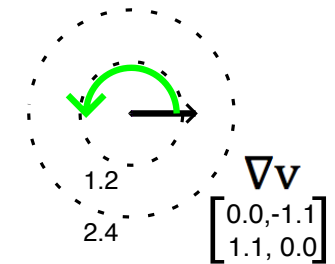
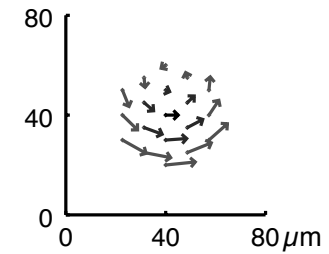
Dilation + translation



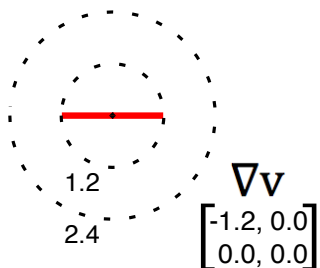
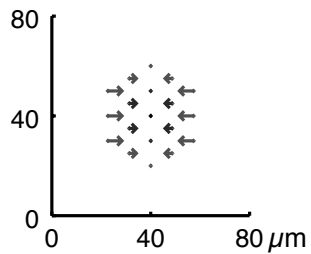
Rotation



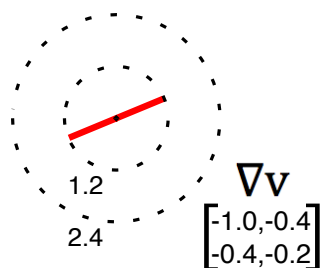
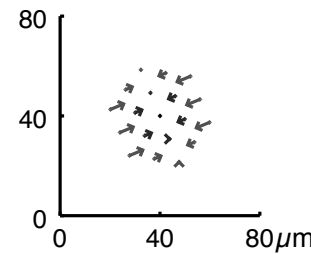
Rotation + translation



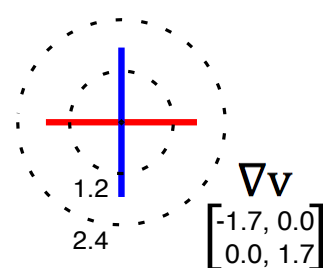
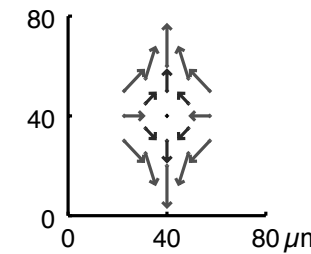
Convergence



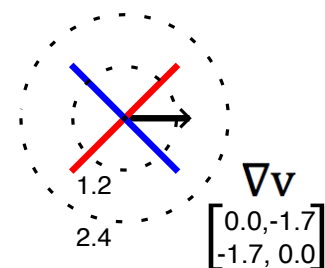
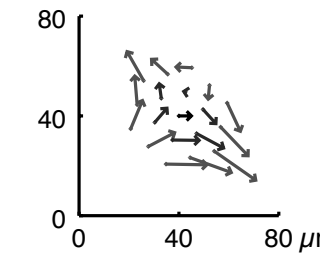
Oblique convergence



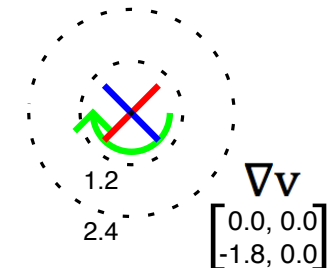
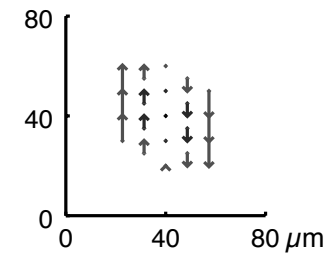
Pure shear (conv. extension)



Oblique pure shear + translation



Simple shear



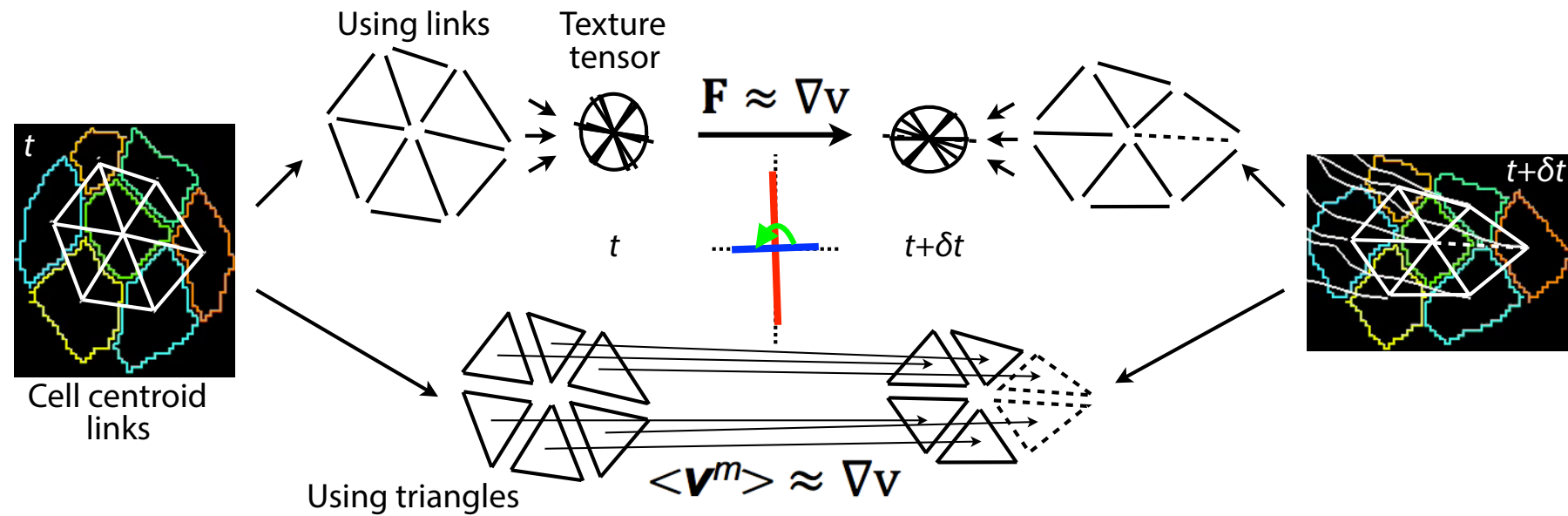
→ translation velocity ($\mu\text{m}/6 \text{ mins}$)

↔ expansion (pp/hr) ↔ contraction (pp/hr) → rotation (radians/hr)

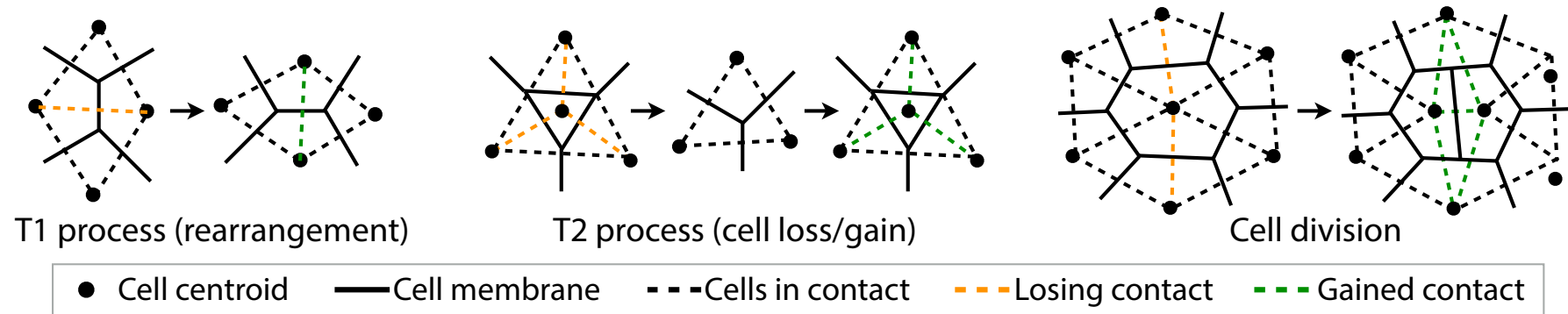


'Discrete' strain rate methods

Using links and triangles to calculate $\nabla \mathbf{v}$



'Discrete' cell behaviours defined by topological changes



Kinematic maps

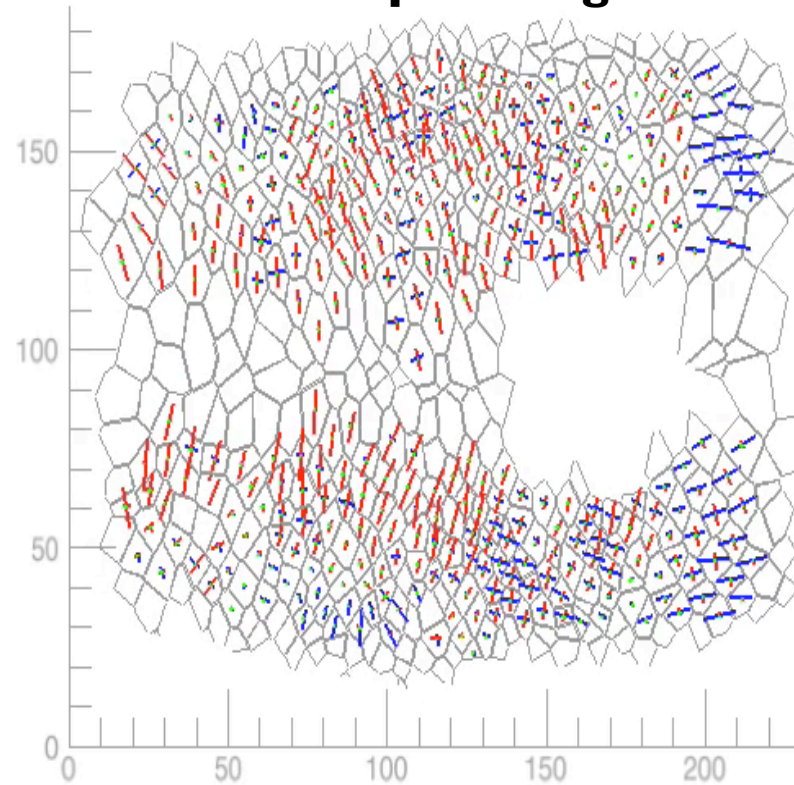
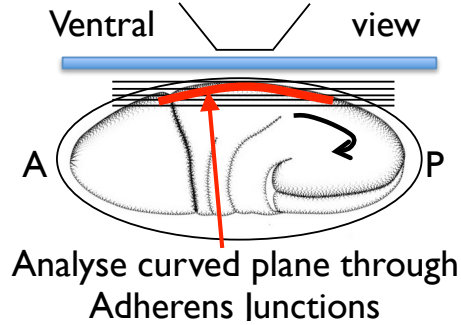
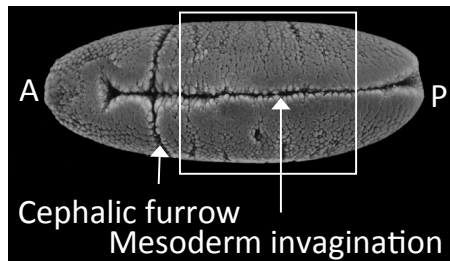
Extension rate

— 0.04 (pp/min)

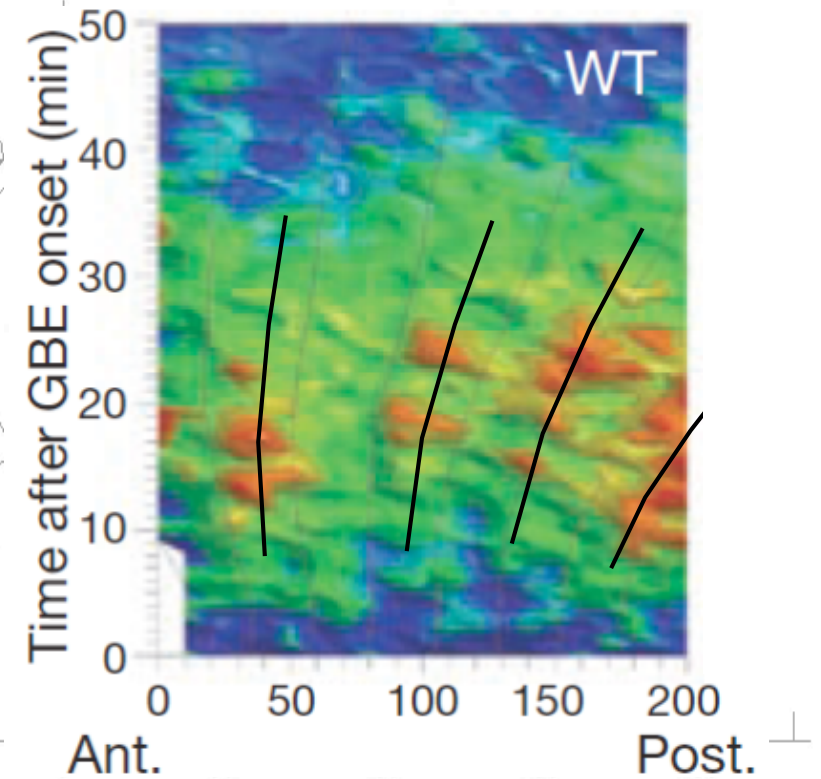
Convergence rate

— -0.04 (pp/min)

Cell shape change



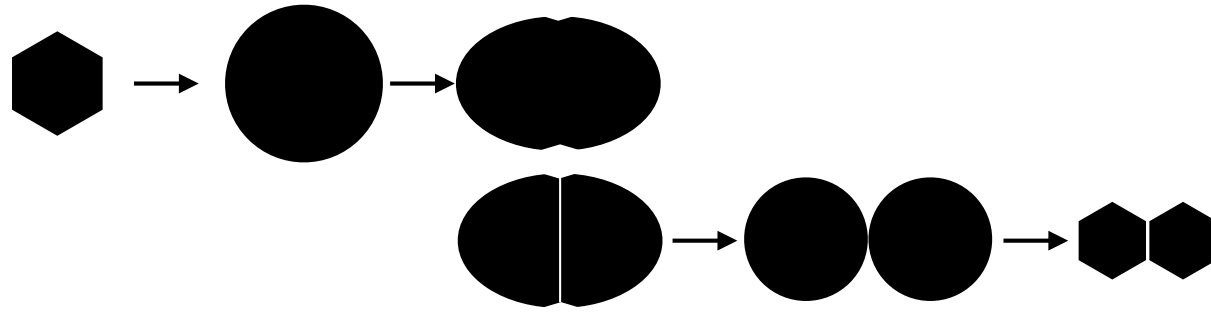
Cell intercalation



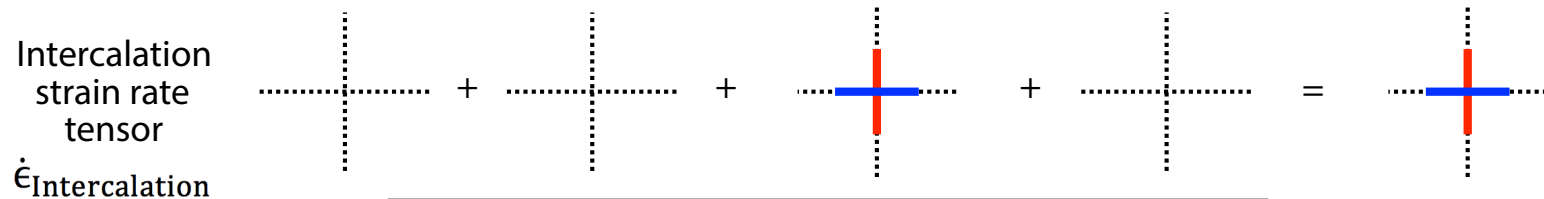
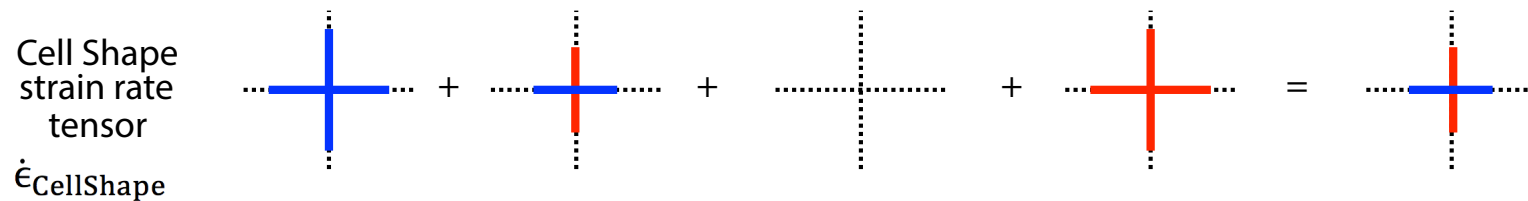
Butler et al. (2009) *Nat. Cell Biol.*



Net strain rates for dividing cell

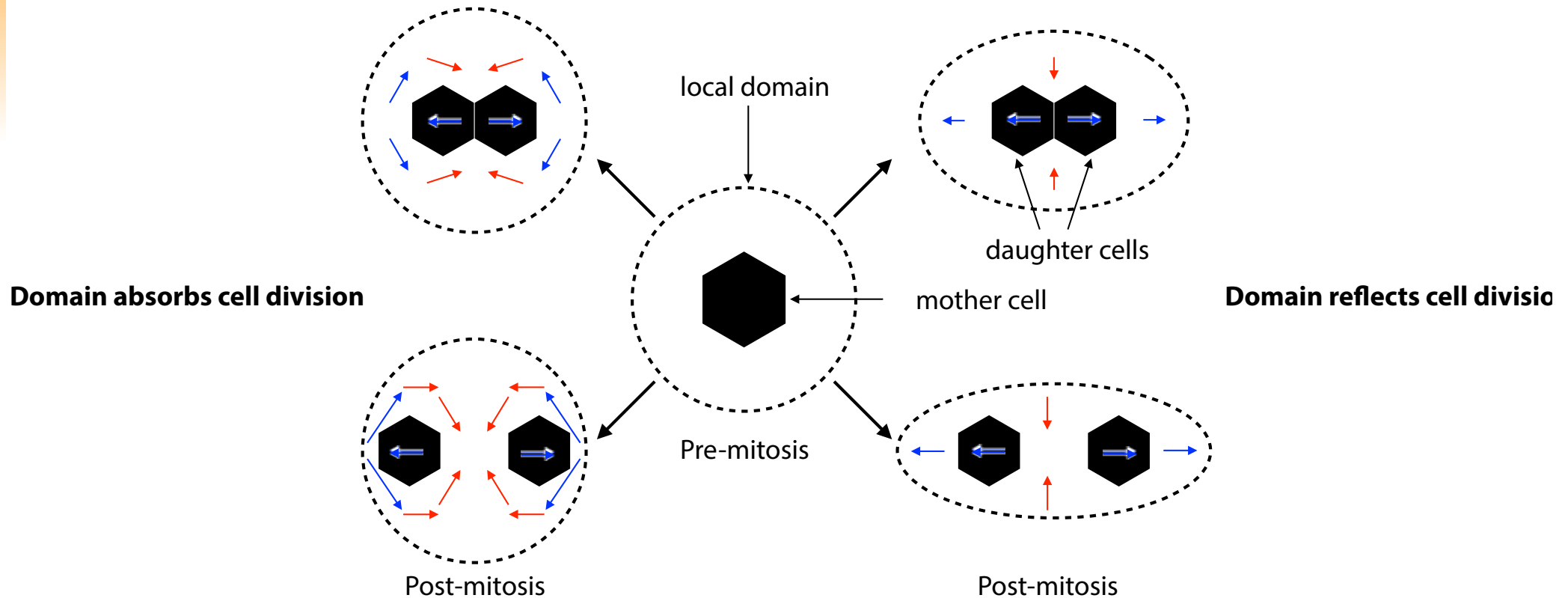


— prophase — anaphase — cytokinesis — relaxation — **Cumulative**



Strain rate: expansion contraction

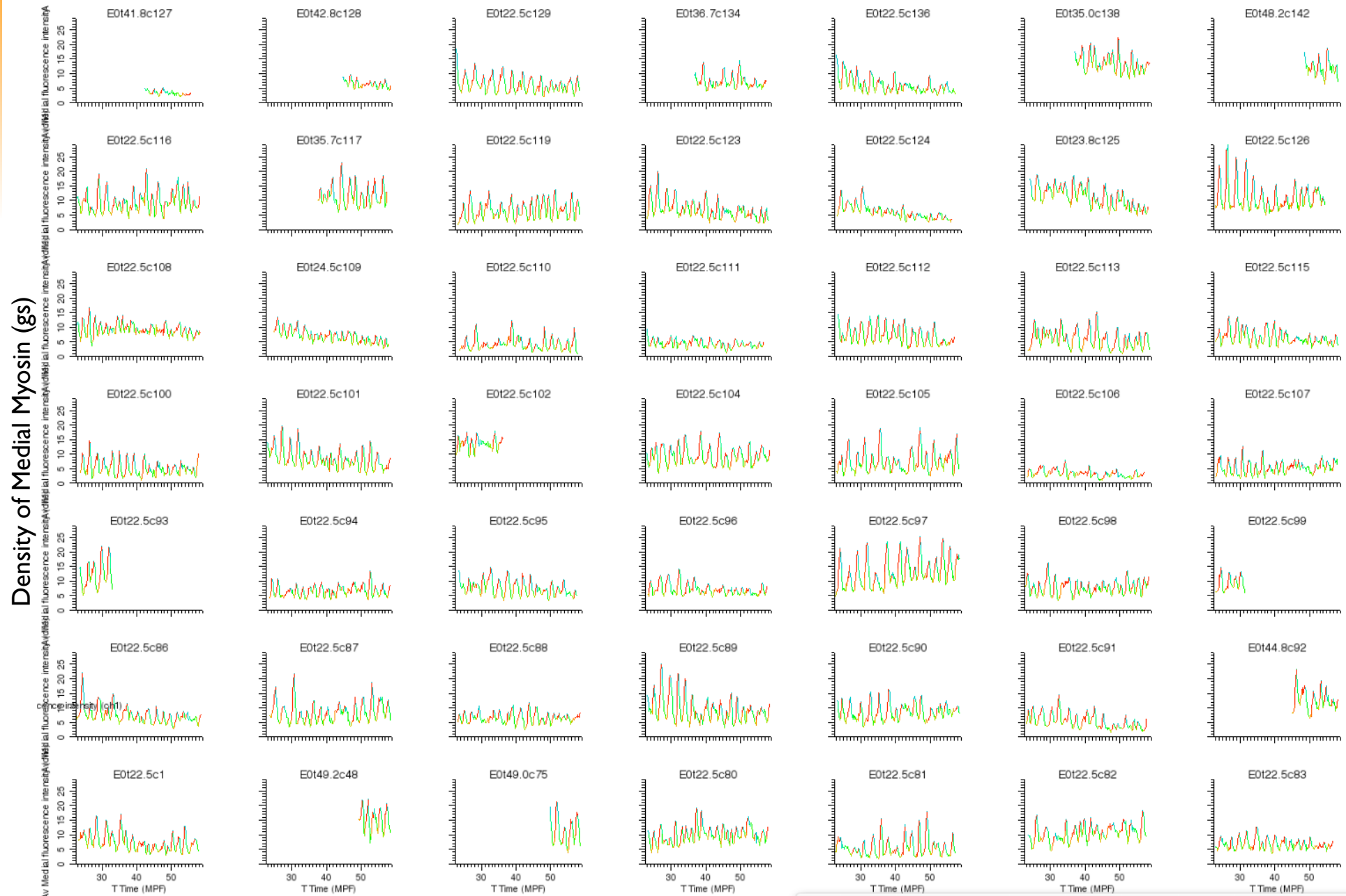
Local impact of cell division



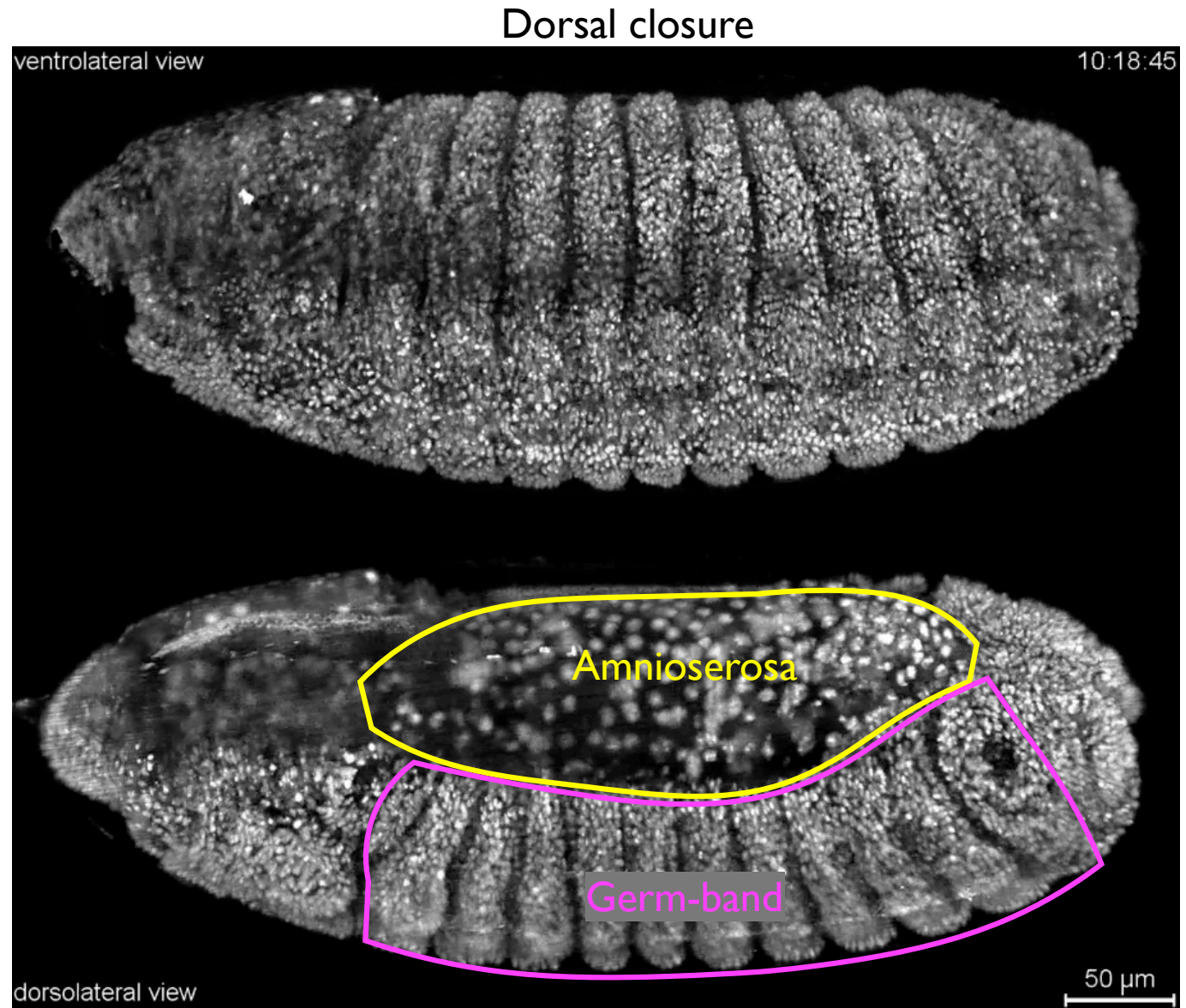
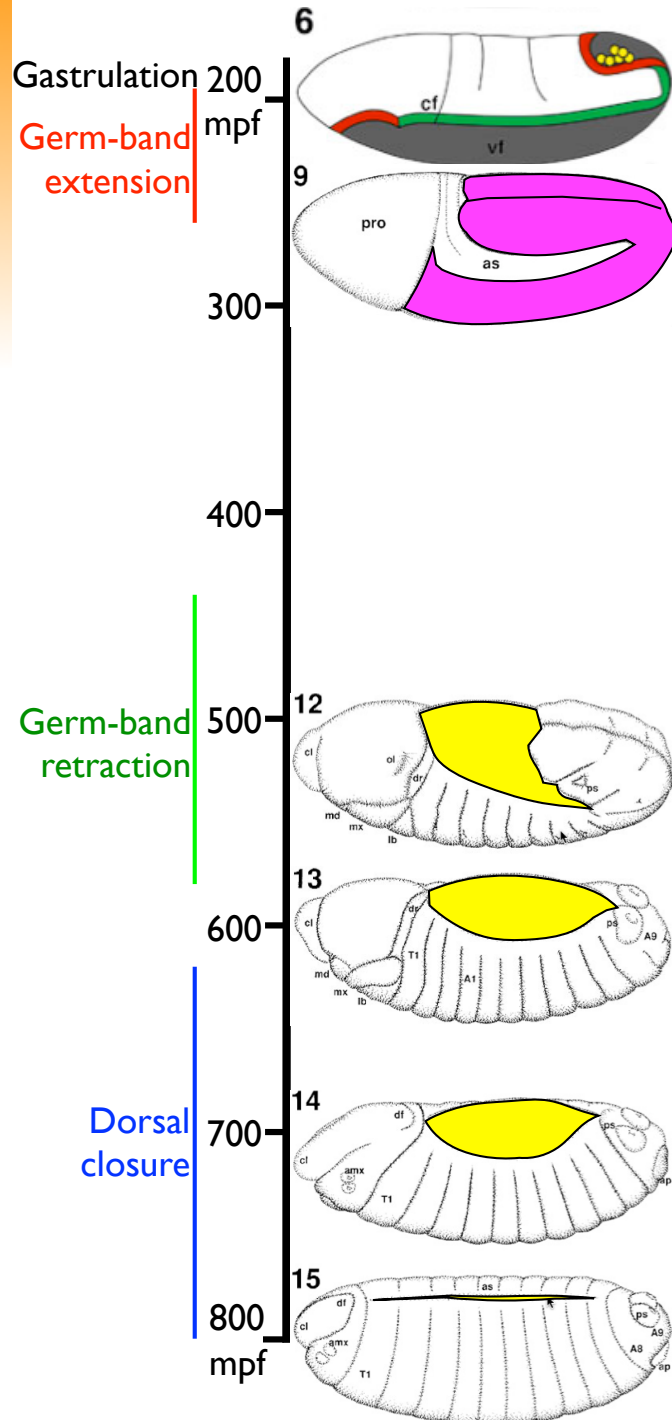
+ Surroundings squash dividing cell (surroundings stiffer than mitosis)

Cell area & Myosin II fluctuations

All cells from example embryo (3LU)



Drosophila embryonic morphogenesis



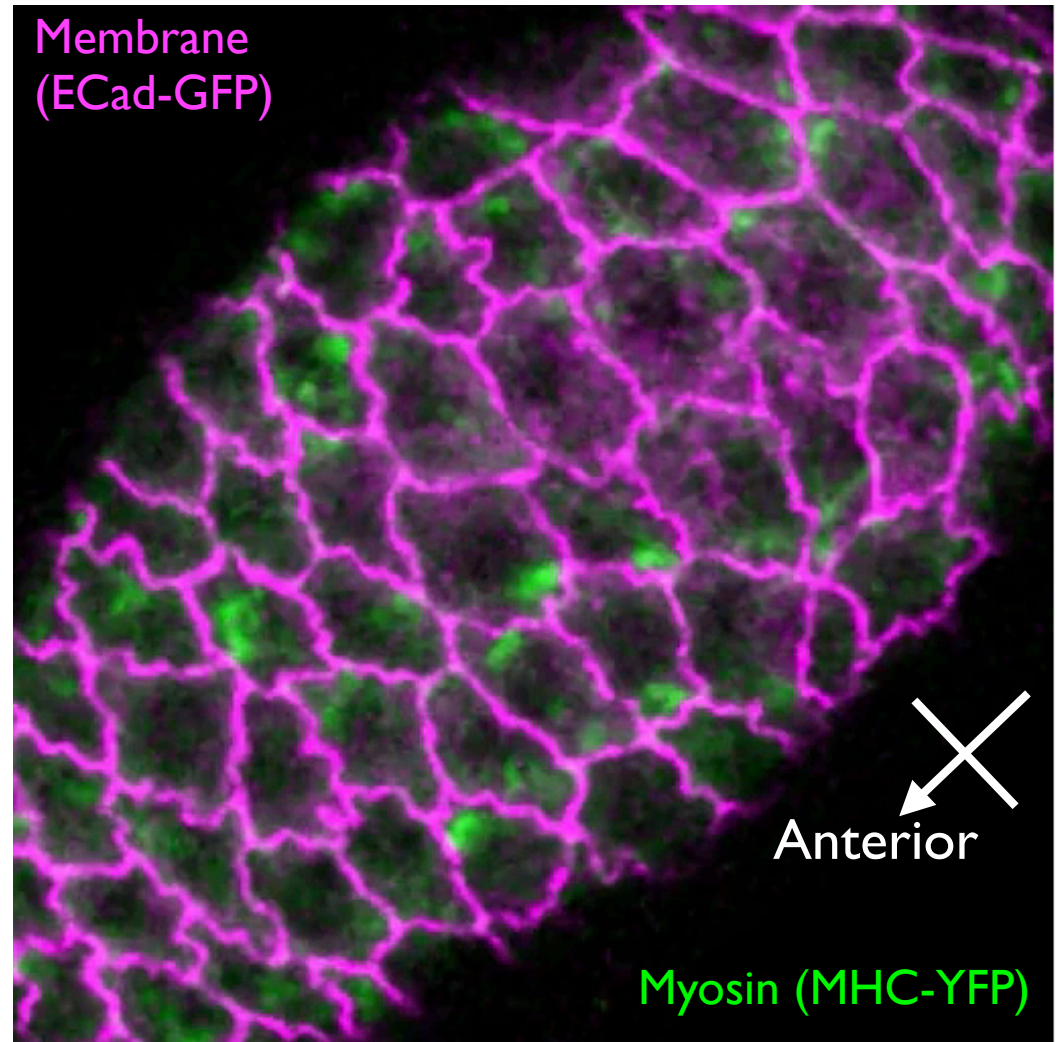
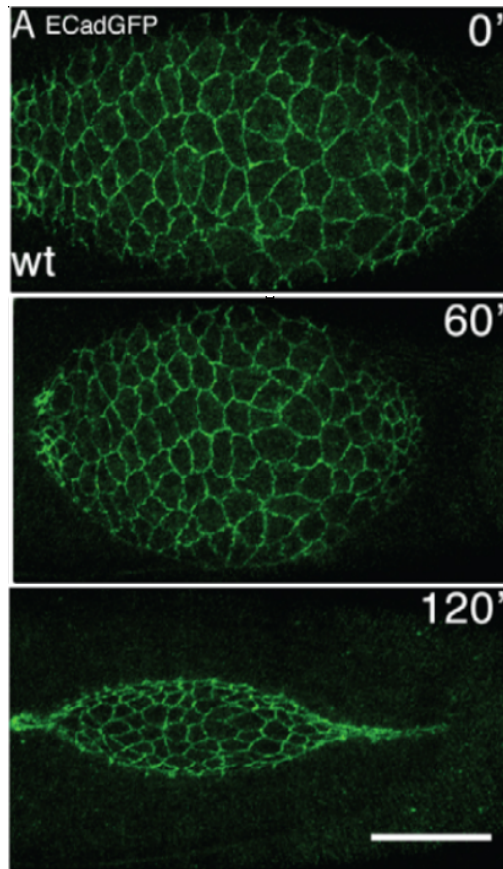
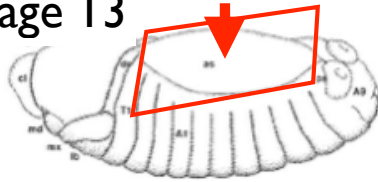
Tomer et al. (2012) *Nat. Methods*



Amnioserosa dorsal closure

Dorsal view of
amnioserosa

stage 13

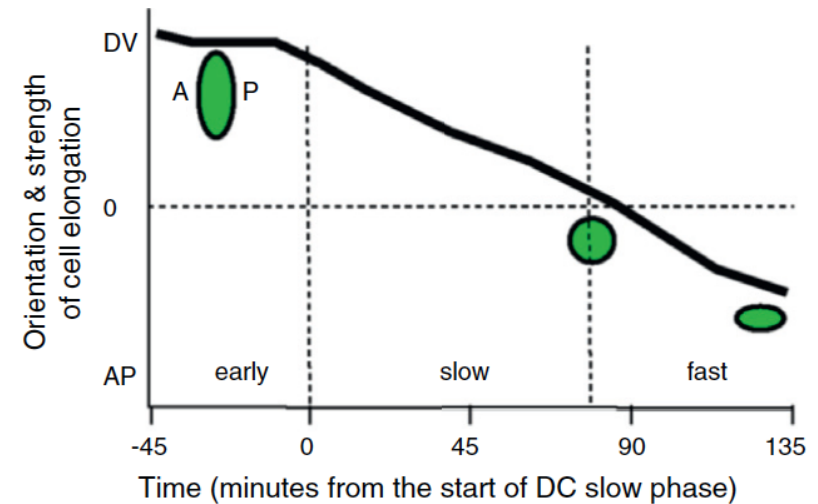
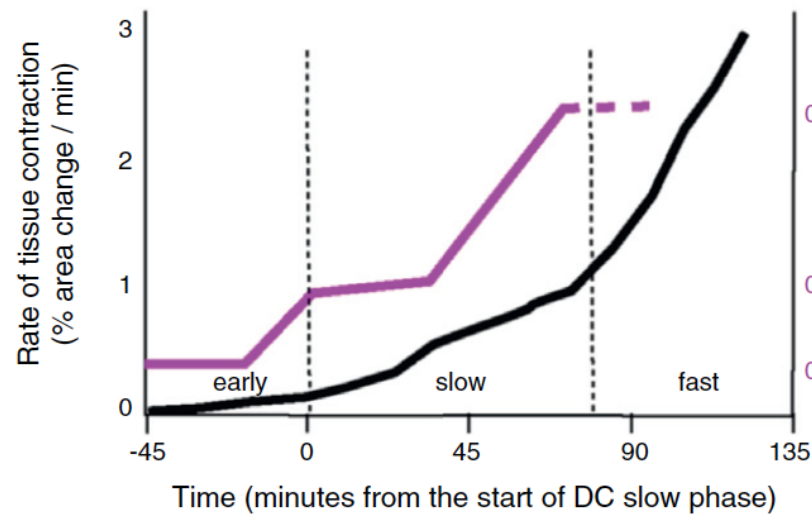
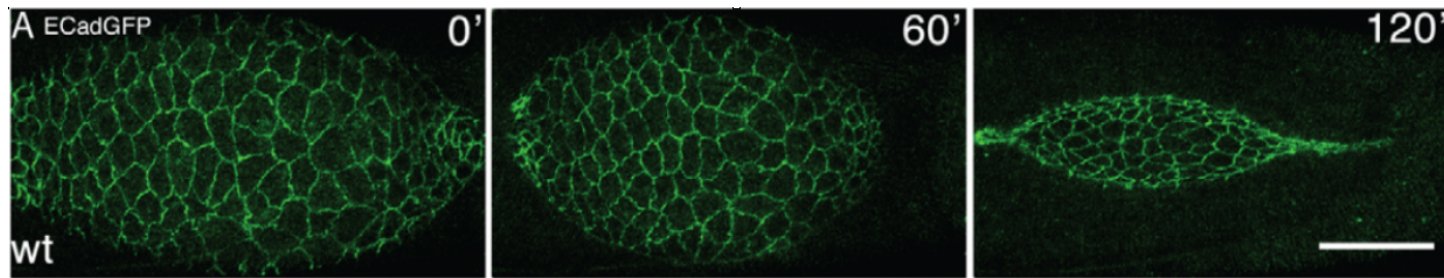


- ★ No cell rearrangement
- ★ Pulsatile Myosin-II is apico-medial, not junctional

Gorfinkiel et al., (2009) *Development*; Blanchard et al., (2010) *Development*

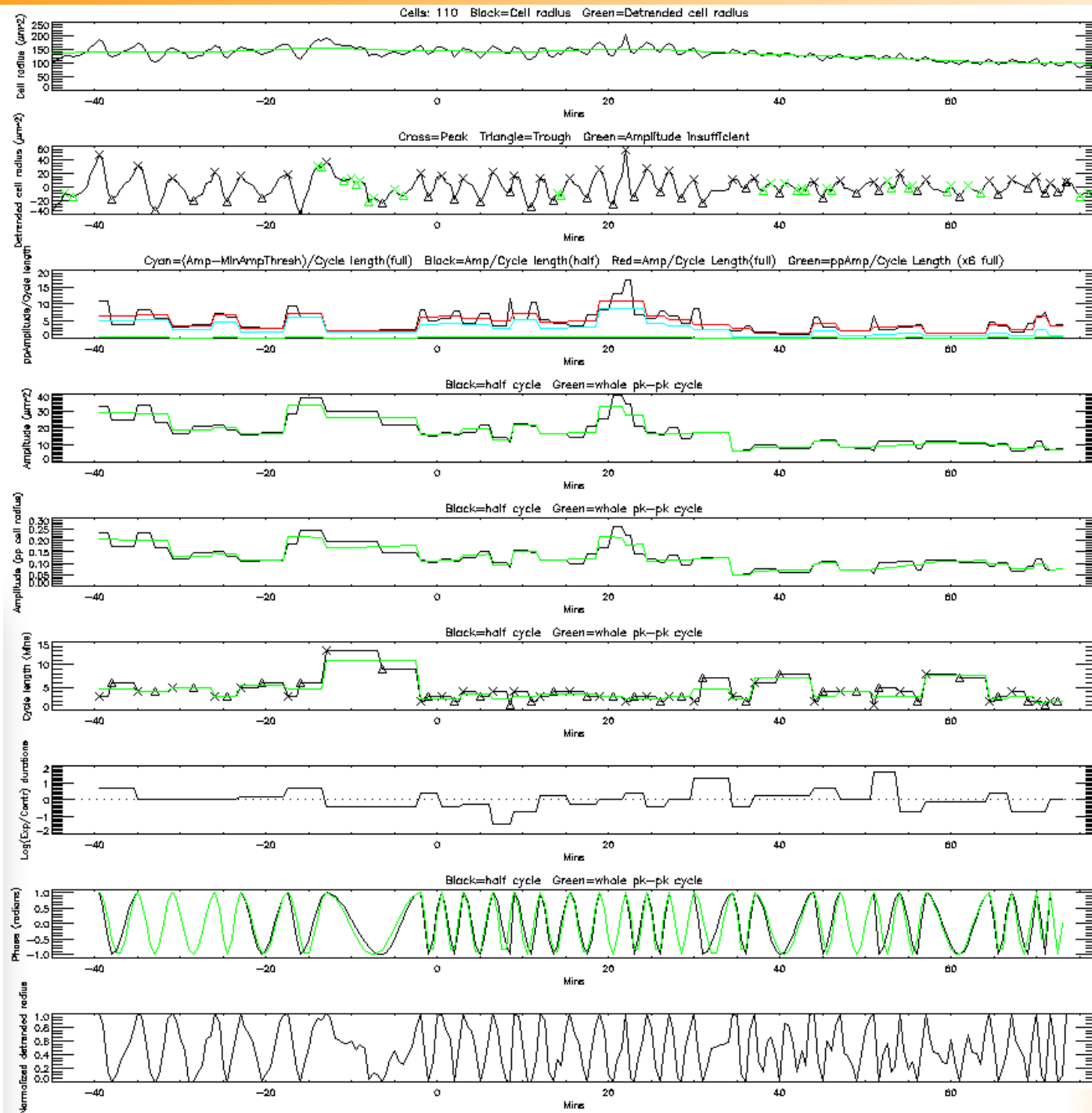


Drosophila dorsal closure trends



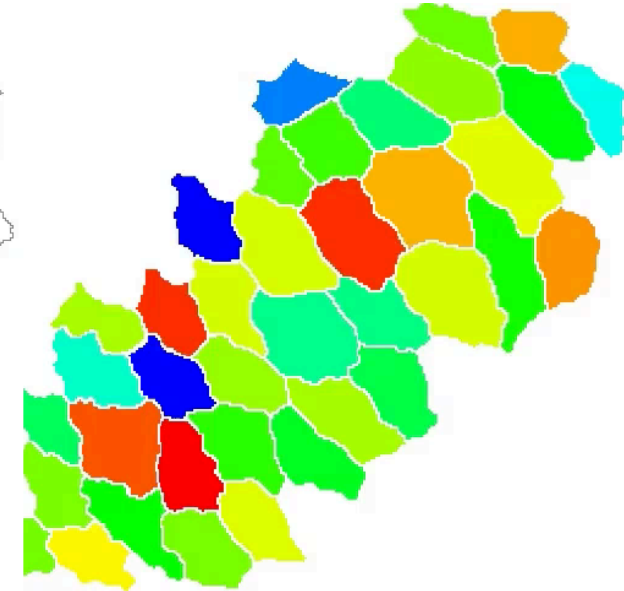
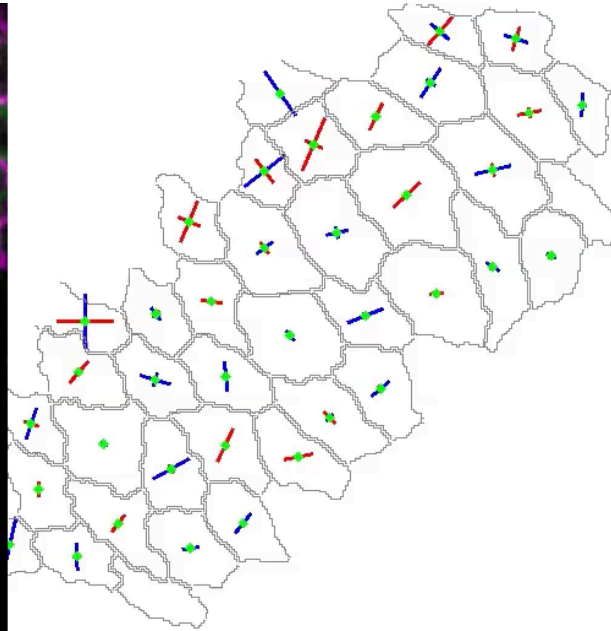
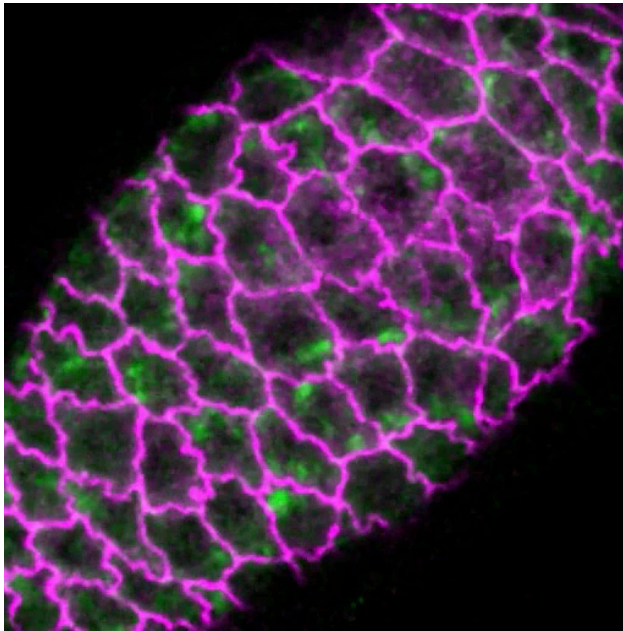
Blanchard & Adams (2011) *COGD*; Gorfinkiel, Schamberg & Blanchard (2011) *Genesis*

Apical area fluctuation of one cell

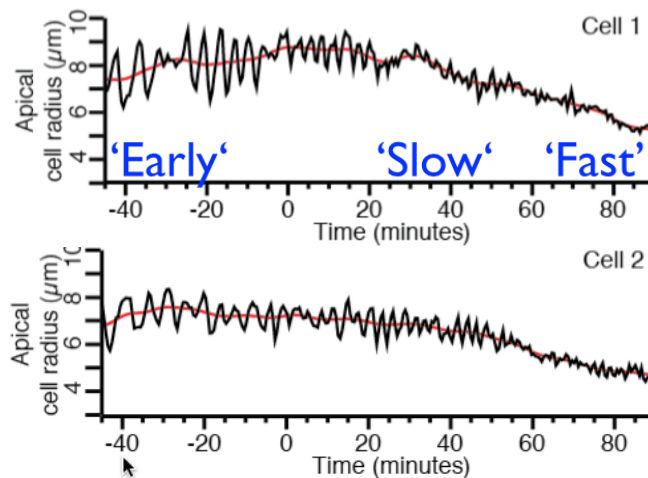


Extract cell contractile stress & strain rates

$$\sigma + \tau_R \dot{\sigma} = \bar{\tau} \kappa c_m + \tau_R \kappa \dot{\epsilon}$$



Mean trends

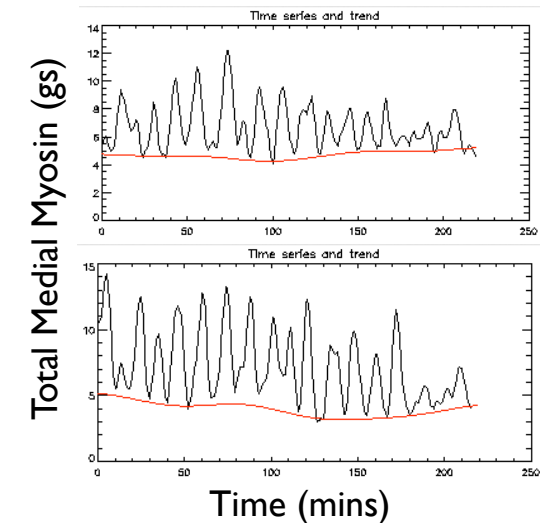


$$\epsilon_{c,t} = \frac{Area_{c,t}}{Area_{0,c,t}} - 1$$

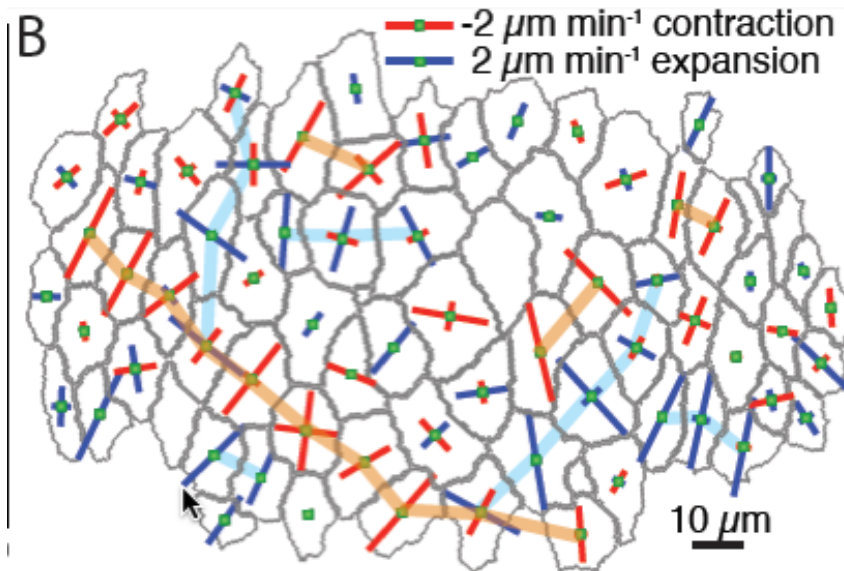
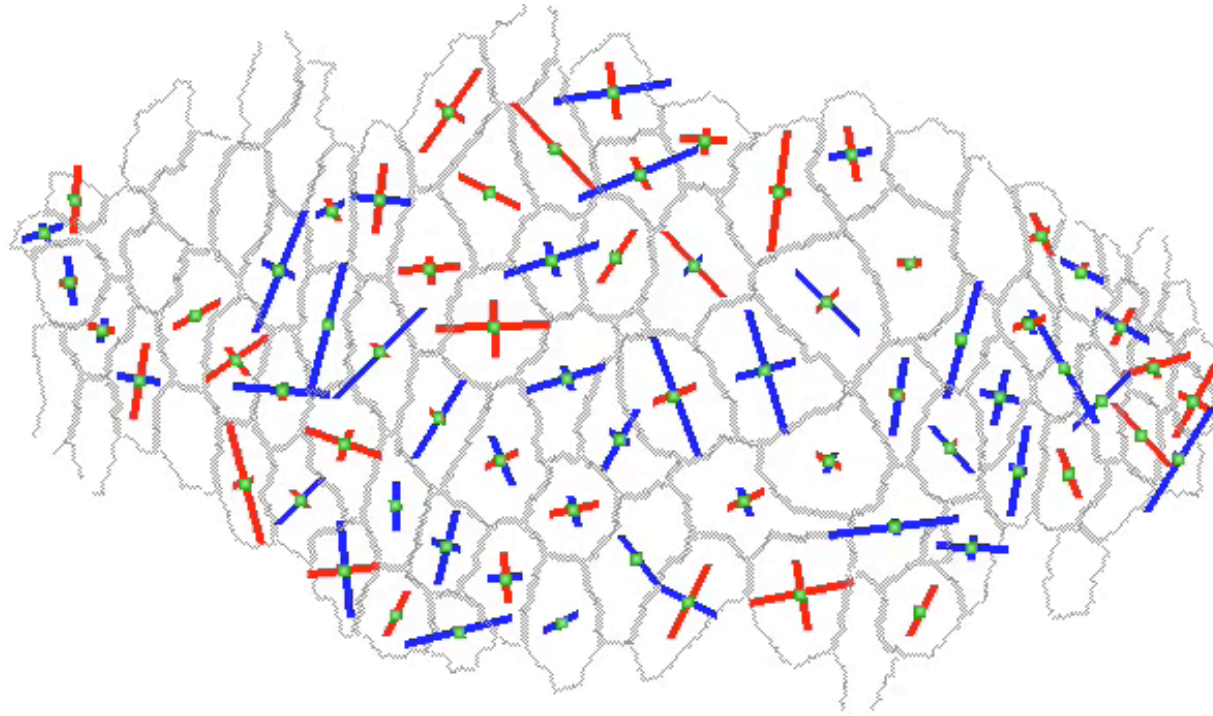
$$myo_{c,t} = \frac{Tot.Med.Myo_{c,t}}{Tot.Med.Myo_{0,c,t}} - 1$$

Trends retrospective
Rates calculated over 10sec time window

Minima trends



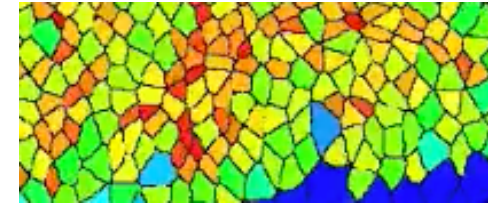
Neighbour accommodation: patterns at intermediate scale



Analysis of Epithelial Morphogenesis

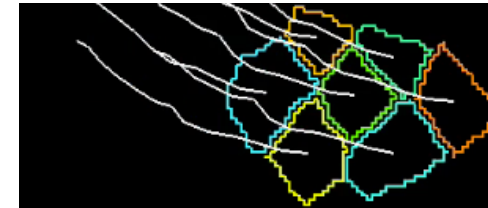
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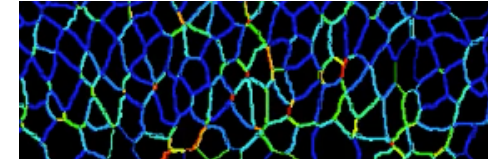
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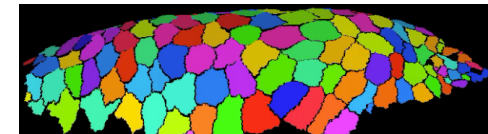
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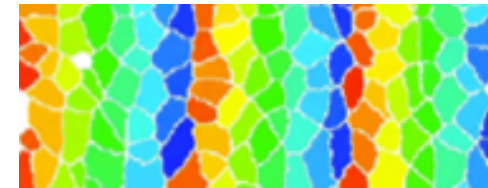
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- Epithelial tilt & curvature
- Combining tracking of apical & basal layers

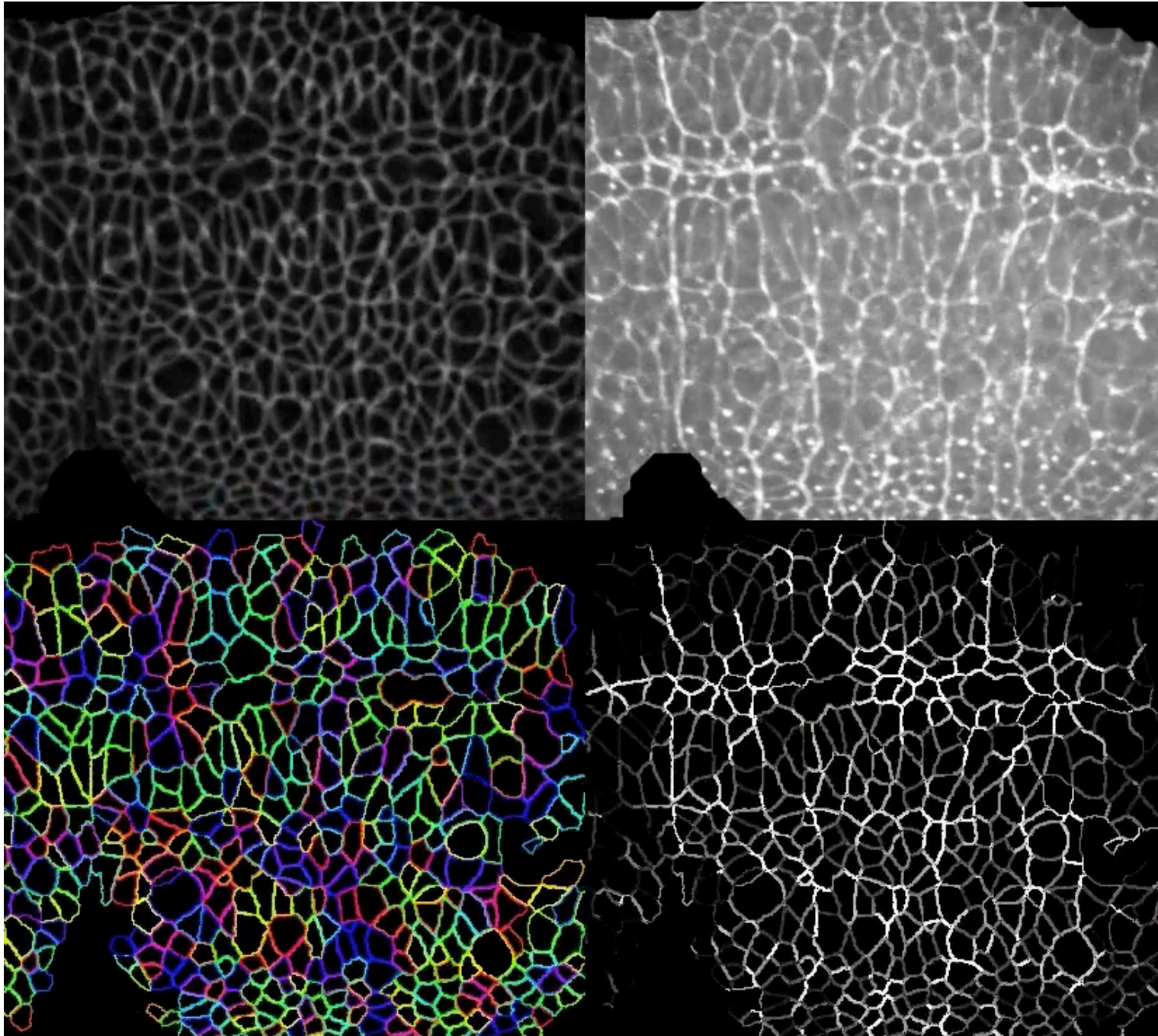


5 Time & space coordinate systems

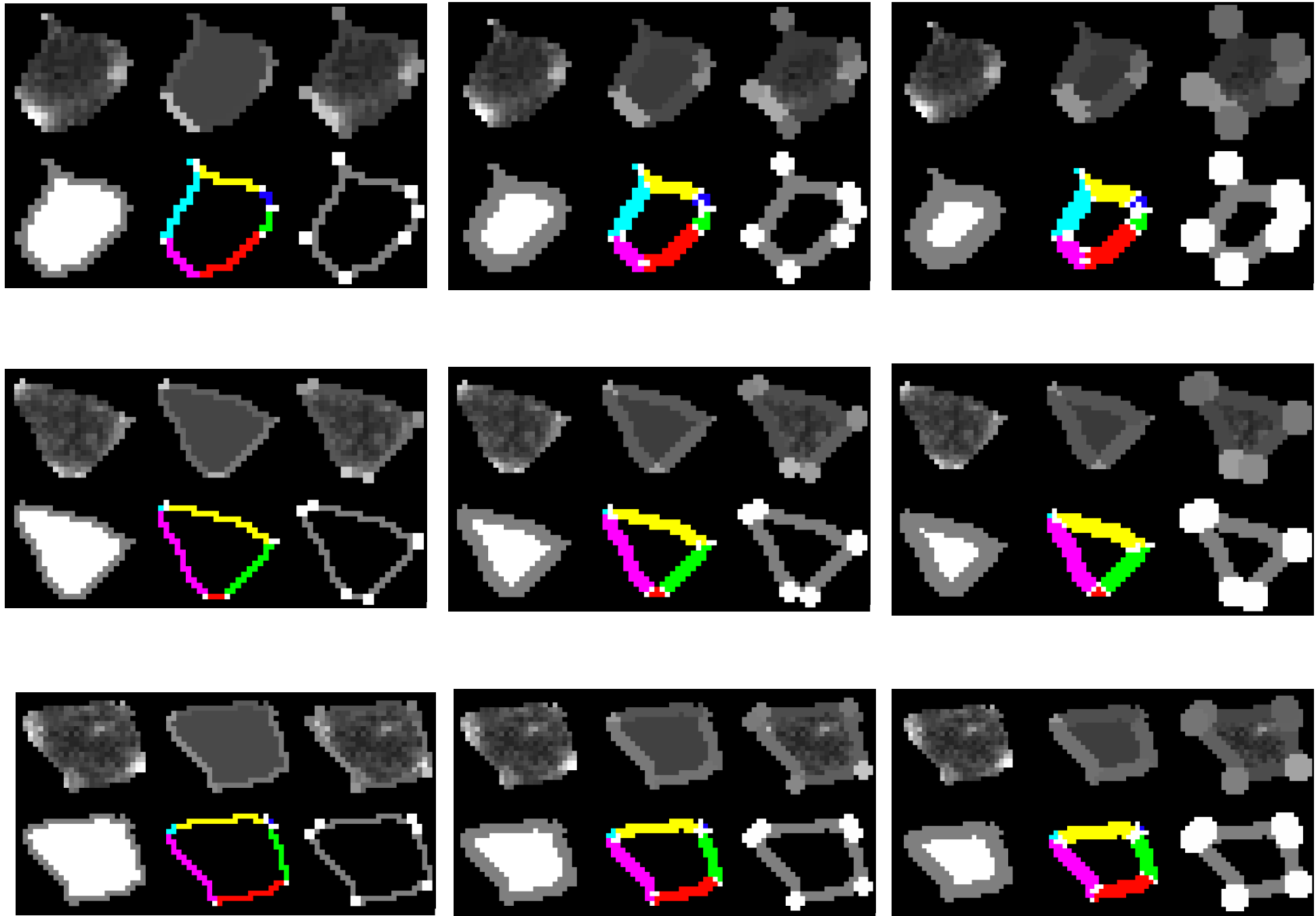
- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries



Cell-cell interface Myosin II fluorescence

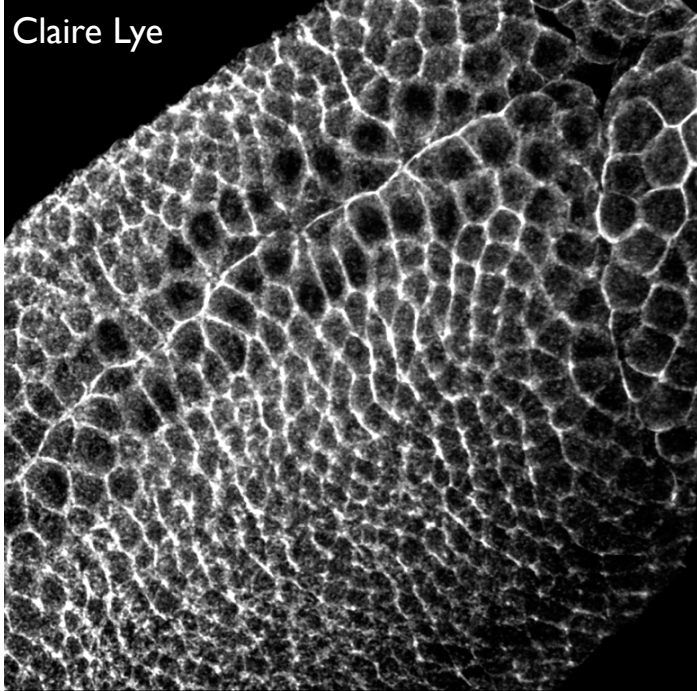


Cell fluorescence quantification

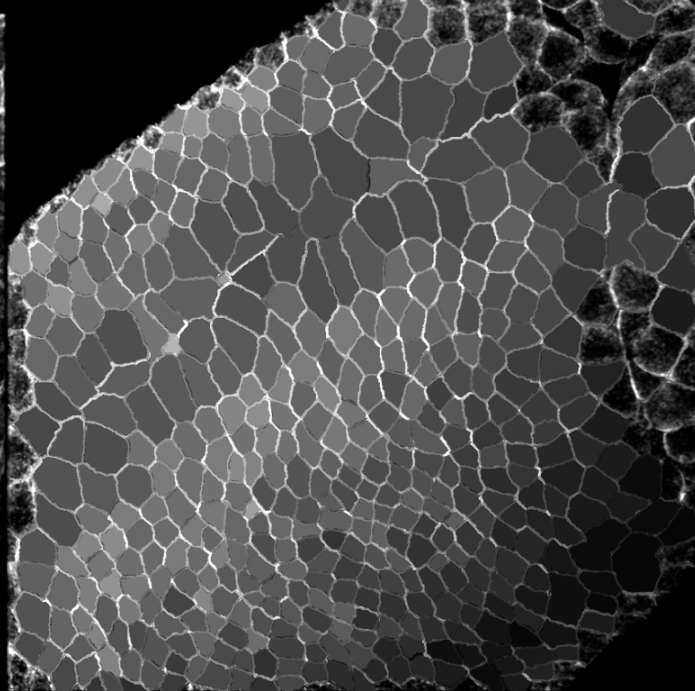


Fluorescence (Planar-)Polarity

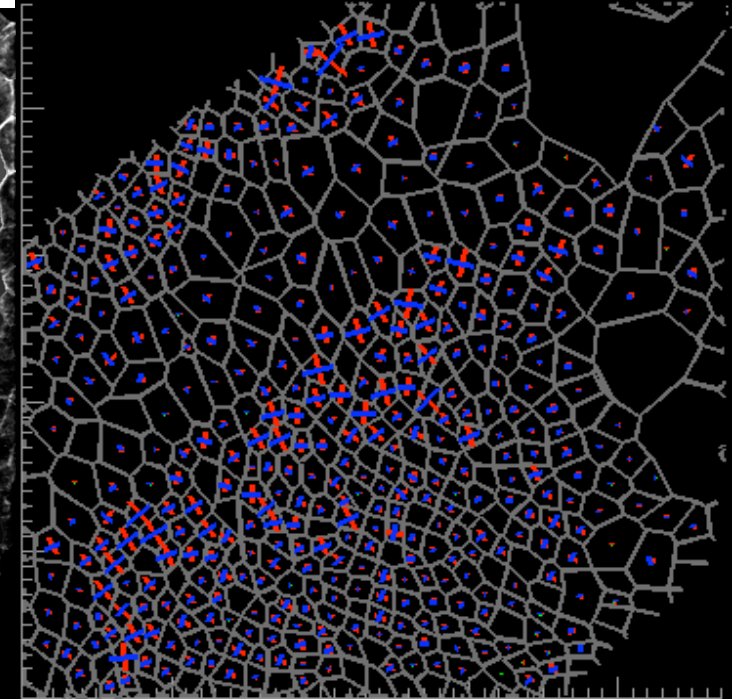
Claire Lye



Myosin staining during early germ-band extension. Cells tracked in Cadherin-GFP channel (not shown).

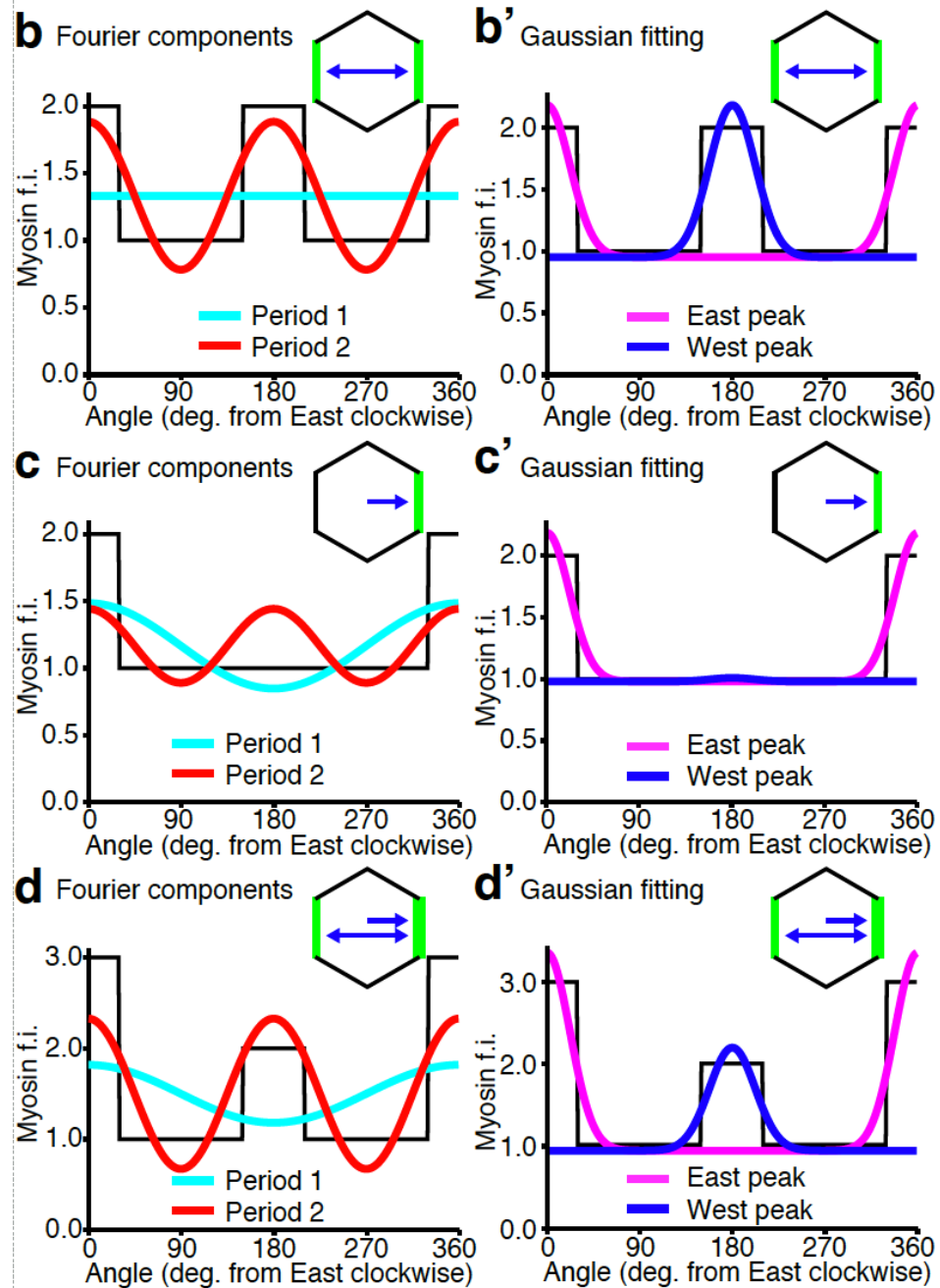


Mean fluorescence has been calculated across cell apices and for each cell-cell interface. Mean intensity for each part of each cell is drawn here.

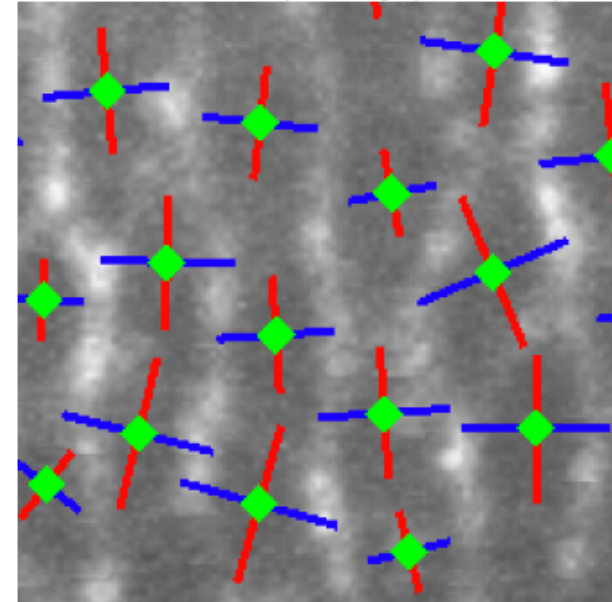


The relative fluorescence intensity of each of a cell's interfaces is compared. Any polarity in the pattern is captured and drawn here as a cross, with red in the orientation of least intensity.

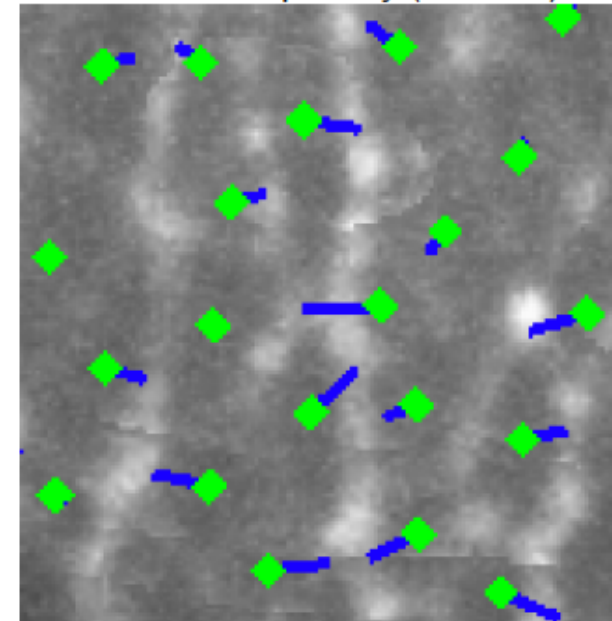
Apical cell myosin polarity



Bidirectional polarity (8.5 mins)

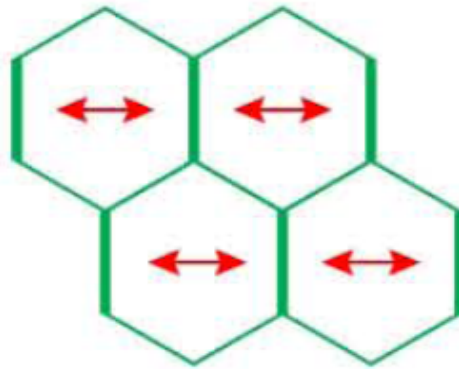
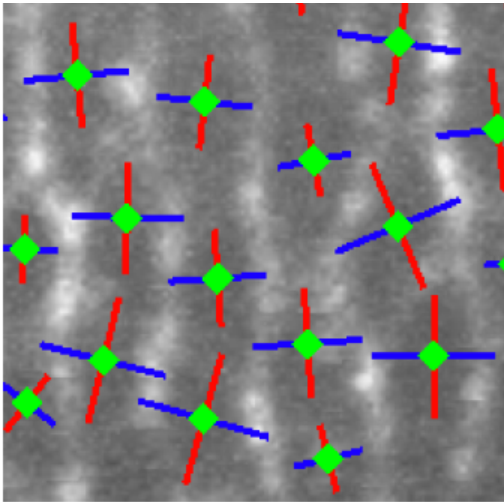


Unidirectional polarity (39 mins)

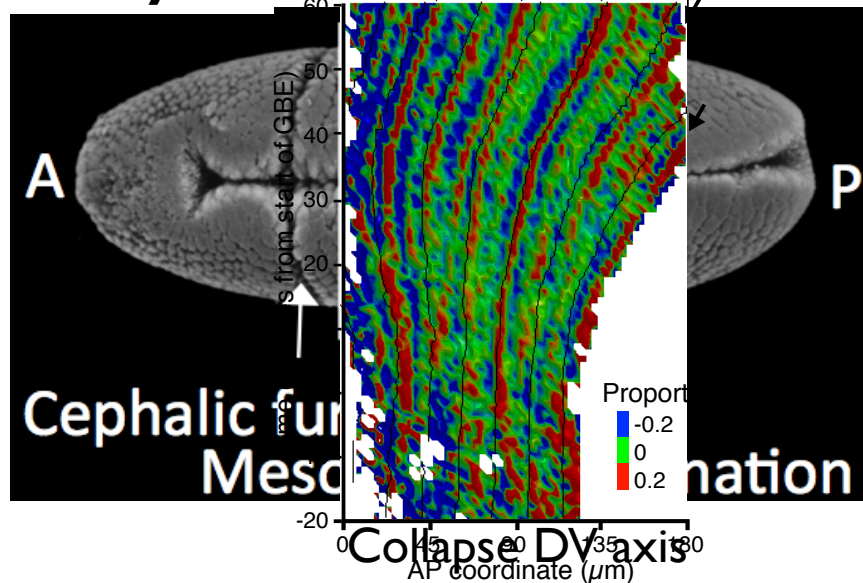
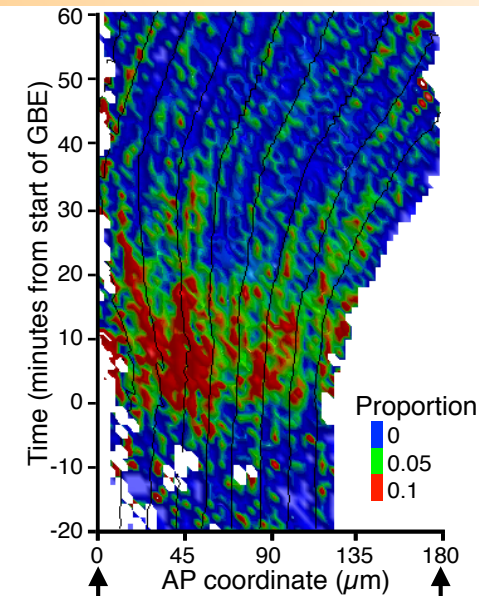
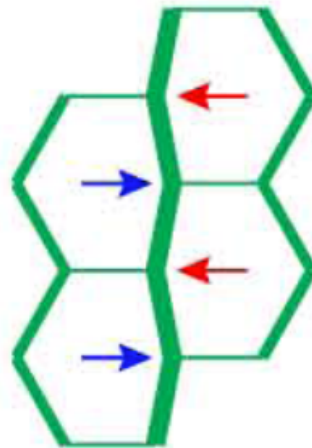
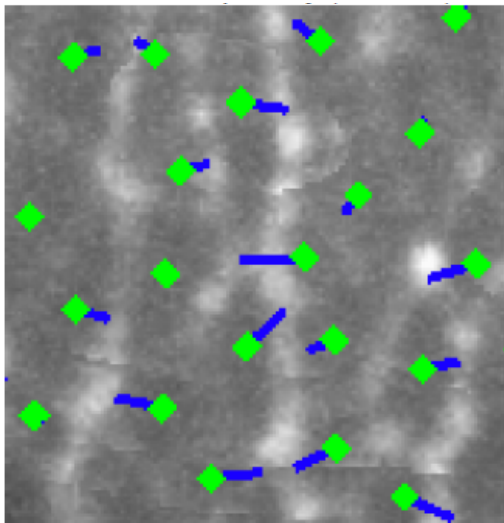


Myosin polarity patterns across AP axis

Bidirectional polarity

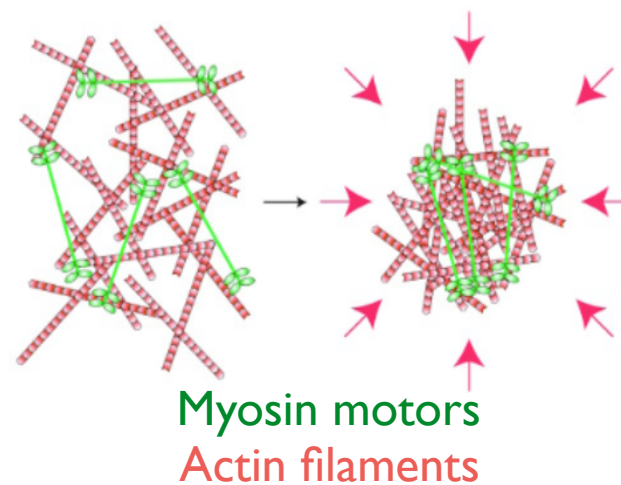
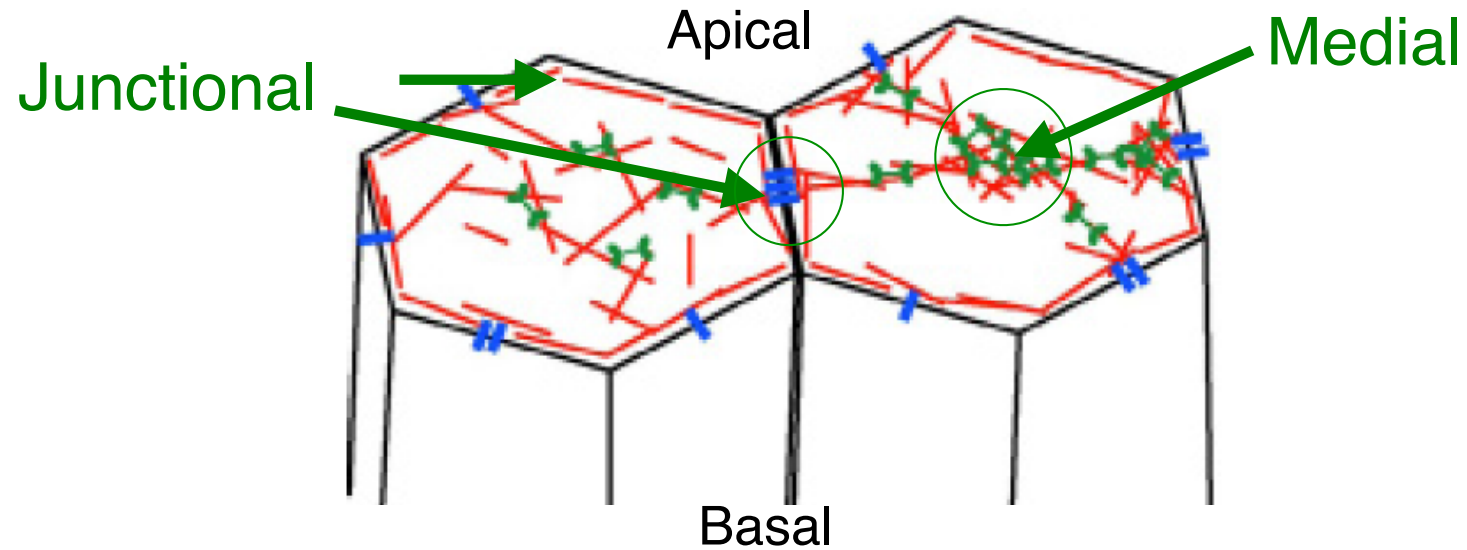


Unidirectional polarity

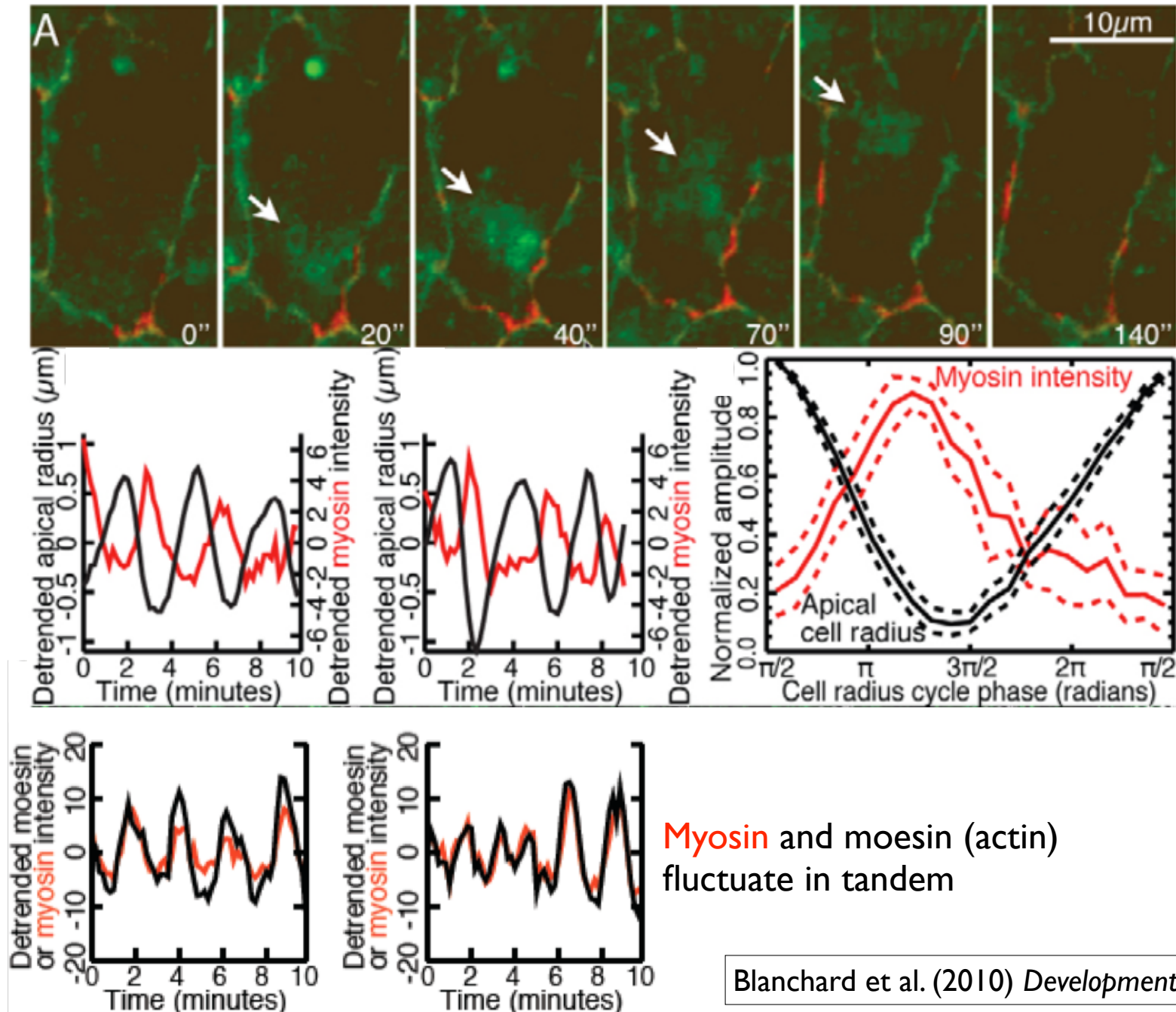


Single embryos. To find stereotypical behaviour want **standardised AP axis coordinate system**.

Actomyosin drives apical cell contractility



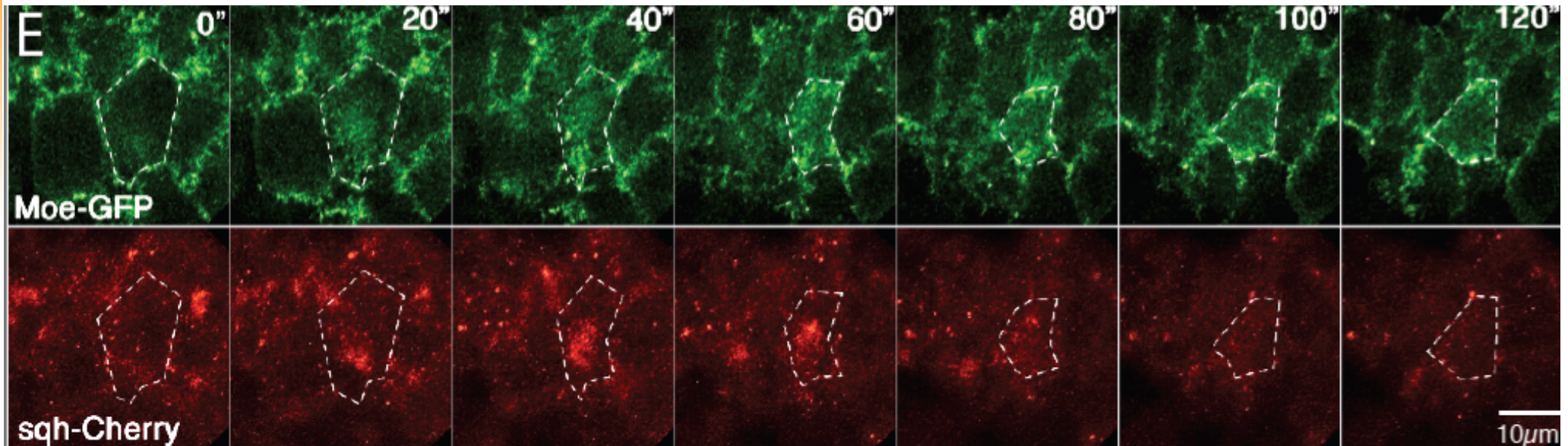
Myosin II fluctuations



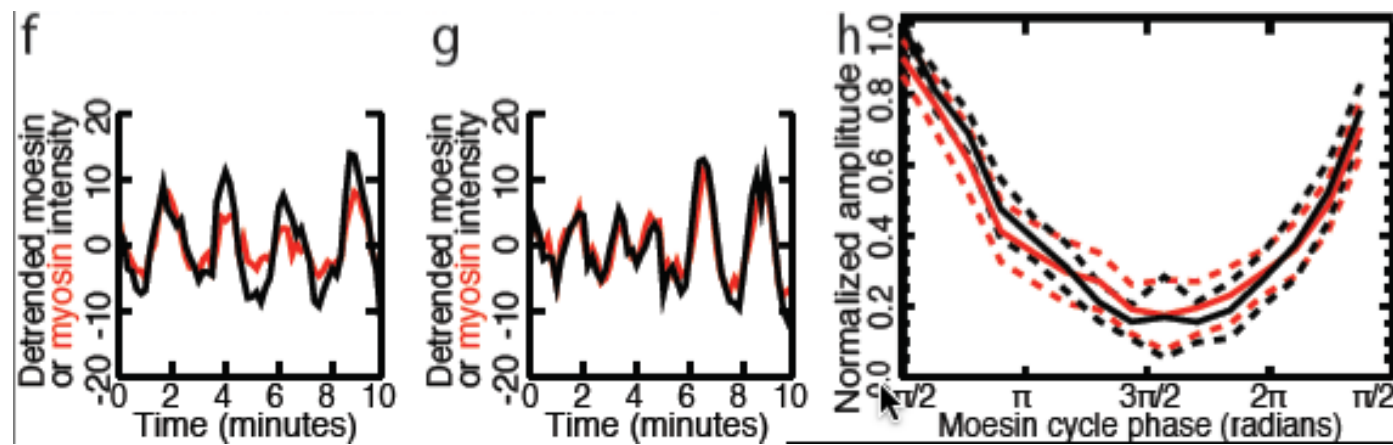
Blanchard et al. (2010) *Development*



Actin and myosin dynamics in cell fluctuations



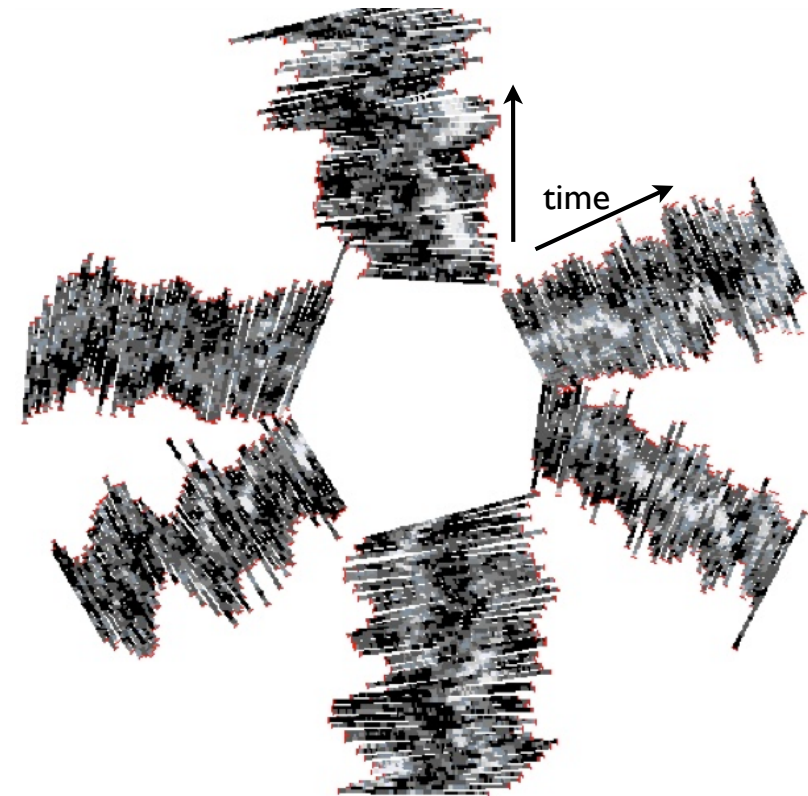
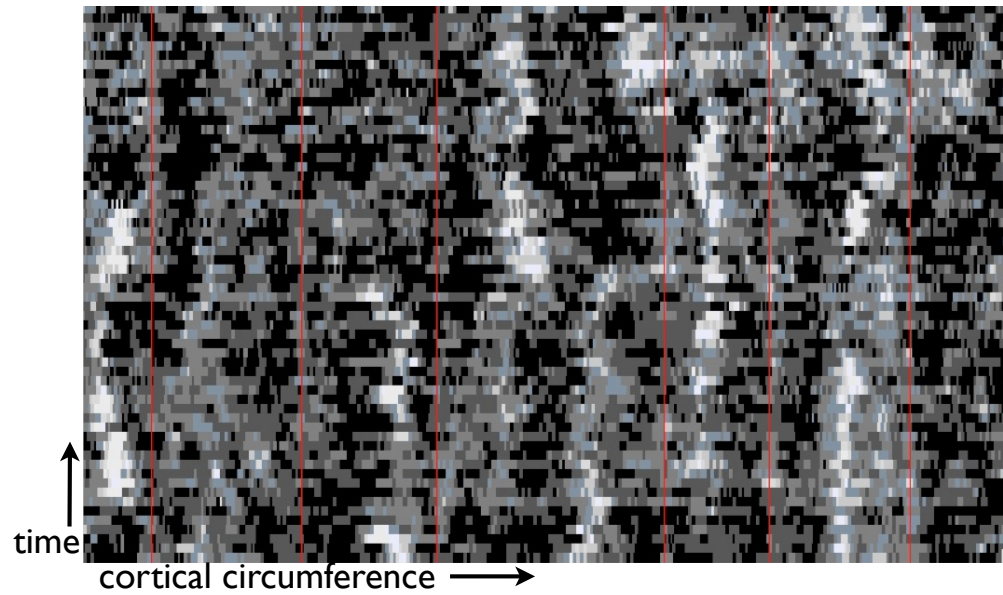
Myosin and **moesin** (actin) fluctuate in tandem



Protein Fluorescence Intensity

Cortical ring fluorescence intensity

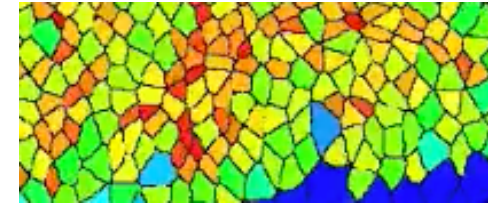
Kymograph



Analysis of Epithelial Morphogenesis

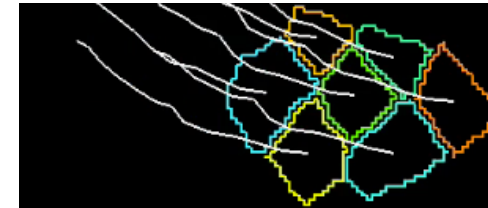
1 Static measures

- Cell shapes
- Expected cell shapes
- Cell centroid arrangement
- Cell-cell interface shapes
- Number of neighbours



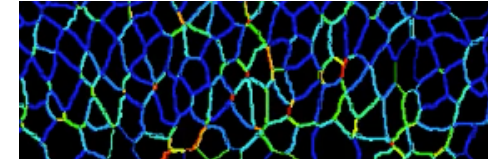
2 Dynamic measures

- Cell-cell interface dynamics
- Small domain deformations
- Impact of cell division
- Measuring fluctuations



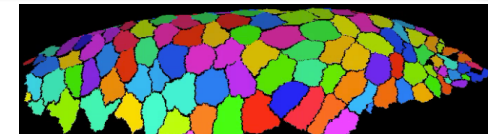
3 Fluorescence intensity

- Quantification of sub-cellular fluorescence intensity
- Medial dynamics
- Polarity of junctional proteins



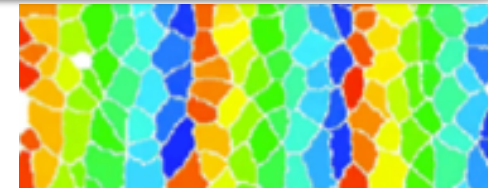
4 Towards 3D

- Epithelial tilt & curvature
- Combining tracking of apical & basal layers

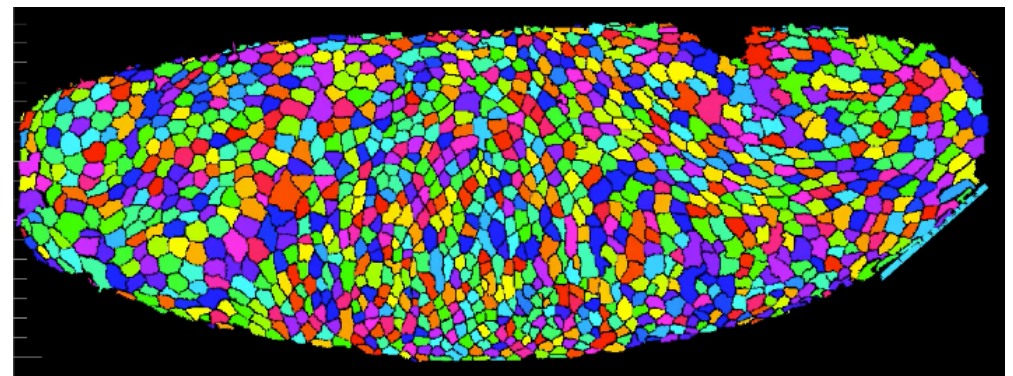


5 Time & space coordinate systems

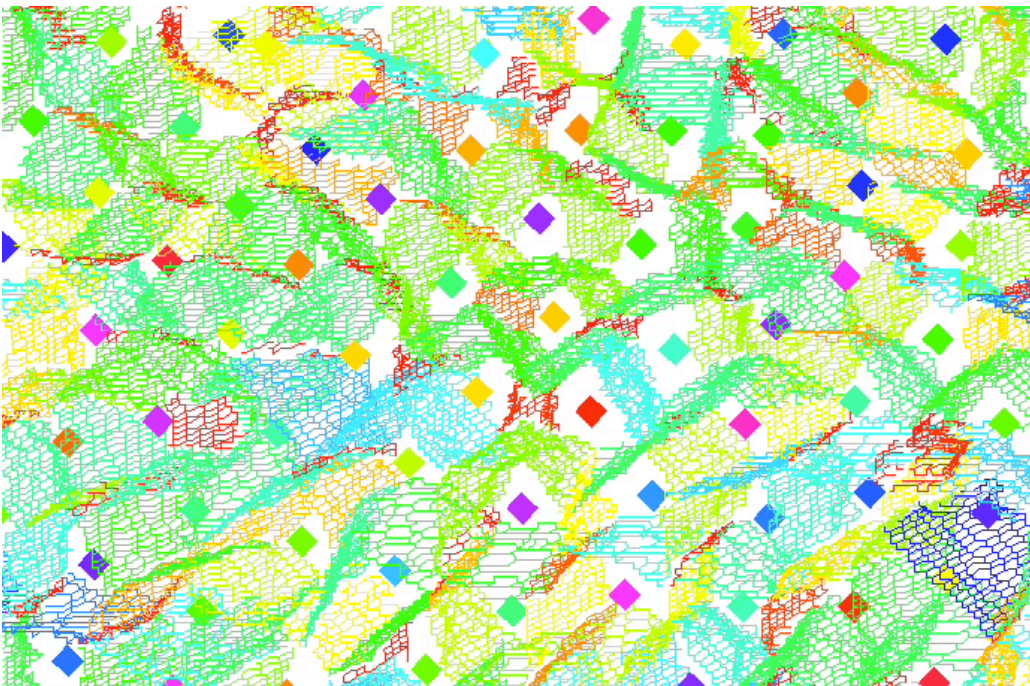
- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries



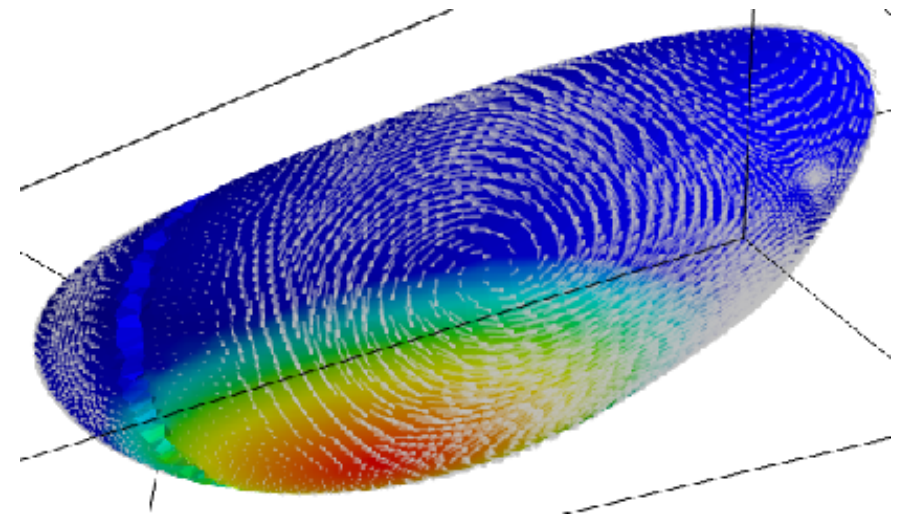
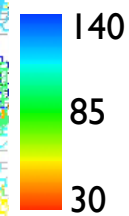
3D and *in toto* tracking & modelling



Claire Lye



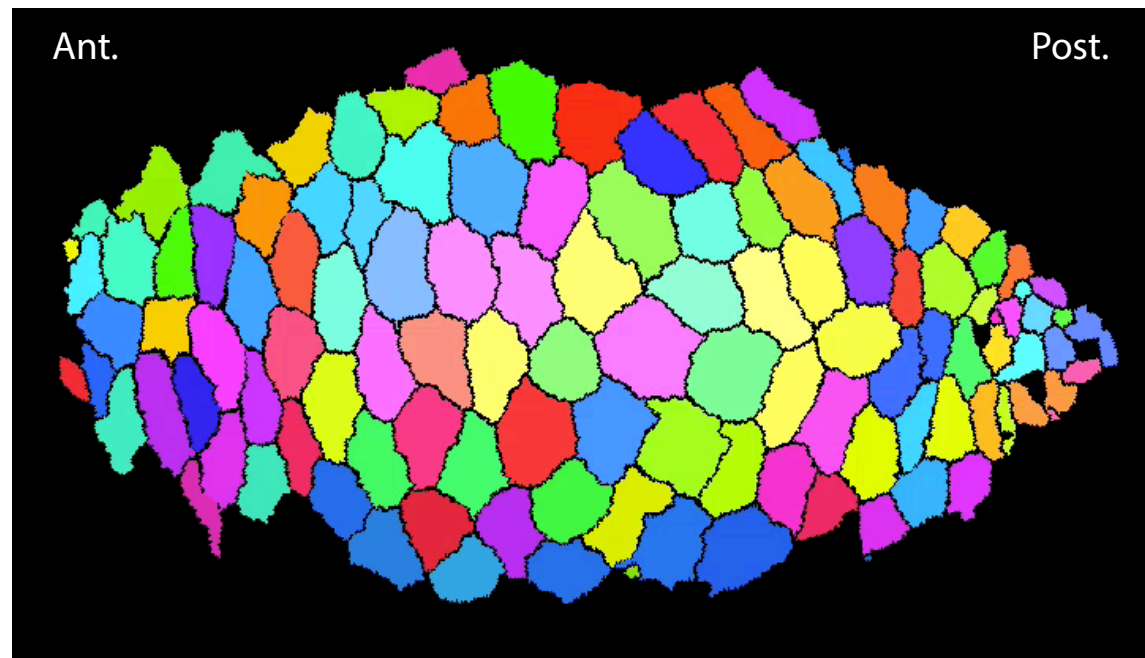
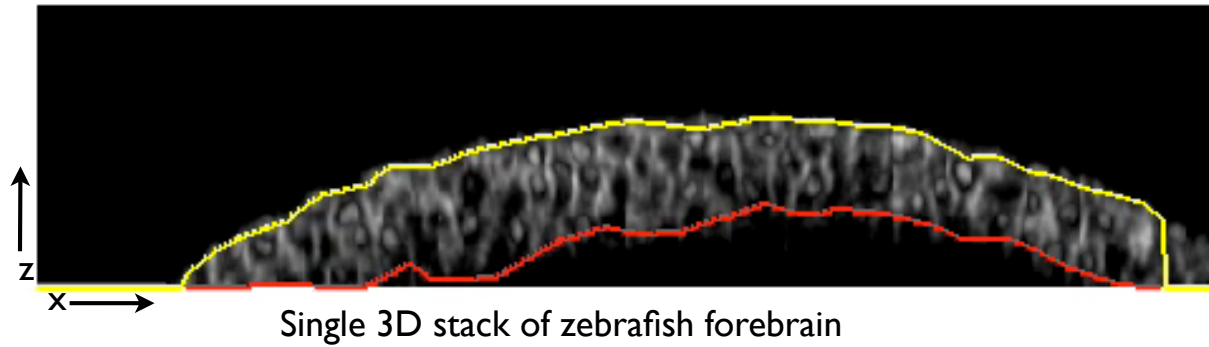
Interface
surface
area (μm^2)



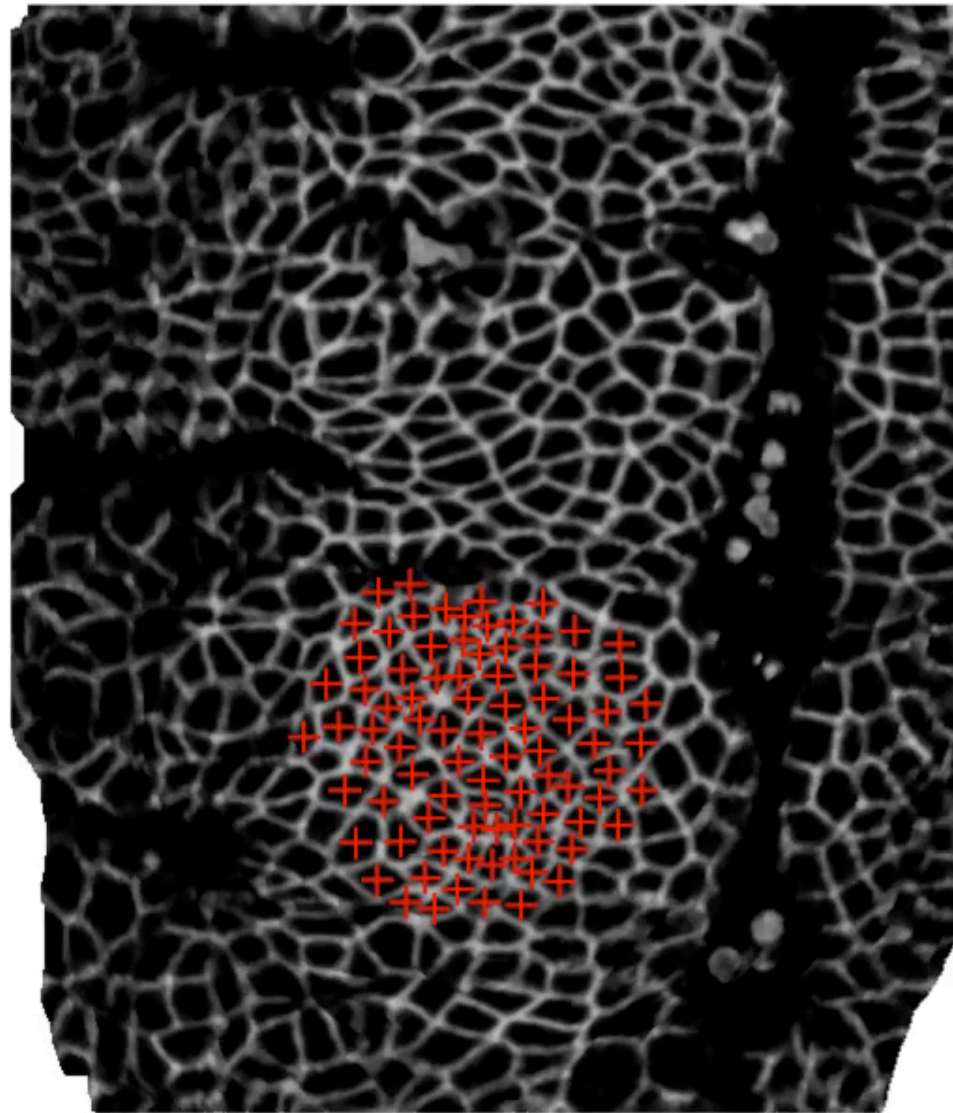
Jocelyn Etienne, Grenoble



Analysis on quasi-2D surfaces



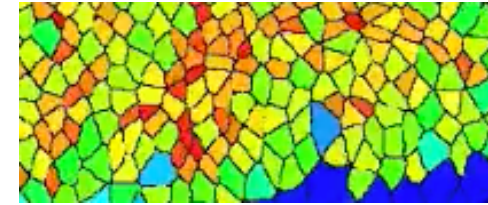
2-layer tracking of salivary placode



Analysis of Epithelial Morphogenesis

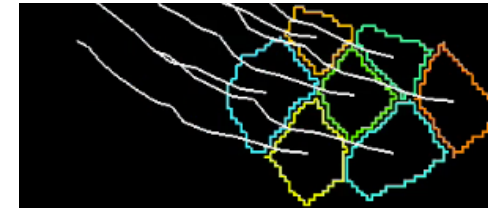
1 Static measures

- Cell shapes
- Expected cell shapes
- Cell centroid arrangement
- Cell-cell interface shapes
- Number of neighbours



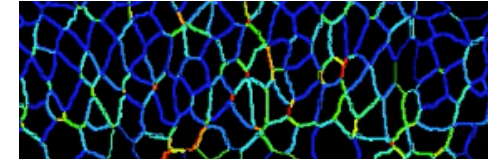
2 Dynamic measures

- Cell-cell interface dynamics
- Small domain deformations
- Impact of cell division
- Measuring fluctuations



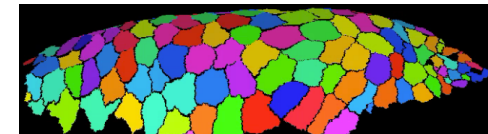
3 Fluorescence intensity

- Quantification of sub-cellular fluorescence intensity
- Medial dynamics
- Polarity of junctional proteins



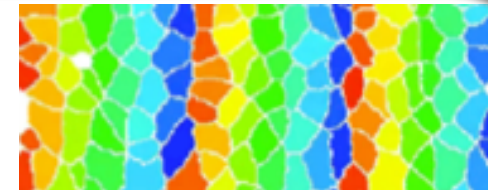
4 Towards 3D

- Epithelial tilt & curvature
- Combining tracking of apical & basal layers

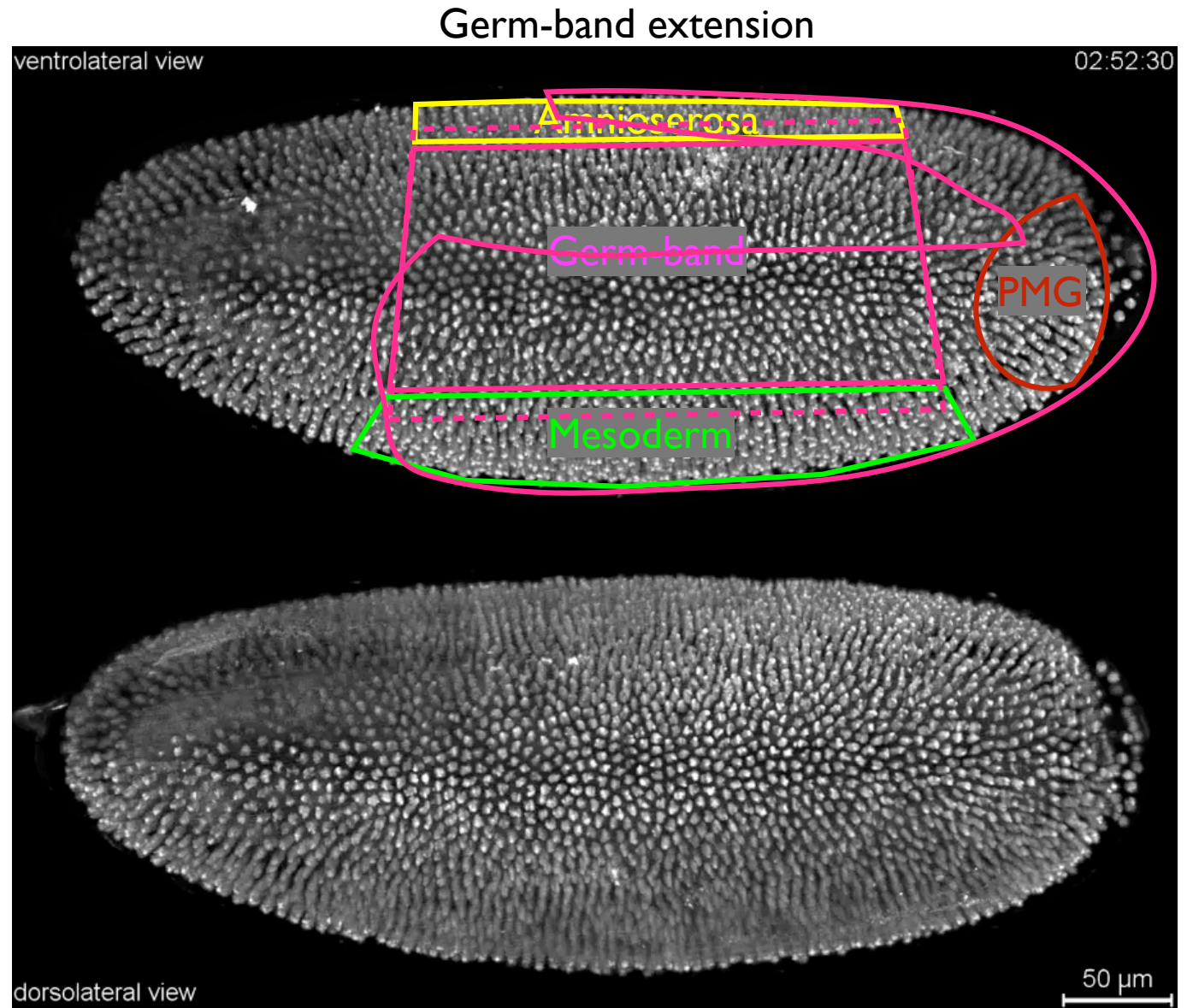
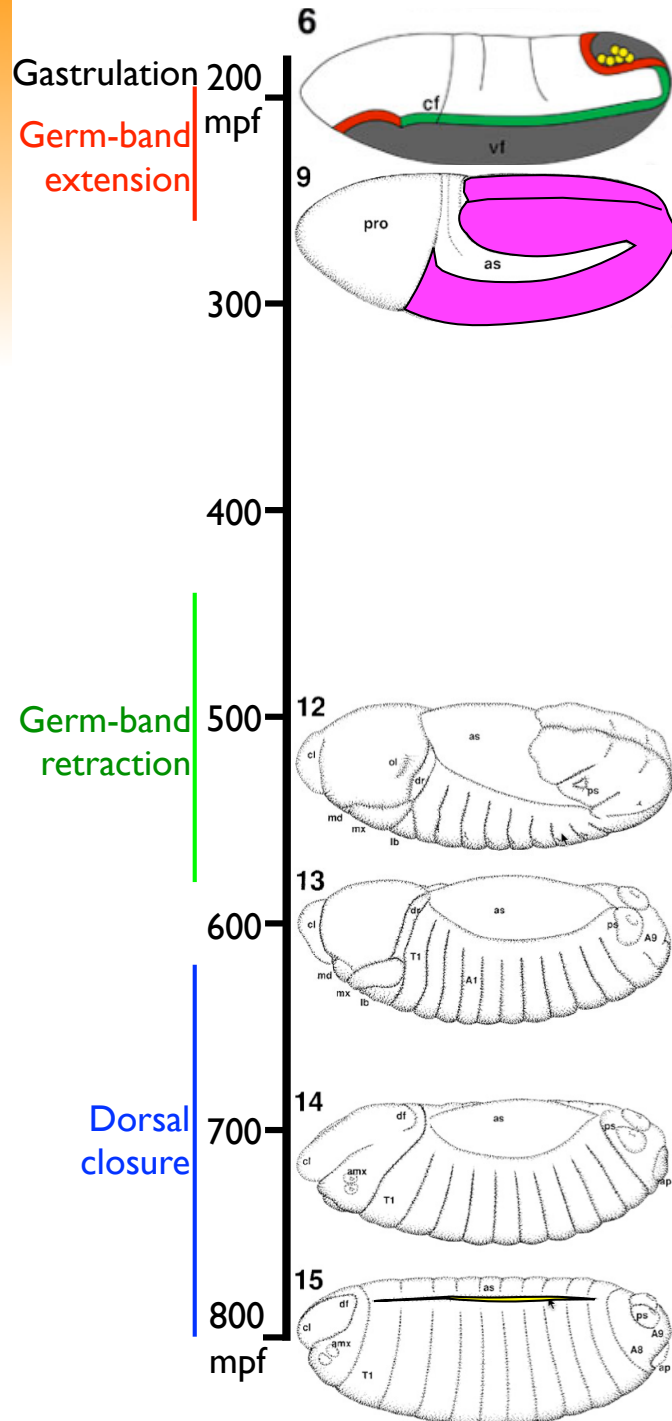


5 Time & space coordinate systems

- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries



Drosophila embryonic morphogenesis

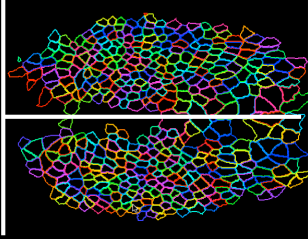
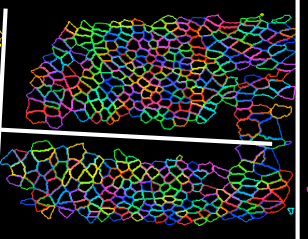
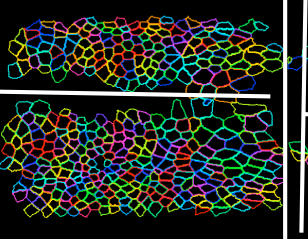
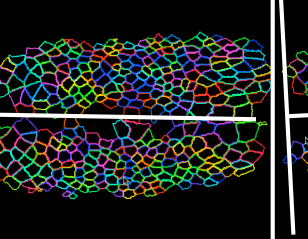
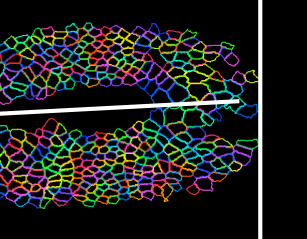
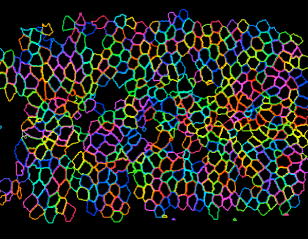
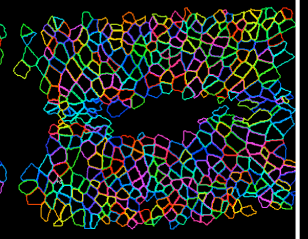
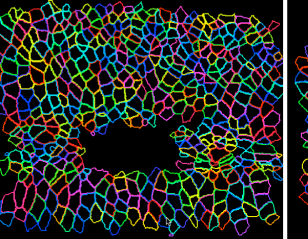
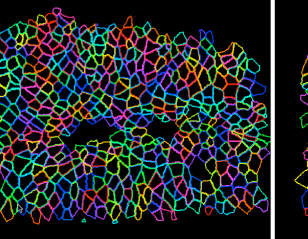
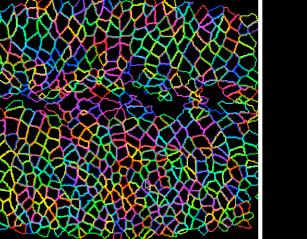


Tomer et al., (2012) *Nat. Methods*



Coordinating space & time in WT and mutant embryos

Drosophila germ-band tracked data sets

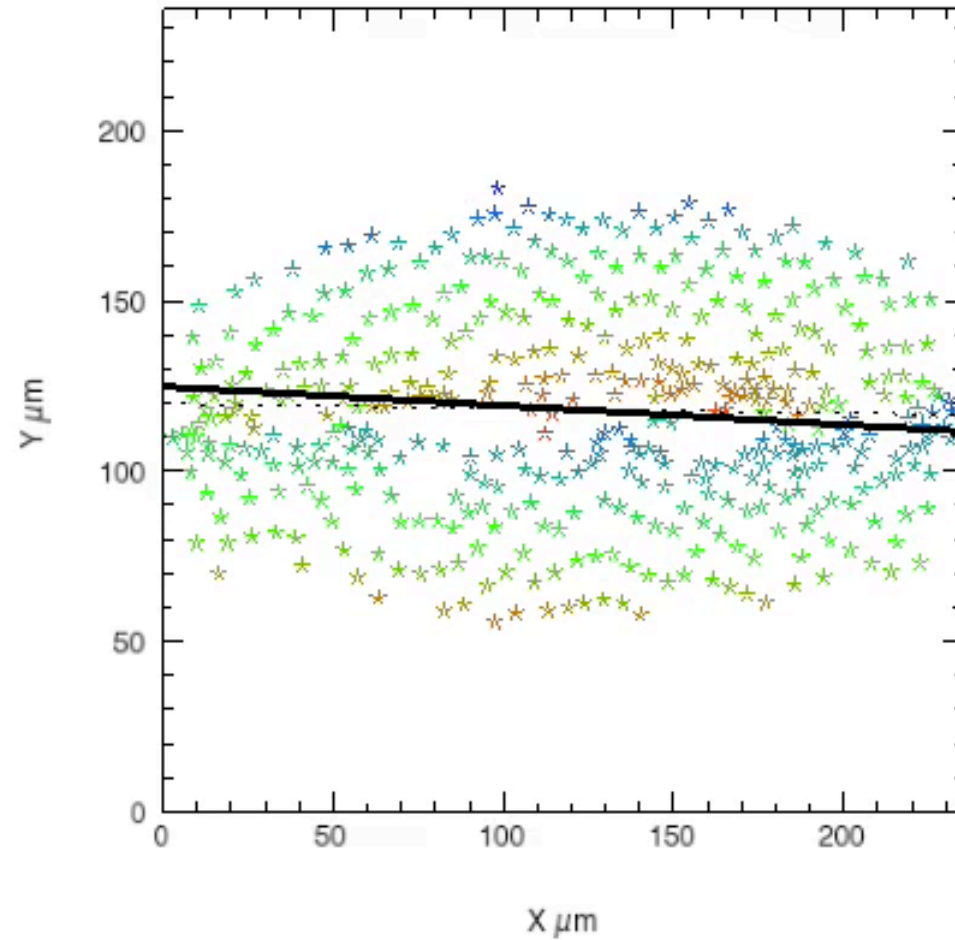
	embryo A	embryo B	embryo C	embryo D	embryo E	Total cell hours (sampled 30s)
wild type	Align embryos by imposing new coordinate system <ul style="list-style-type: none"> • x-axis: distance from AP feature (e.g. cephalic furrow) • y-axis: distance from embryonic mid-line • time: relative to stage (e.g. start of germ-band elongation) 					~2100
Kruppel						~1500
Twist						~2000

All movies cover at least the first 50 minutes of germ-band extension

with Lucy Butler & Benedicte Sanson

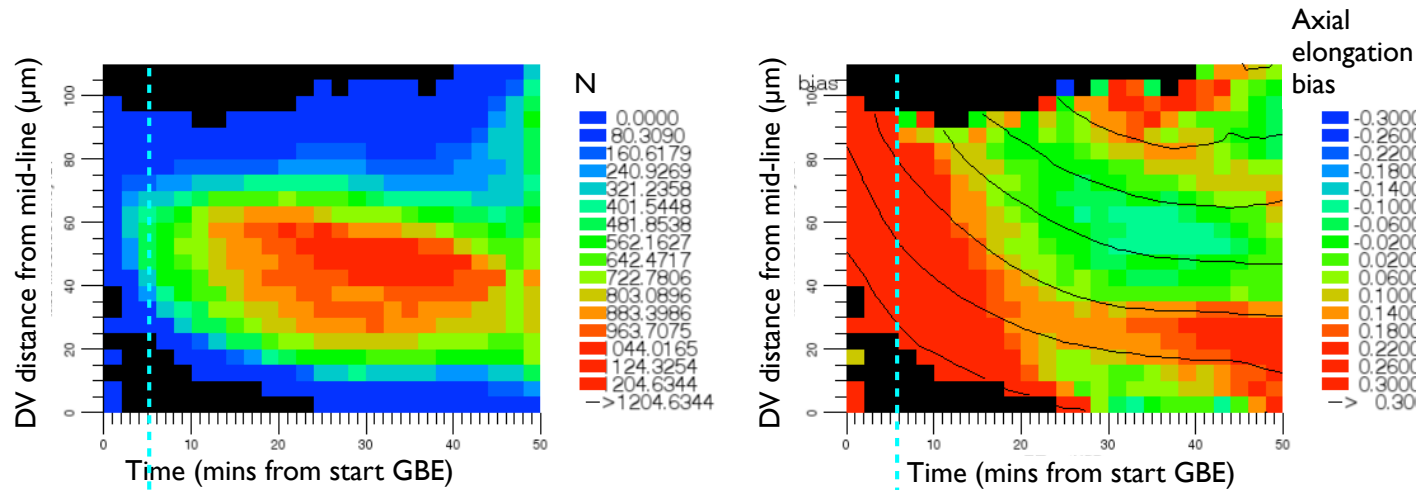
Moving embryonic axes

Midlines vs t (red->blue in time)

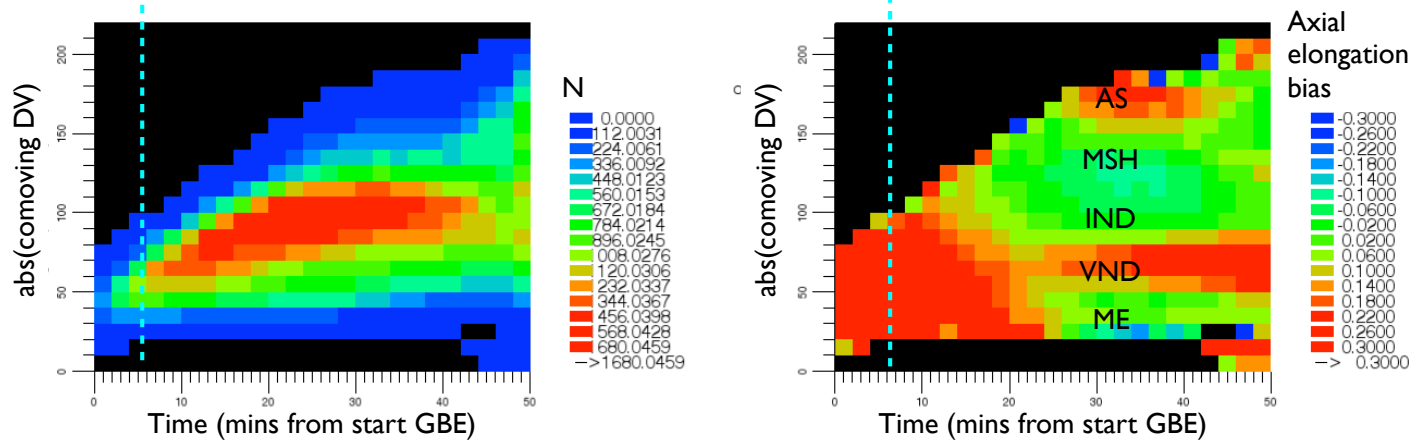


Fixed and comoving coordinate systems

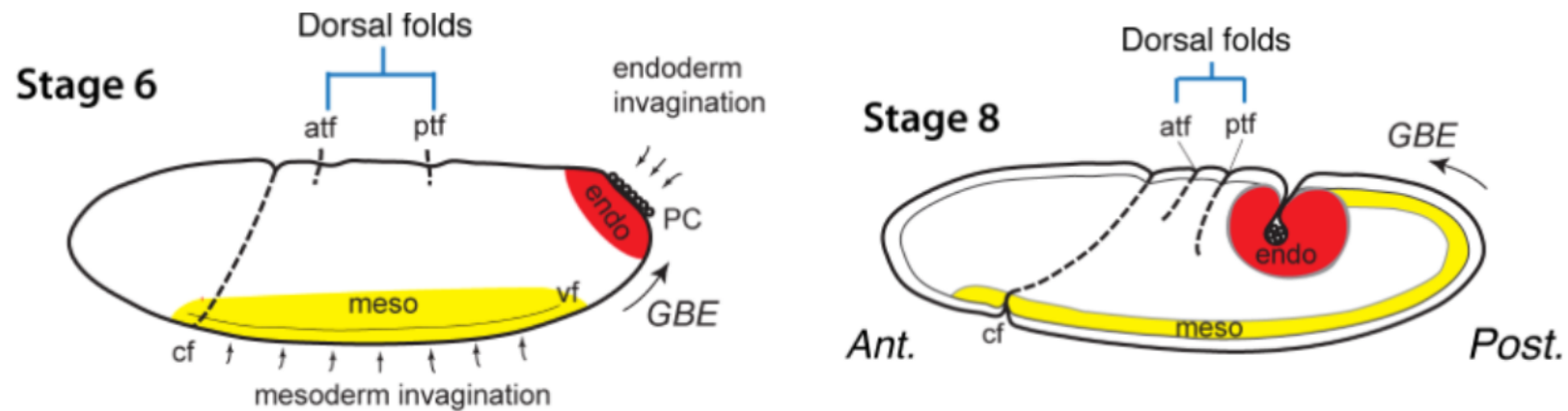
Fixed coordinate system, μm from ventral midline



Comoving coordinate system, 'painted' on at 5 minutes

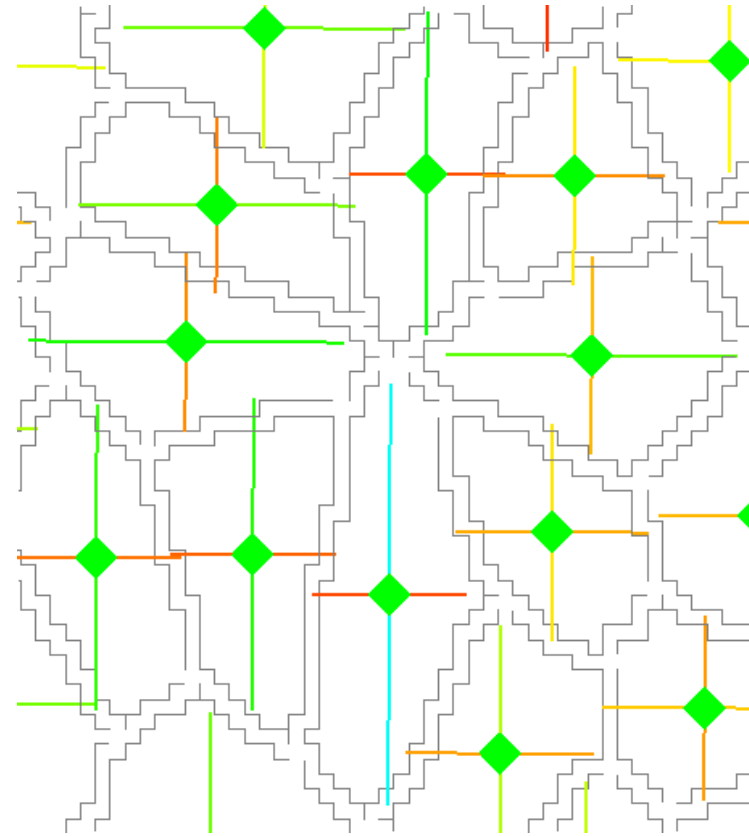
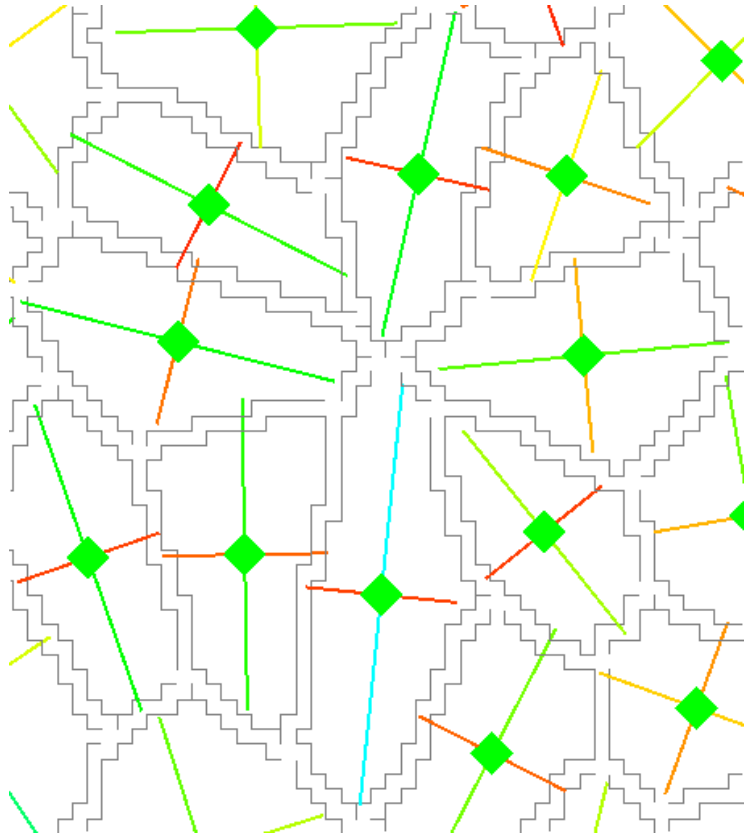


Posterior mid-gut pulls the germ-band

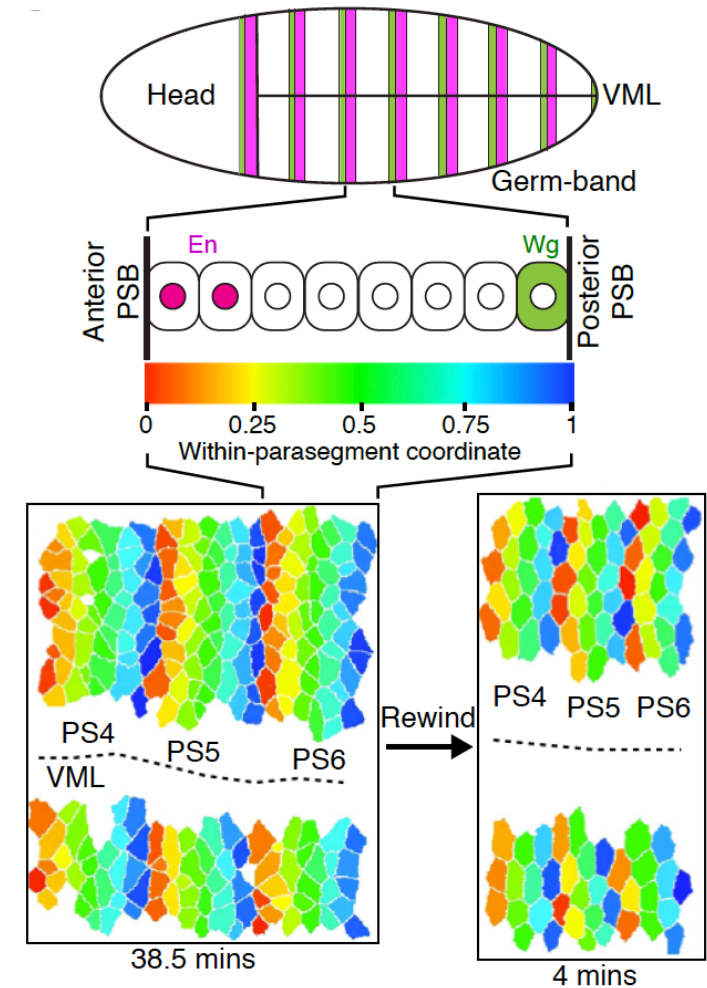
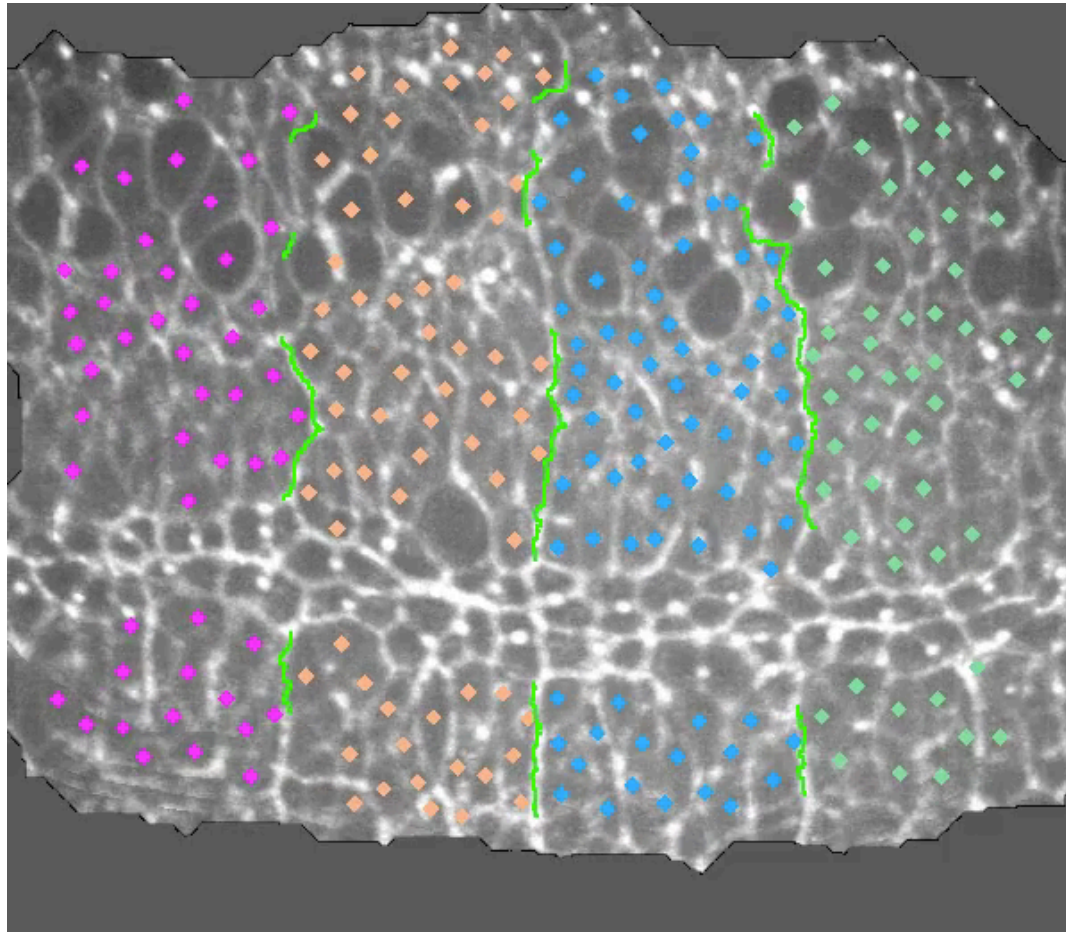


Butler et al., (2009) *Nat. Cell Biol.*; Lye et al., (2015) *PLOS Biology*;
see also Collinet et al., (2015) *Nat. Cell Biol.*

Project 2D or vectorial quantities onto defined axes



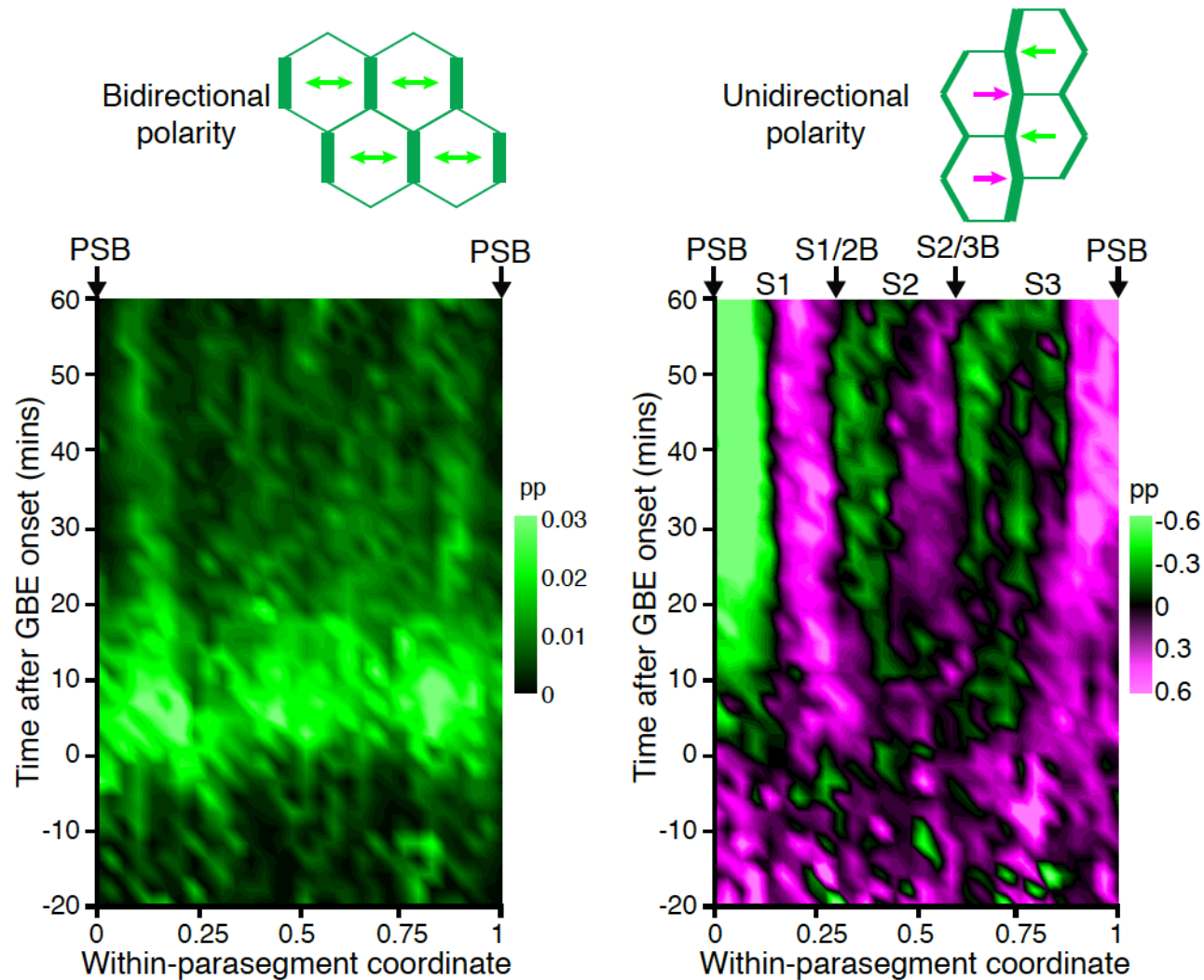
Assigning within-parasegment coordinate system



Define cell parasegment membership by location of PSBs at ends of movies
Back-track PSBs to starts of movies



Within-parasegment patterns



6 embryos, 3-4 parasegments each

Stereotypical within-parasegmental 'cable' locations



WikiTracks

The screenshot shows the WikiTracks website interface. The top navigation bar includes 'Page', 'Discussion', 'Read', 'Edit', 'View history', and a search box. The main content area is titled 'WikiTracks' and is a redirect from the 'Main Page'. Below this, a breadcrumb trail reads: 'Pretacking > Detrend Brightness > AutoTracker > Tracking > Membrane Tracking > TrackAnalysis > Calculating parameters > Depth from shell > WikiTracks'. A 'Contents' table of contents is visible, listing '1 Cell Tracking and Analysis Protocols' with sub-items '1.1 Programs', '1.2 Discussion', and '1.3 Help with editing the wiki'. The main heading is 'Cell Tracking and Analysis Protocols'. The text explains that manual and automated cell tracking is run in 'IDL' (Interactive Data Language) and the 'R' statistical package. It lists three programs: 1. browser (for viewing and screening movies), 2. tracker (for manual tracking), and 3. otracks (for automated tracking). A 'Discussion' section is also present at the bottom.

Example pages from WikiTracks web-site

The screenshot shows the WikiTracks website interface for the 'Depth from shell' page. The top navigation bar is identical to the previous page. The main content area is titled 'Depth from shell' and is a redirect from the 'Main Page'. Below this, a breadcrumb trail reads: 'AutoTracker > Pretacking > Detrend Brightness > AutoTracker > Tracking > Membrane Tracking > TrackAnalysis > Calculating parameters > Depth from shell'. A 'How to calculate 'Depth from shell' parameter' section is visible, followed by a 'Go to 'Processes' page:' section with three steps: 1. Select processes 'Depth from shell', 2. Make sure no rules apply, and 3. Calculate. An 'Output' section follows, explaining that the parameter records the depth of the centroid from the outer surface of the embryo. It also includes a 'Plot->histogram with Parameter selected as 'depth from shell'' section, which shows a histogram of depth from shell for 3D germband tracking. The histogram has a title 'Histogram of depth from shell' and a subtitle 's:FP271010ds: frames 1-105 Time: ~23.00-59.00 MPI N=48350 Mean=6.97 Med=7.42 StDev=4.94'. The x-axis is 'depth from shell' and the y-axis is 'Count'. To the right of the histogram is a 3D scatter plot showing the first frame of the same movie, color-coded by depth from shell, with good cells in green, bad surface cell shards in red (0 µm), and aberrant deep cells in blue (20 µm).

wiki-based resource for sharing tracking and analysis methods

- provides downloads of manual and automated 2- and 3D tracking programs
- downloads of analysis programs
- users can add functionality by writing and adding their own analysis modules
- documentation for all programs
- all users can improve the wiki documentation and contribute to discussions
- currently used and tested by collaborators, to be rolled out for general use



Collaborators & funding

Amnioserosa

- **Pedro Machado**
- **A. Martinez Arias**



- **Nicole Gorfinkiel**
- **Julia Duque**

Biophysics



- **Jocelyn Etienne**

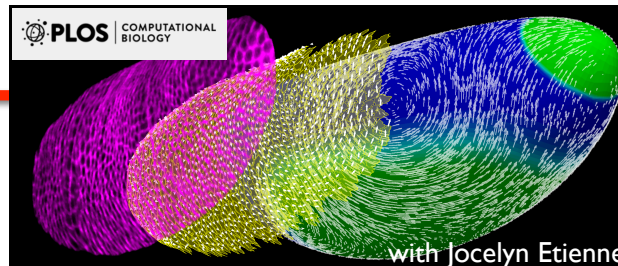
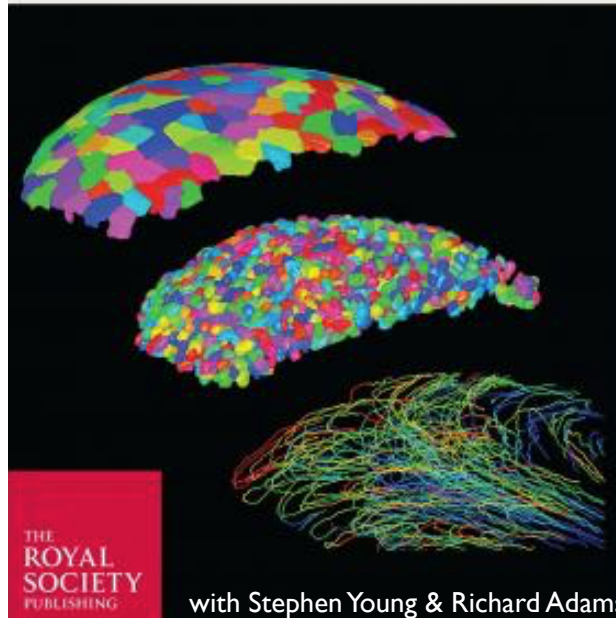
Salivary placode

- **Katja Röper**
- **Alex Booth**
- **Yara Sanchez-Corrales**



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Systems morphodynamics: understanding the development of tissue hardware
Theme issue compiled and edited by Helen Mox, Corinne M. Nelson and Jeremy B.A. Jones



Germ-band extension

- **Bénédicte Sanson**
- **Rob Tetley**
- **Claire Lye**
- **Huw Naylor**
- **Elena Scarpa**
- **Tara Finnegan**
- **Nathan Hervieux**
- **Thomas Sharrock**

Vertex-based modelling

- **Alex Fletcher**



Zebrafish

- **Richard Adams**
- **Nora Schultz**
- **Stephen Young**
- **Alexandre Kabla**
- **Joel Jennings**

