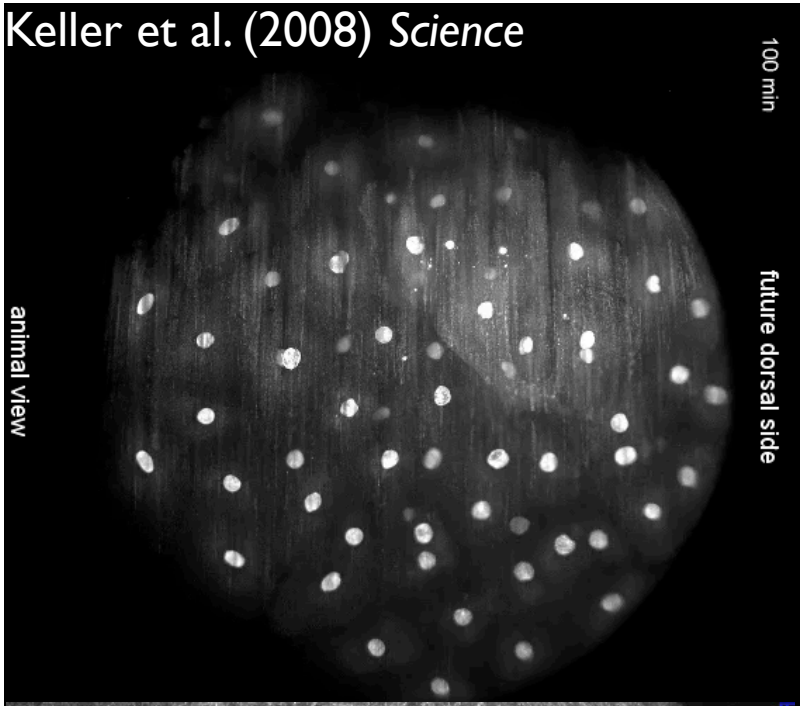
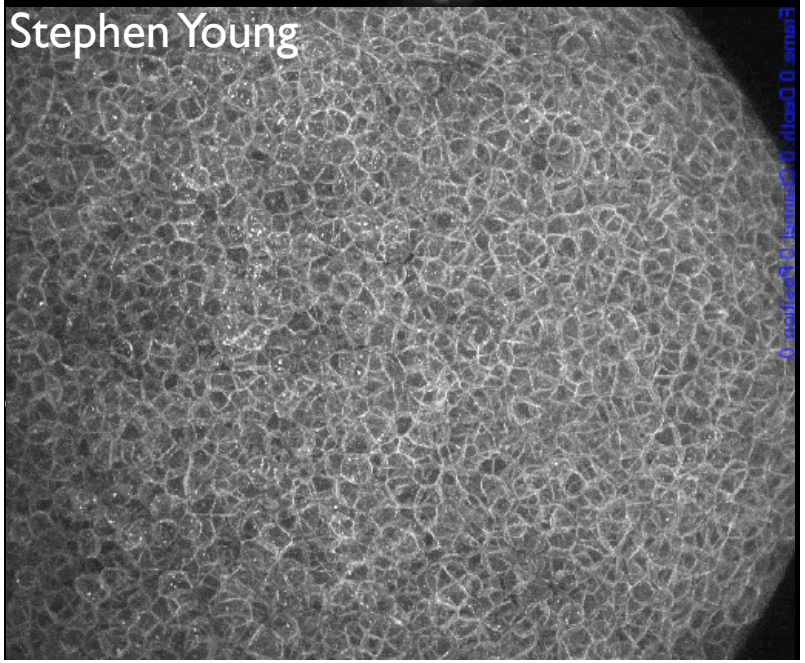
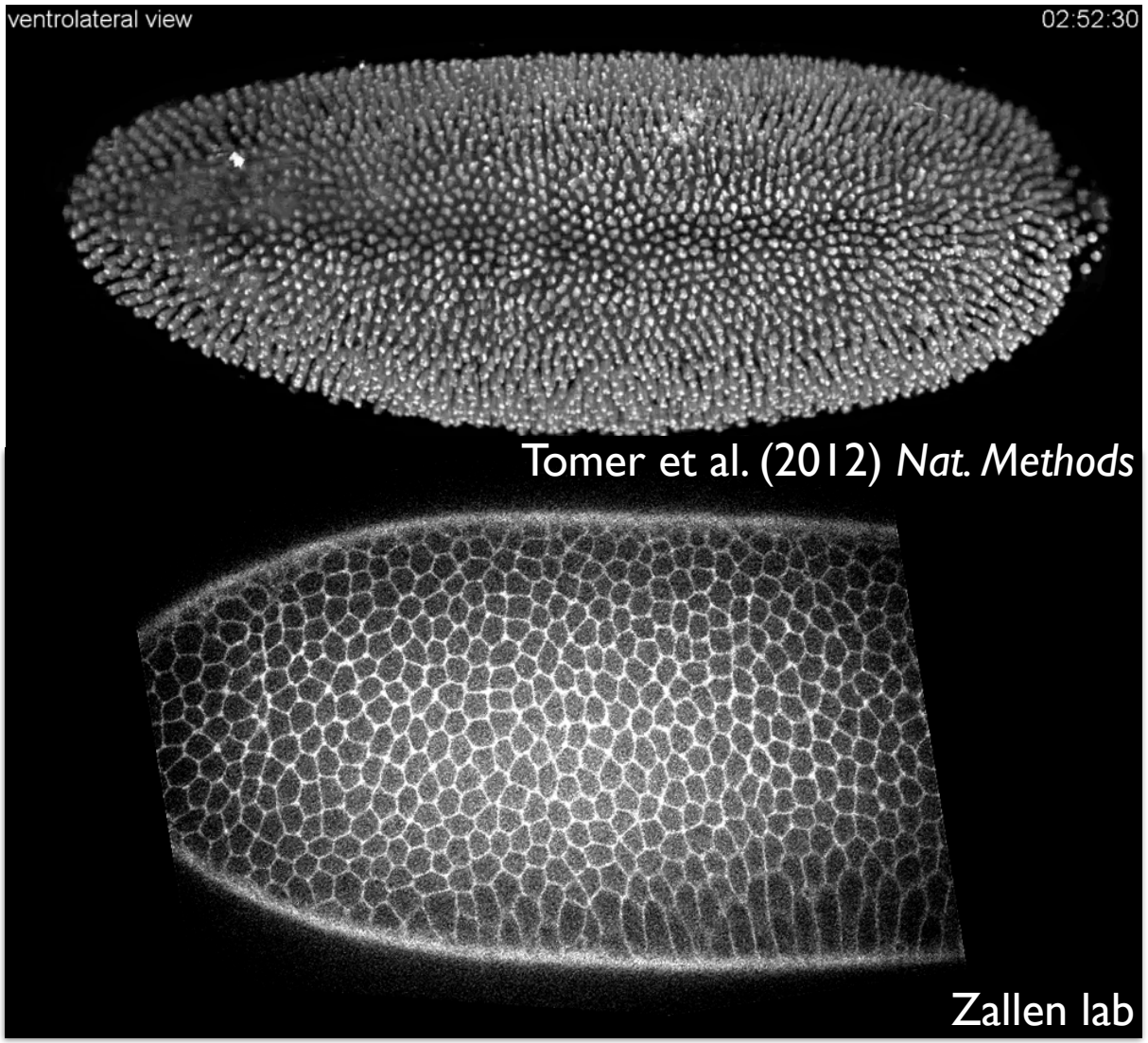
Bio-imaging revolution

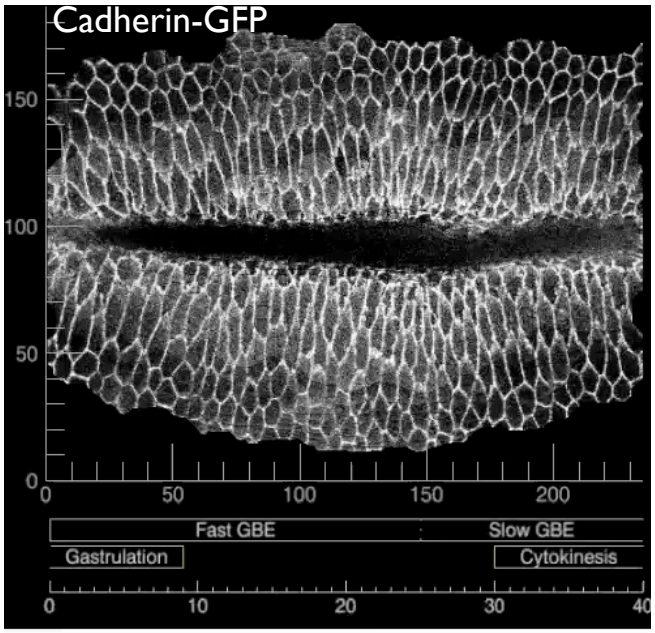


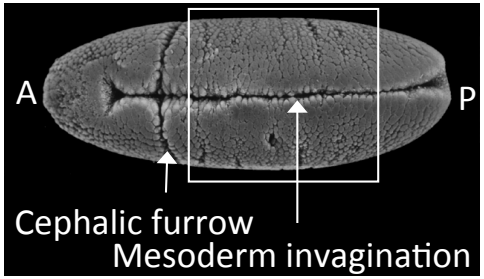


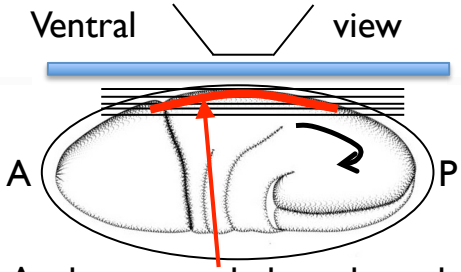


Cell tracking & analysis

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





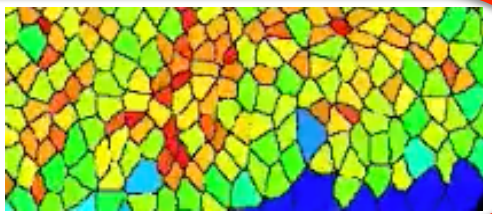


Analyse curved plane through
Adherens Junctions

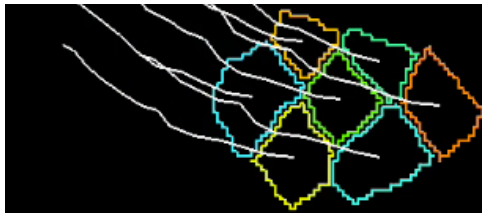
Blanchard et al. (2009) *Nat. Methods*

Analysis of Epithelial Morphogenesis

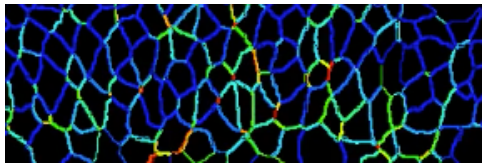
1 Static measures
- Cell shapes
- Expected cell shapes
- Cell-cell interface shapes
- Number of neighbours



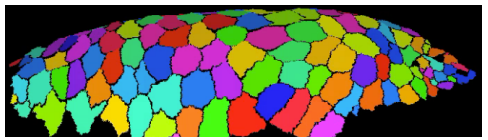
2 Dynamic measures
- Cell-cell interface dynamics
- Small domain deformations
- Impact of cell division
- Measuring fluctuations



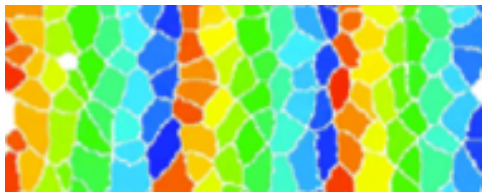
3 Fluorescence intensity
- Quantification of sub-cellular fluorescence intensity
- Medial dynamics
- Polarity of junctional proteins



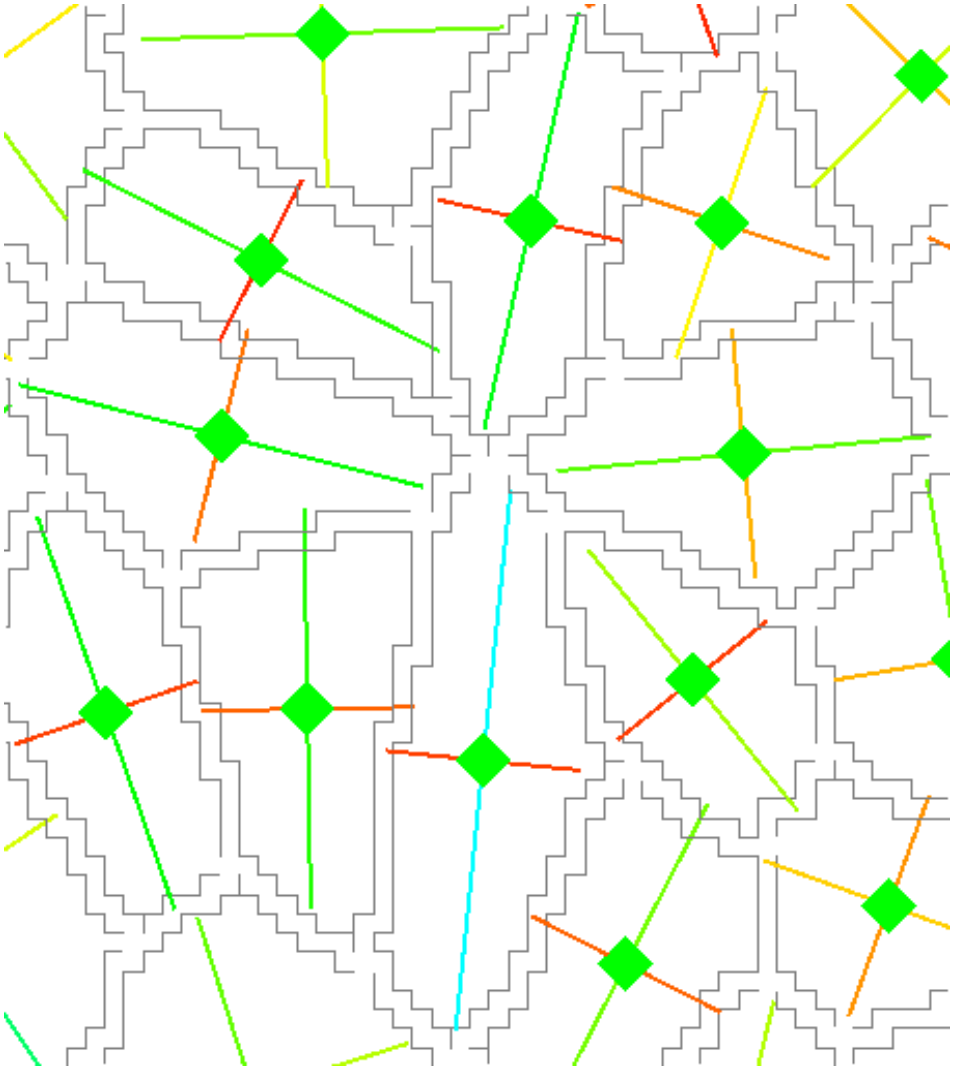
4 Towards 3D
- Epithelial tilt & curvature
- Combining tracking of apical & basal layers



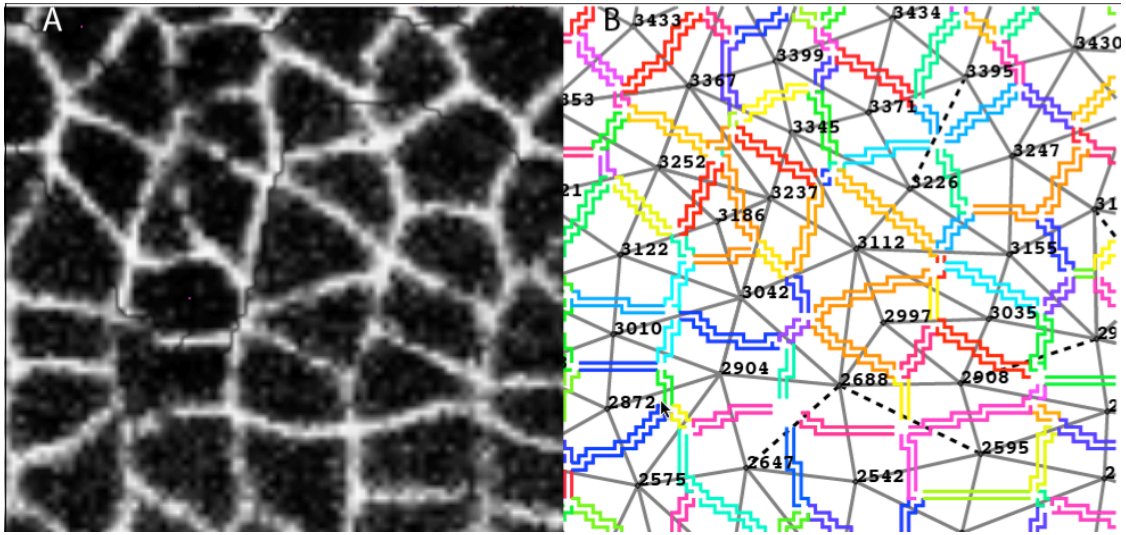
5 Time & space coordinate systems
- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries

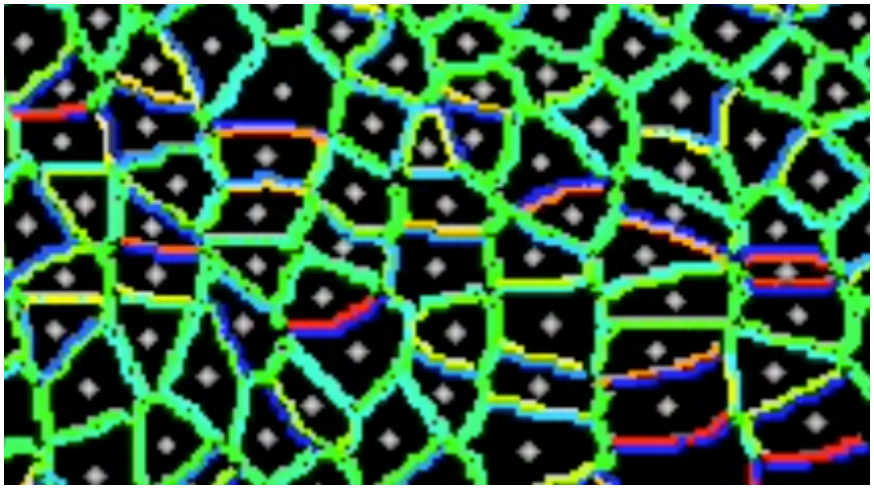


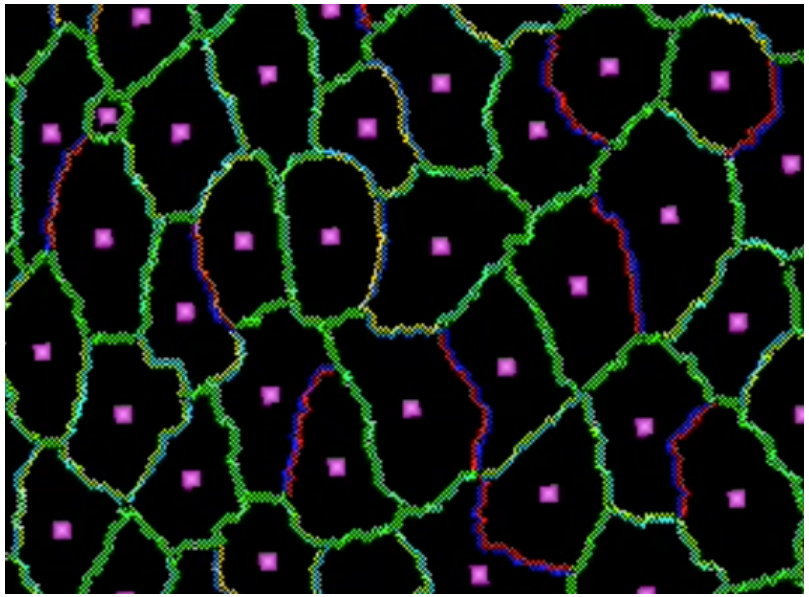
Cell shapes



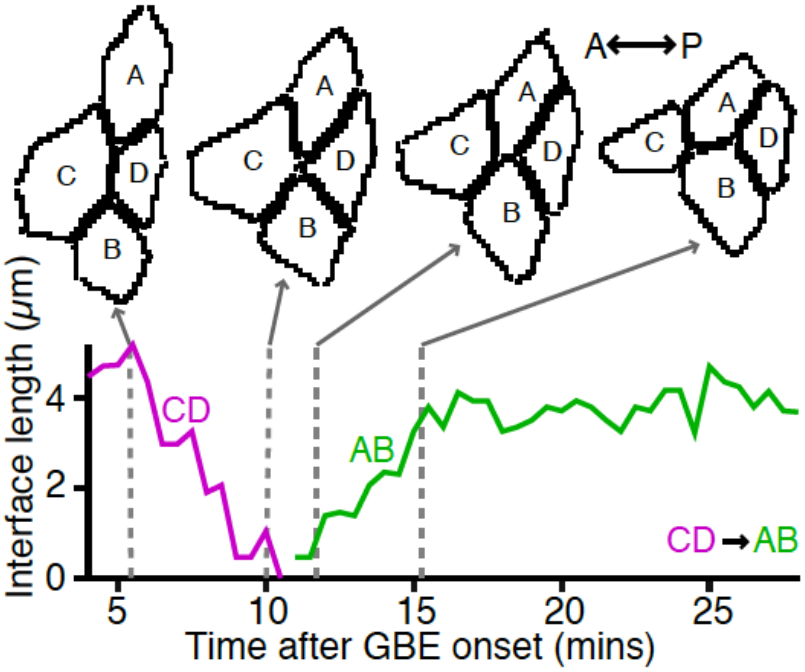
Cell-cell interfaces

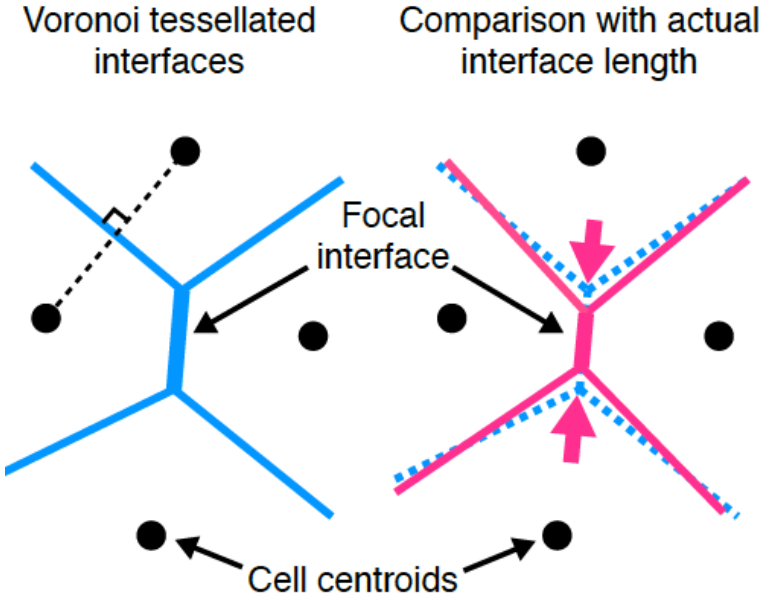


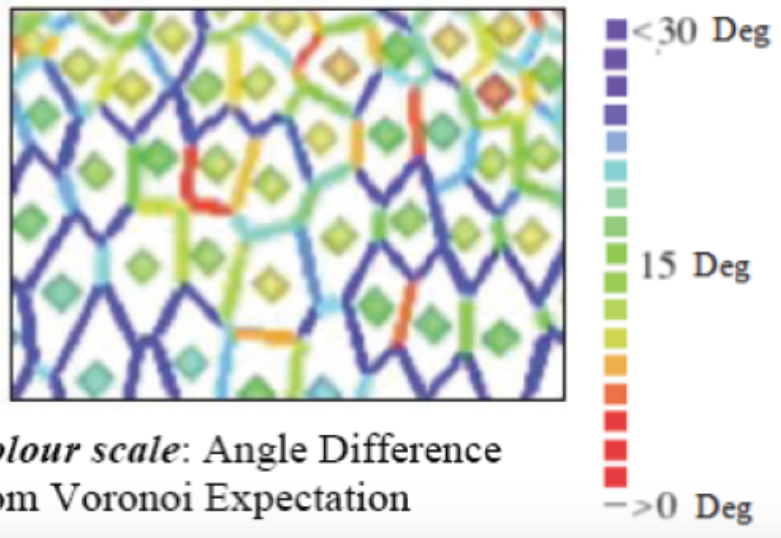


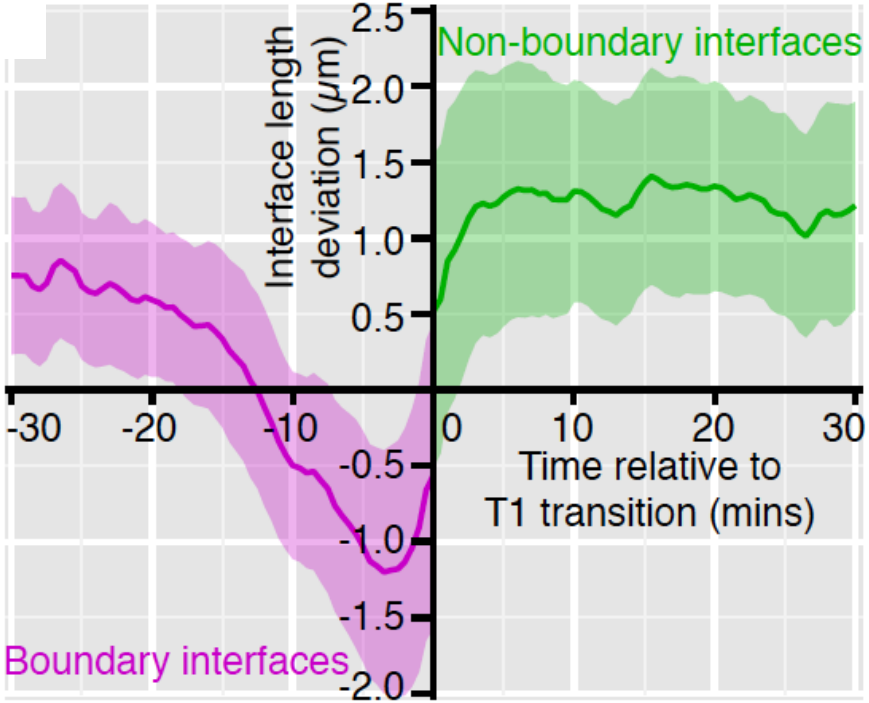


Cell-cell interface geometric stress



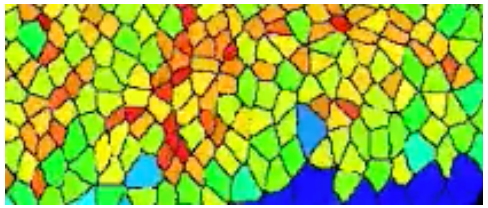




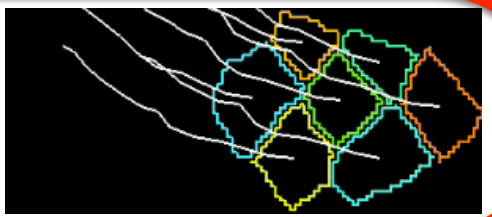


Analysis of Epithelial Morphogenesis

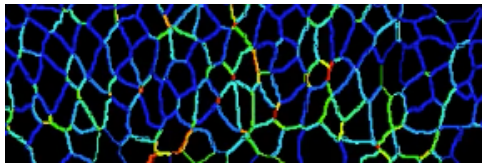
1 Static measures
- Cell shapes
- Expected cell shapes
- Cell centroid arrangement
- Cell-cell interface shapes
- Number of neighbours



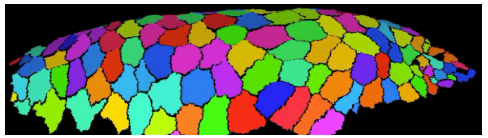
2 Dynamic measures
- Cell-cell interface dynamics
- Small domain deformations
- Impact of cell division
- Measuring fluctuations



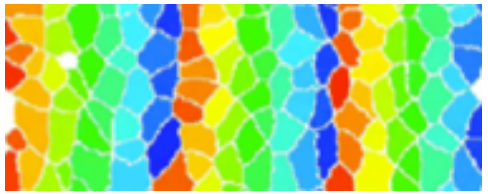
3 Fluorescence intensity
- Quantification of sub-cellular fluorescence intensity
- Medial dynamics
- Polarity of junctional proteins



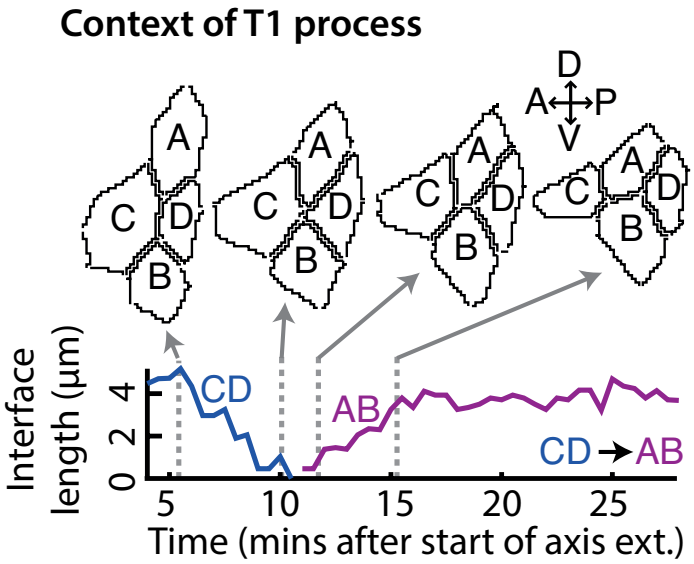
4 Towards 3D
- Epithelial tilt & curvature
- Combining tracking of apical & basal layers

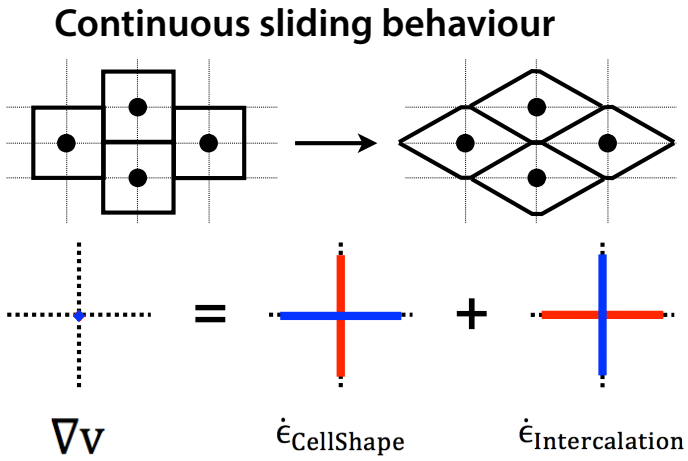


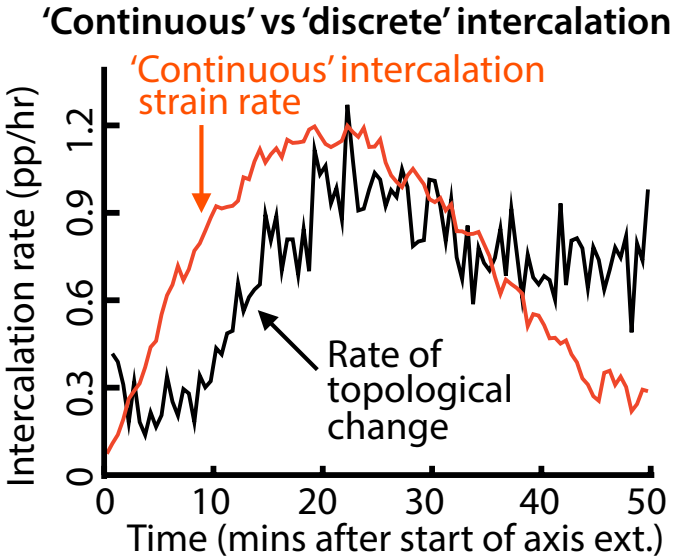
5 Time & space coordinate systems
- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries



Cell-cell interface dynamics

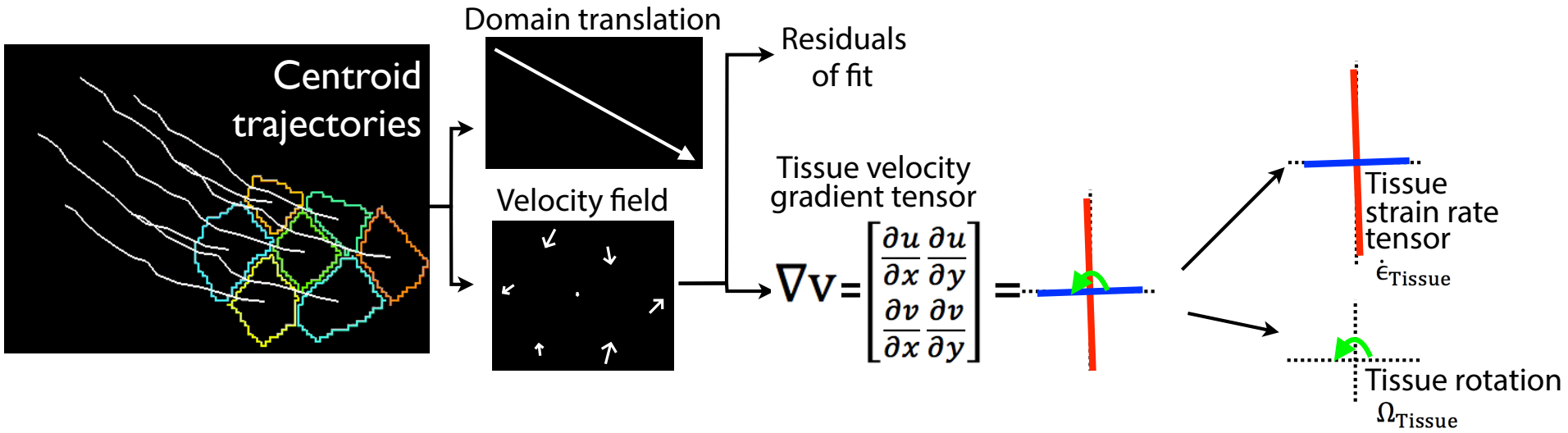






Blanchard (2017) *Phil. Trans. R. Soc. B*

Small domain deformation rates

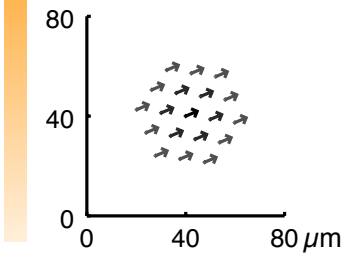


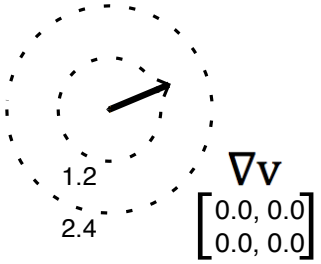
Strain rate:
- expansion
- contraction
- rotation

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

Tissue tectonics

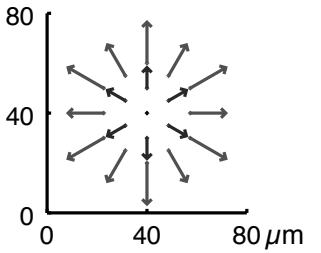
Translation

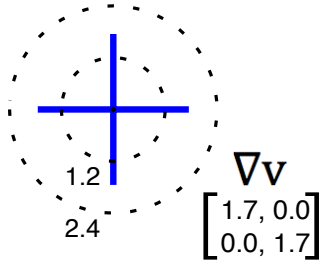




$$\nabla v = \begin{bmatrix} 0.0, & 0.0 \\ 0.0, & 0.0 \end{bmatrix}$$

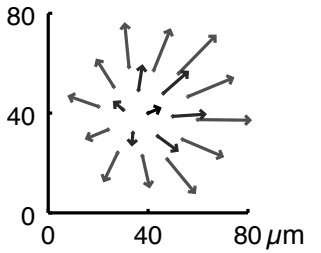
Dilation

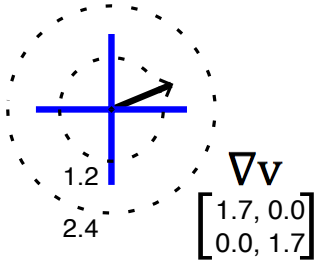




$$\nabla v = \begin{bmatrix} 1.7, & 0.0 \\ 0.0, & 1.7 \end{bmatrix}$$

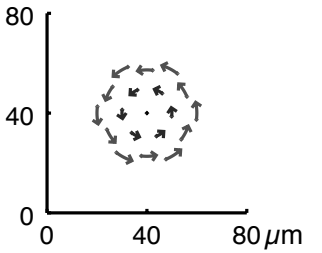
Dilation + translation

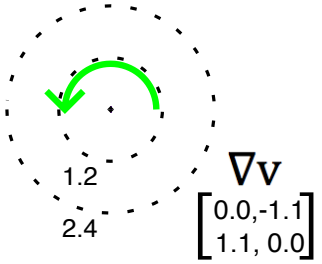




$$\nabla v = \begin{bmatrix} 1.7, & 0.0 \\ 0.0, & 1.7 \end{bmatrix}$$

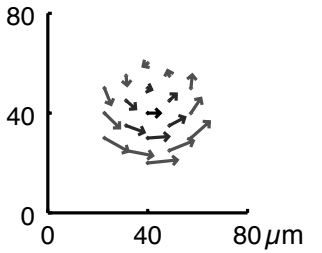
Rotation

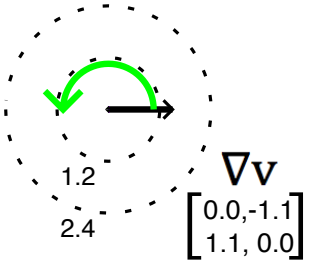




$$\nabla v = \begin{bmatrix} 0.0, & -1.1 \\ 1.1, & 0.0 \end{bmatrix}$$

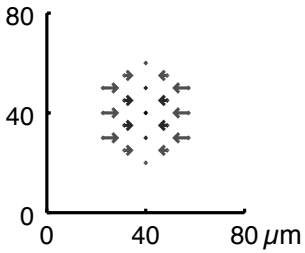
Rotation + translation

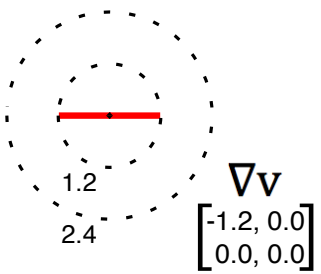




$$\nabla v = \begin{bmatrix} 0.0, & -1.1 \\ 1.1, & 0.0 \end{bmatrix}$$

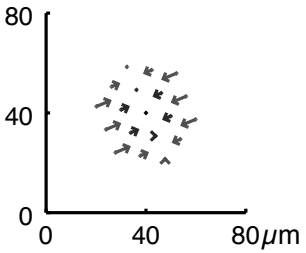
Convergence

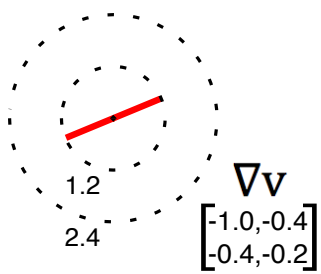




$$\nabla v = \begin{bmatrix} -1.2, & 0.0 \\ 0.0, & 0.0 \end{bmatrix}$$

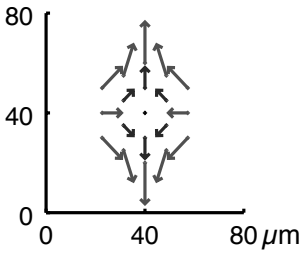
Oblique convergence

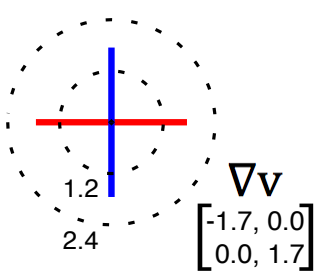




$$\nabla v = \begin{bmatrix} -1.0, & -0.4 \\ -0.4, & -0.2 \end{bmatrix}$$

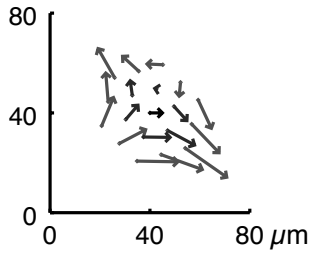
Pure shear (conv. extension)

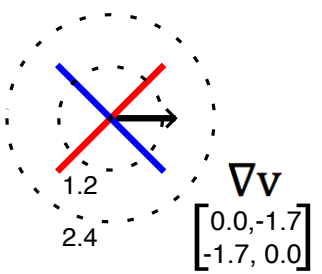




$$\nabla v = \begin{bmatrix} -1.7, & 0.0 \\ 0.0, & 1.7 \end{bmatrix}$$

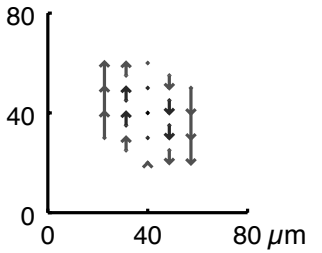
Oblique pure shear + translation

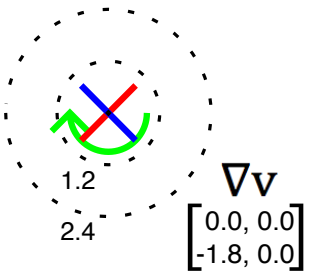




$$\nabla v = \begin{bmatrix} 0.0, & -1.7 \\ -1.7, & 0.0 \end{bmatrix}$$

Simple shear





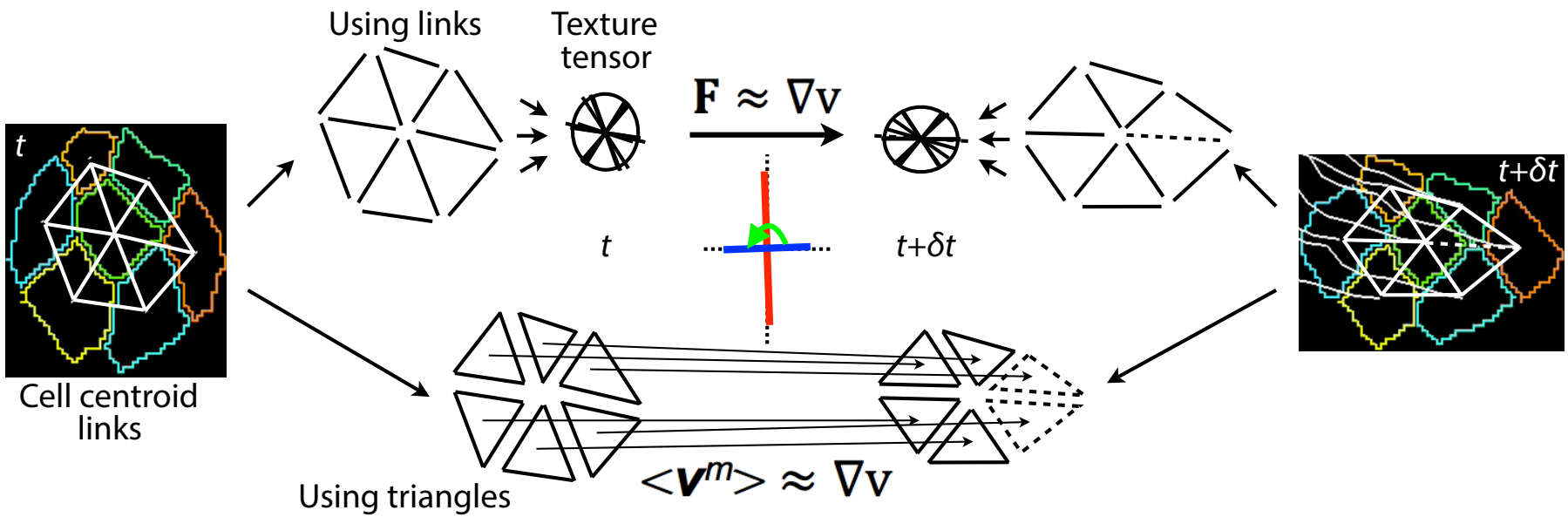
$$\nabla v = \begin{bmatrix} 0.0, & 0.0 \\ -1.8, & 0.0 \end{bmatrix}$$

translation velocity (μm/6 mins)

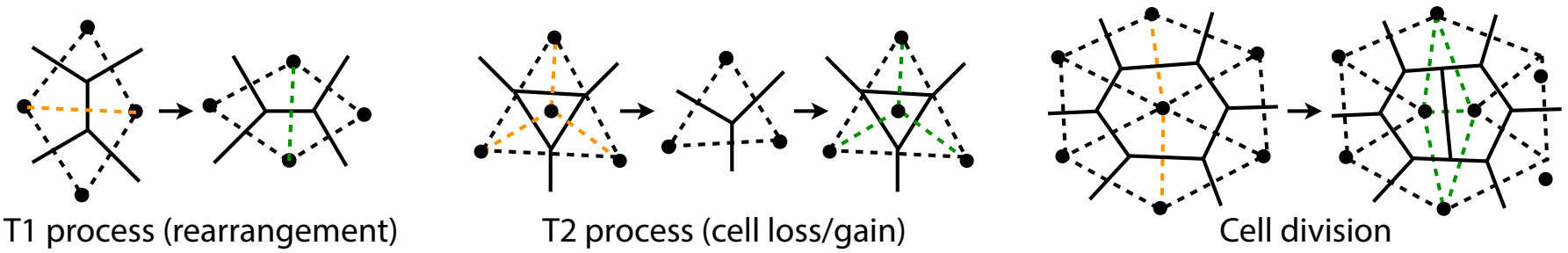
expansion (pp/hr) contraction (pp/hr) rotation (radians/hr)

‘Discrete’ strain rate methods

Using links and triangles to calculate ∇v



‘Discrete’ cell behaviours defined by topological changes



Guirao et al., (2015) *eLife*; Etournay et al., (2015, 2016) *eLife*; Blanchard (2017) *Phil.Trans. R. Soc. B*

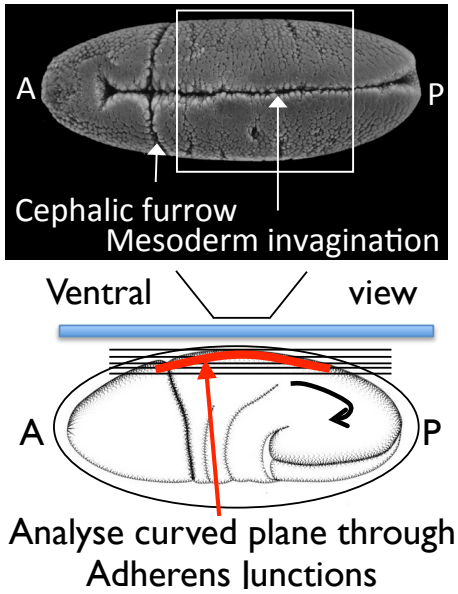
Kinematic maps

Extension rate

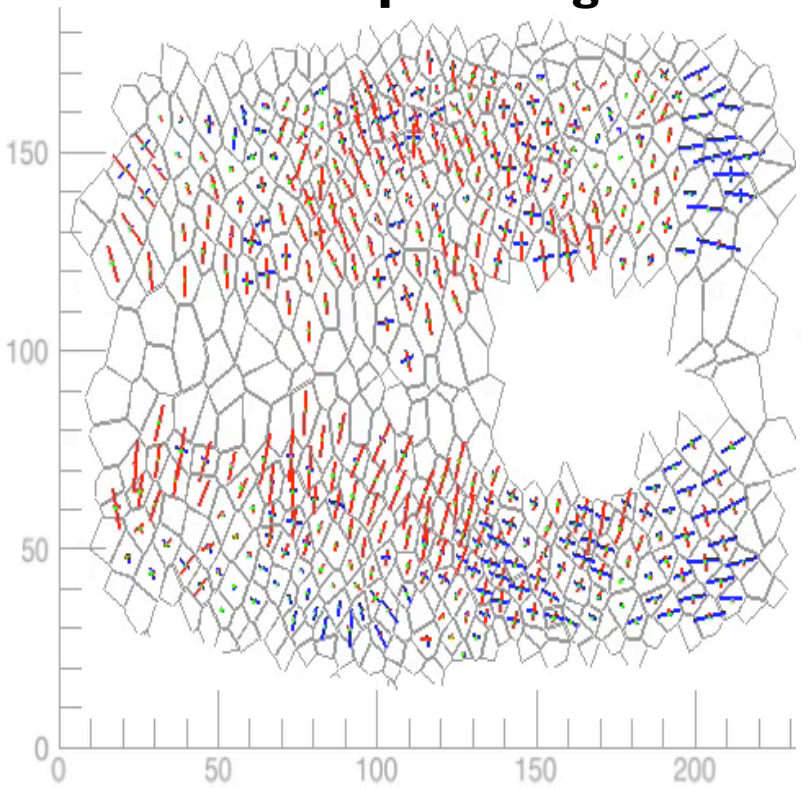
0.04 (pp/min)

Convergence rate

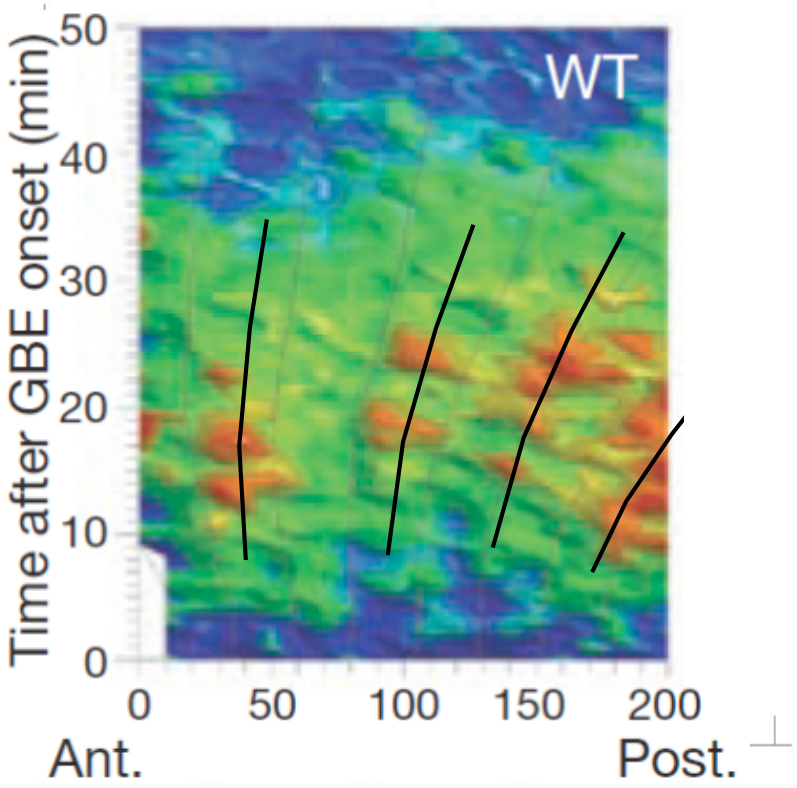
-0.04 (pp/min)



Cell shape change

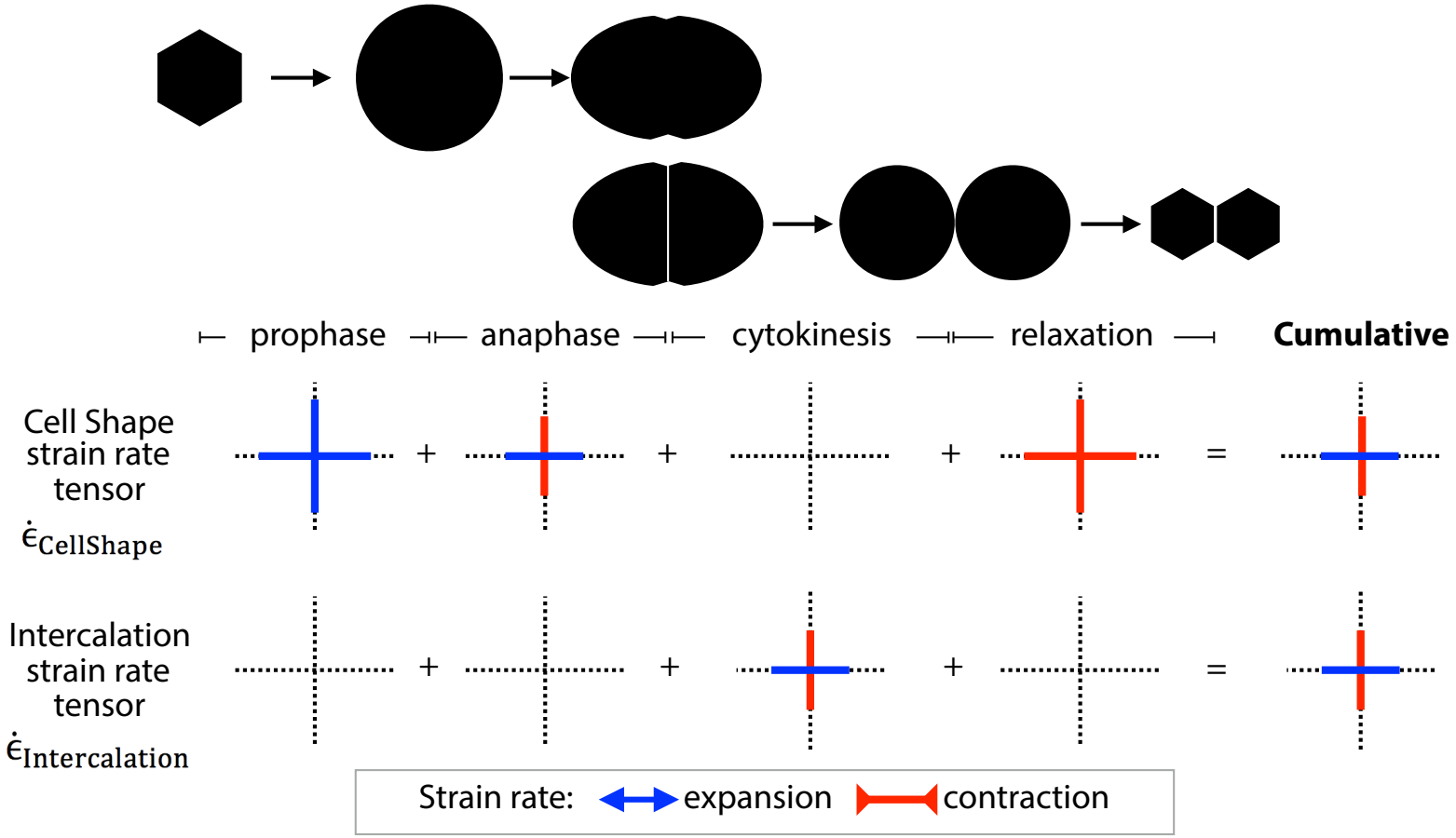


Cell intercalation

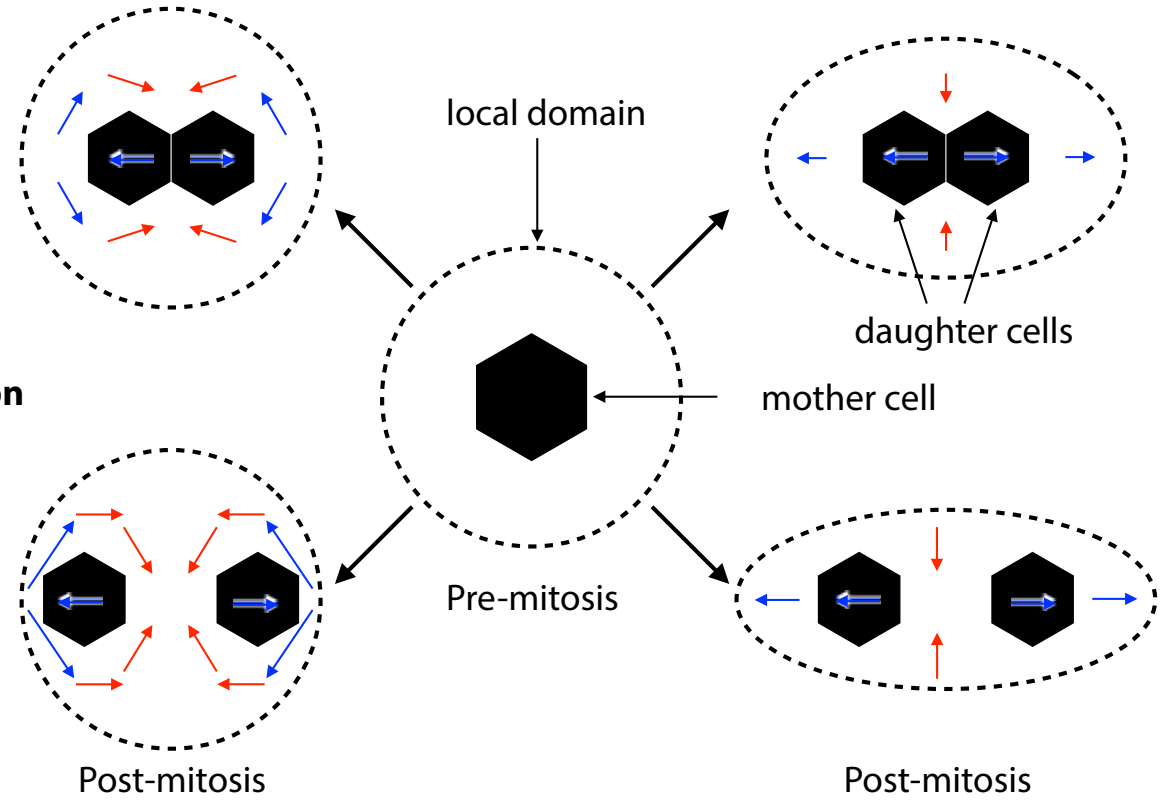


Butler et al. (2009) *Nat. Cell Biol.*

Net strain rates for dividing cell



Local impact of cell division



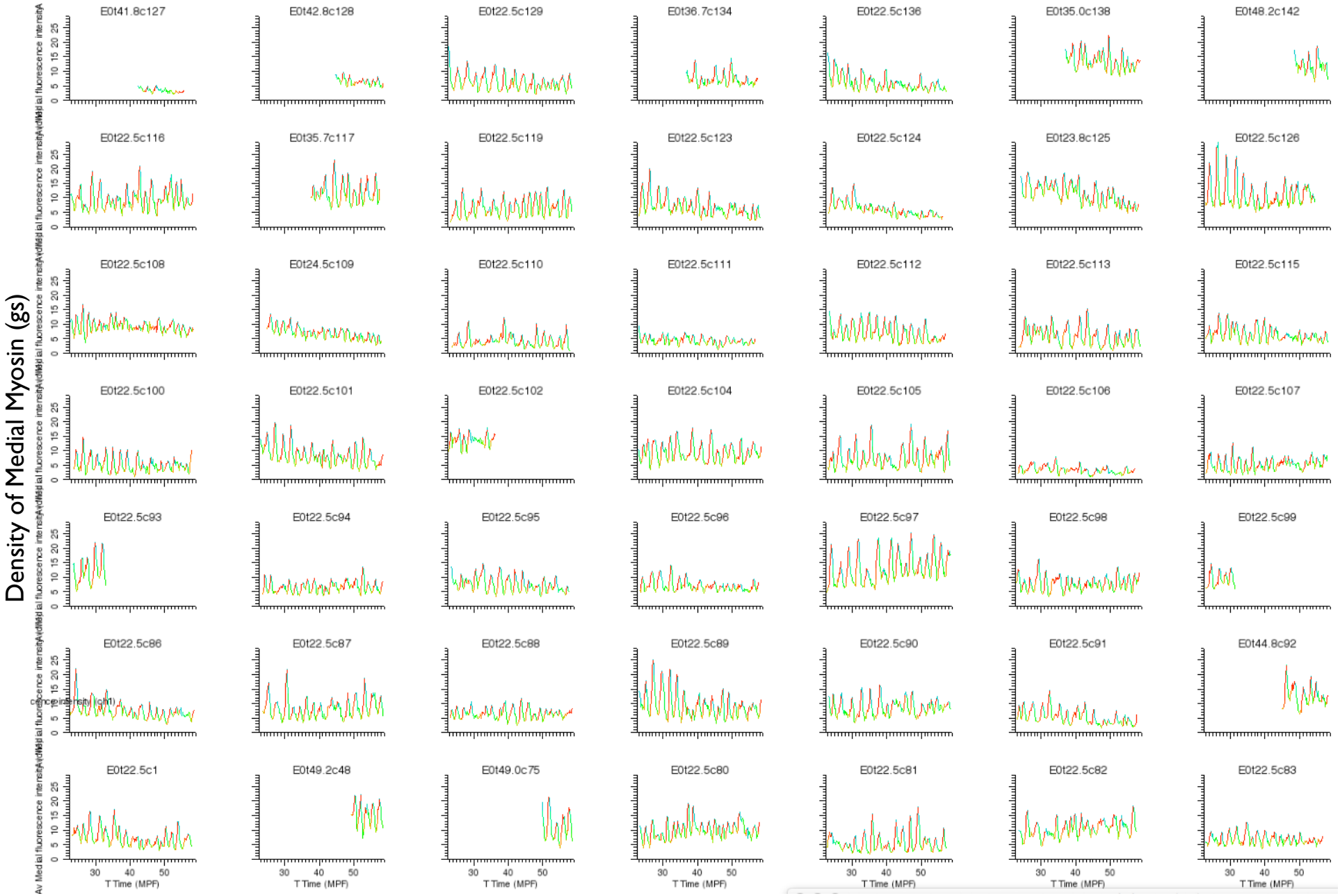
Domain absorbs cell division

Domain reflects cell divisio

+ Surroundings squash dividing cell (surroundings stiffer than mitosis)

Cell area & Myosin II fluctuations

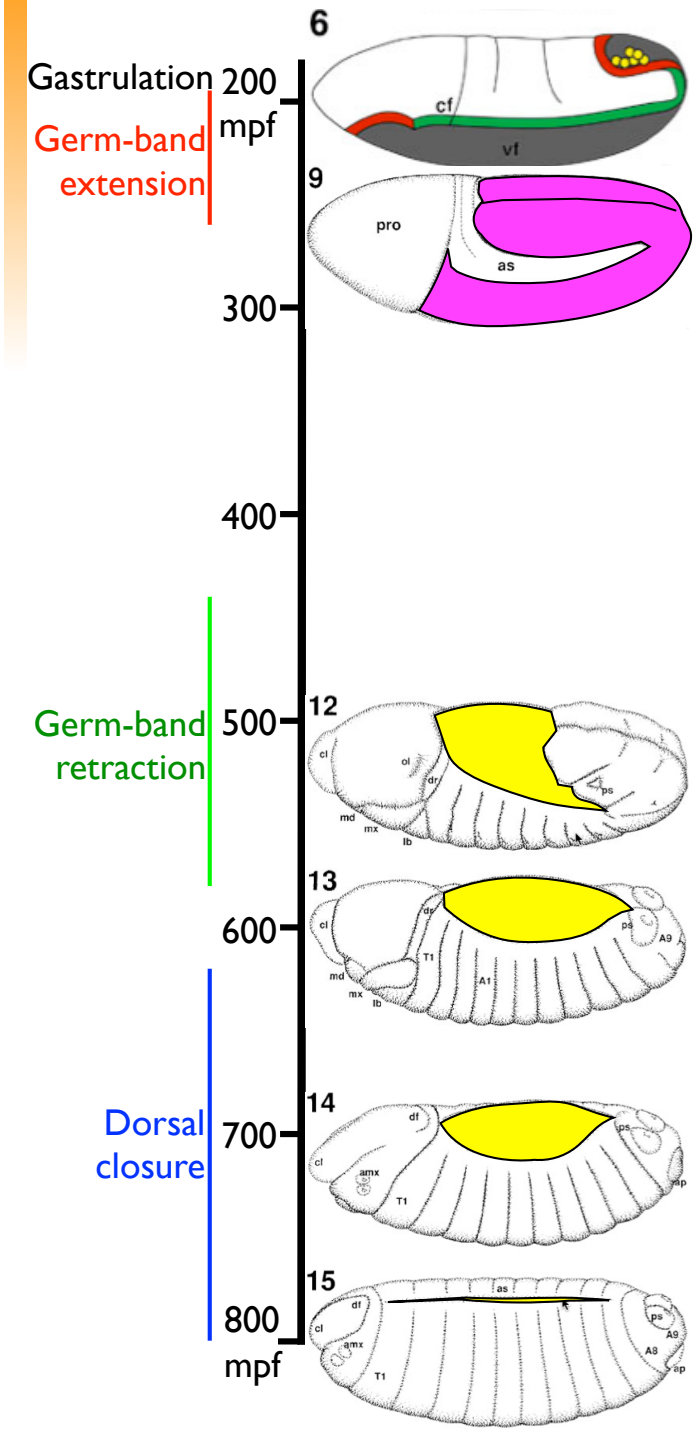
All cells from example embryo (3LU)



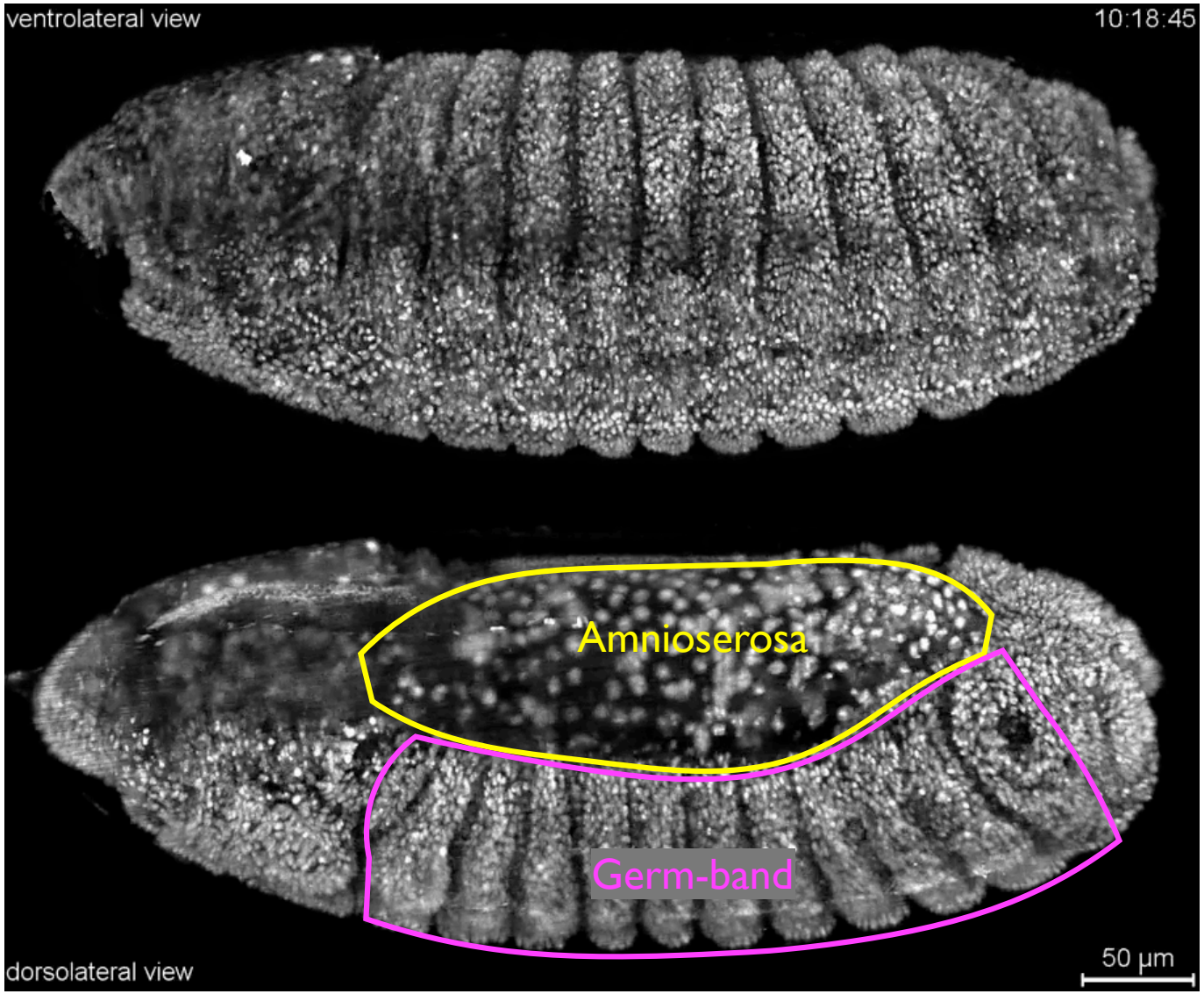
Density of Medial Myosin (gs)

Time (mins)

Drosophila embryonic morphogenesis



Dorsal closure

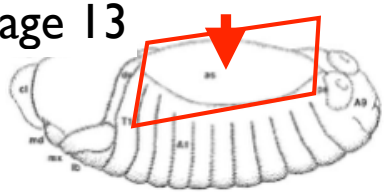


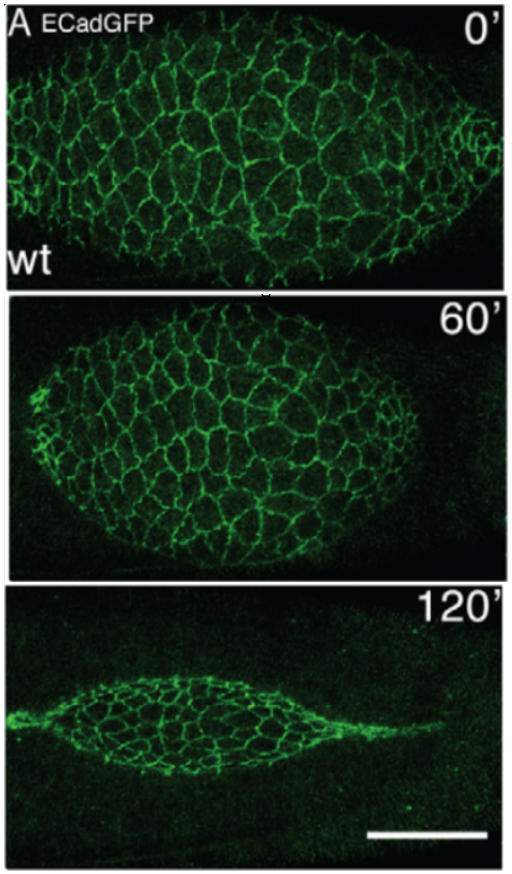
Tomer et al. (2012) *Nat. Methods*

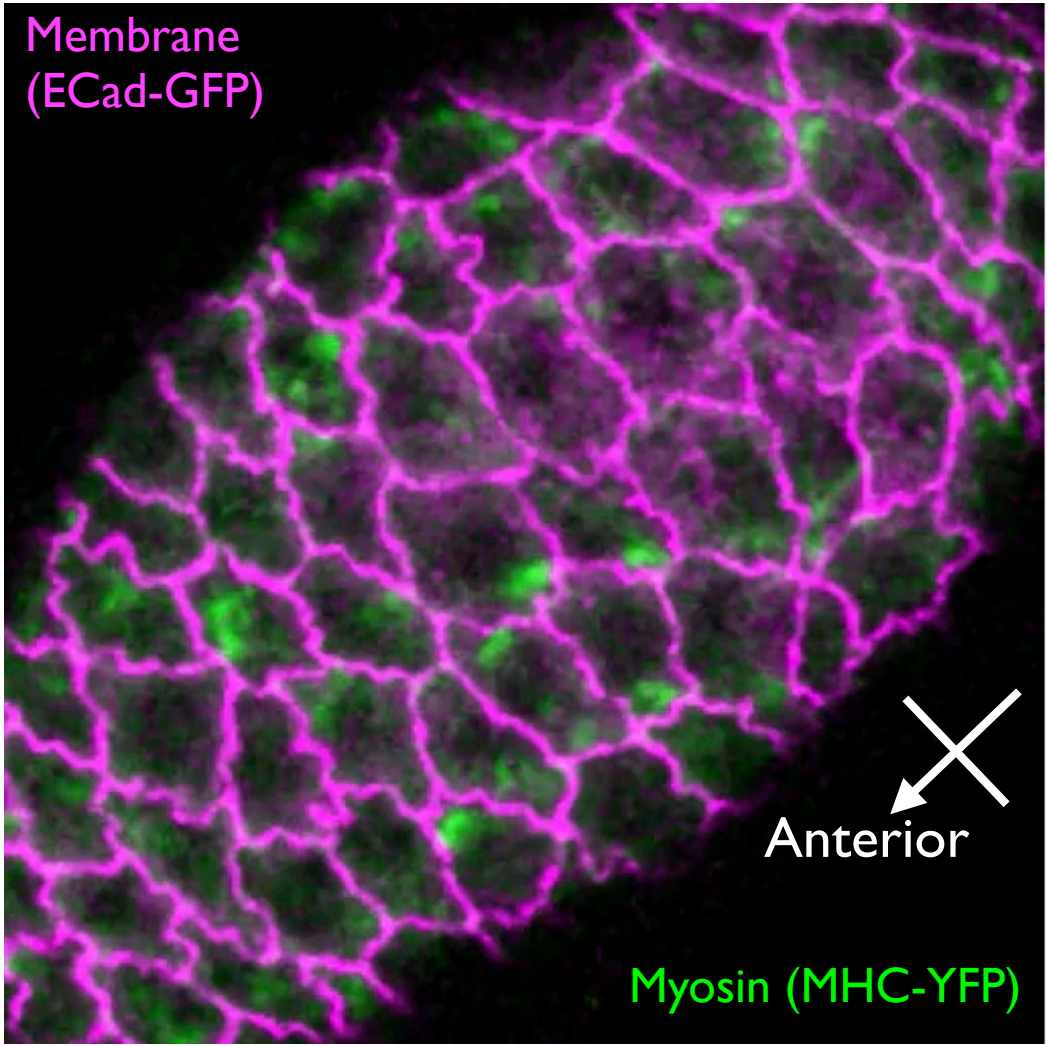
Amnioserosa dorsal closure

Dorsal view of amnioserosa

stage 13



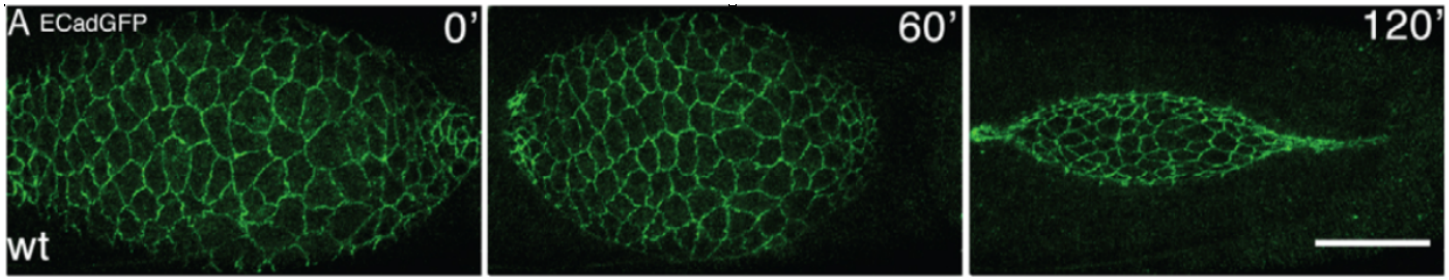


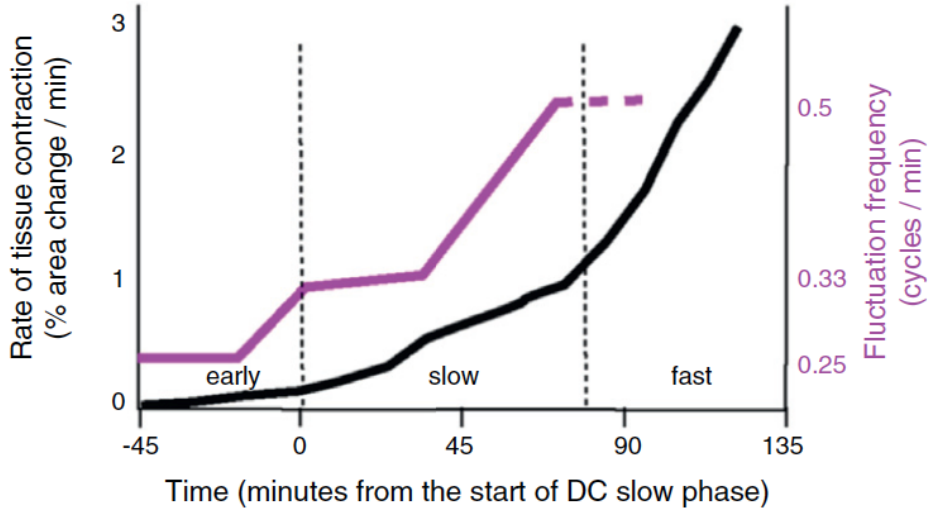


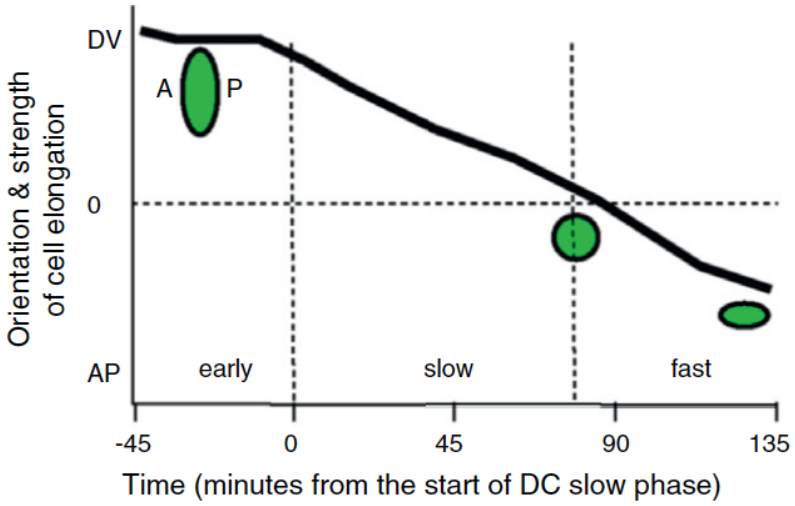
★No cell rearrangement
★Pulsatile Myosin-II is apico-medial, not junctional

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

Drosophila dorsal closure trends

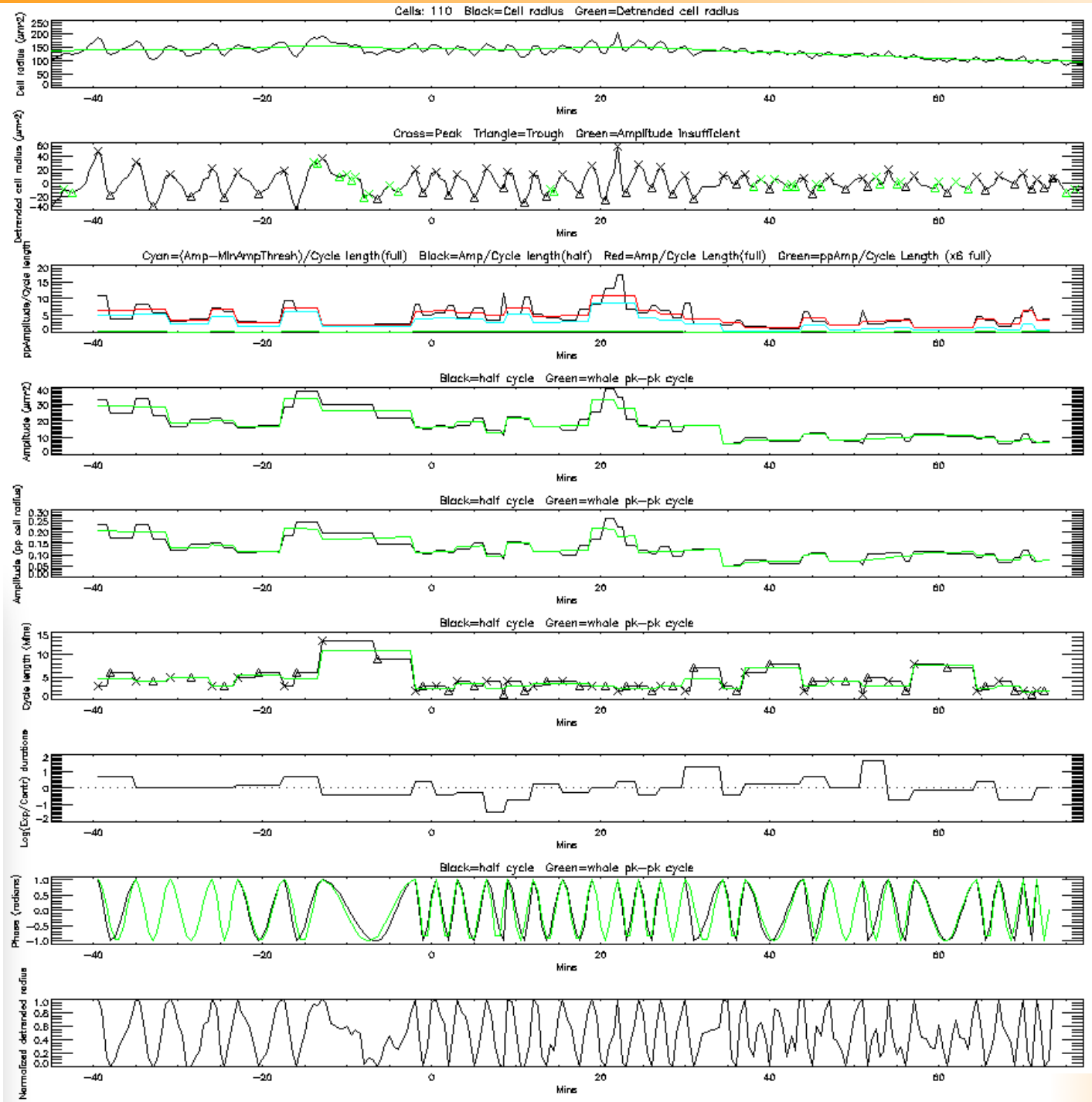






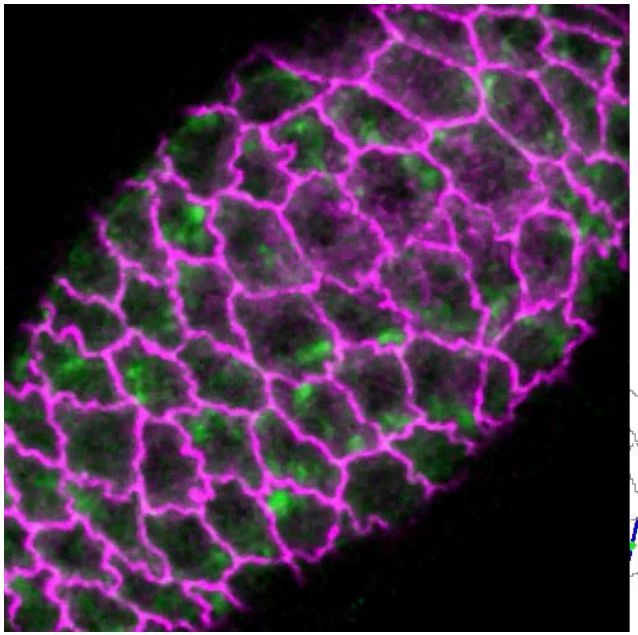
Blanchard & Adams (2011) *COGD*; Gorfinkiel, Schamberg & Blanchard (2011) *Genesis*

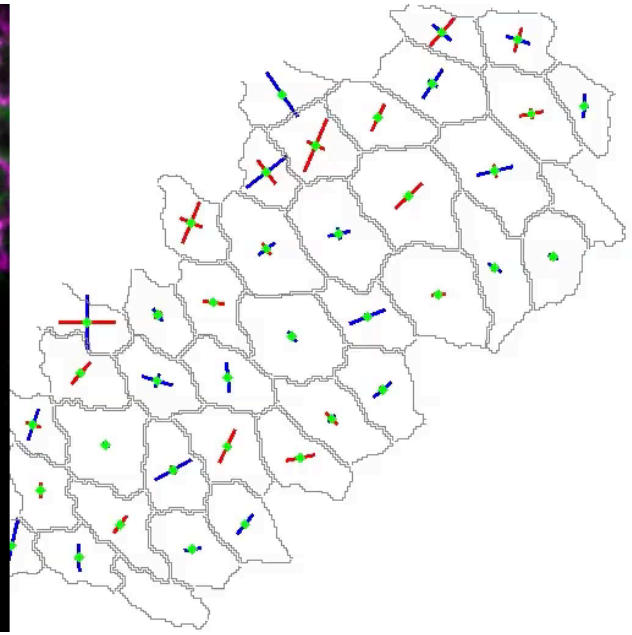
Apical area fluctuation of one cell



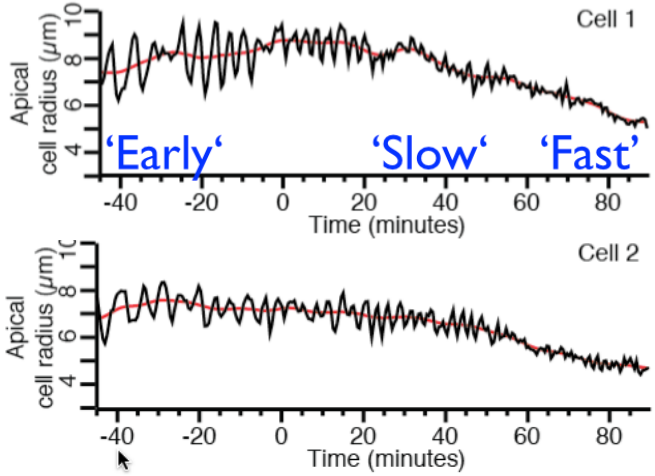
Extract cell contractile stress & strain rates

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





Mean trends

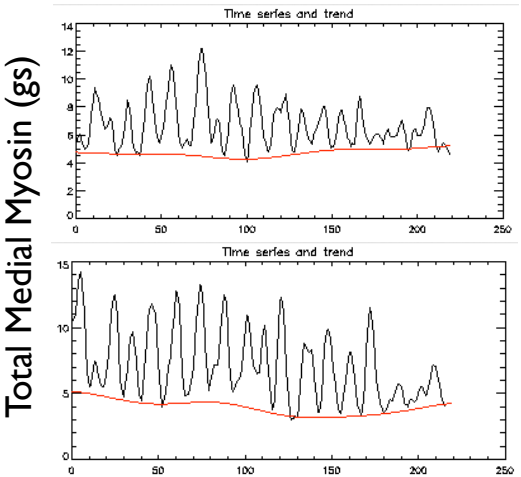


$$\epsilon_{c,t} = \frac{Area_{c,t}}{Area0_{c,t}} - 1$$

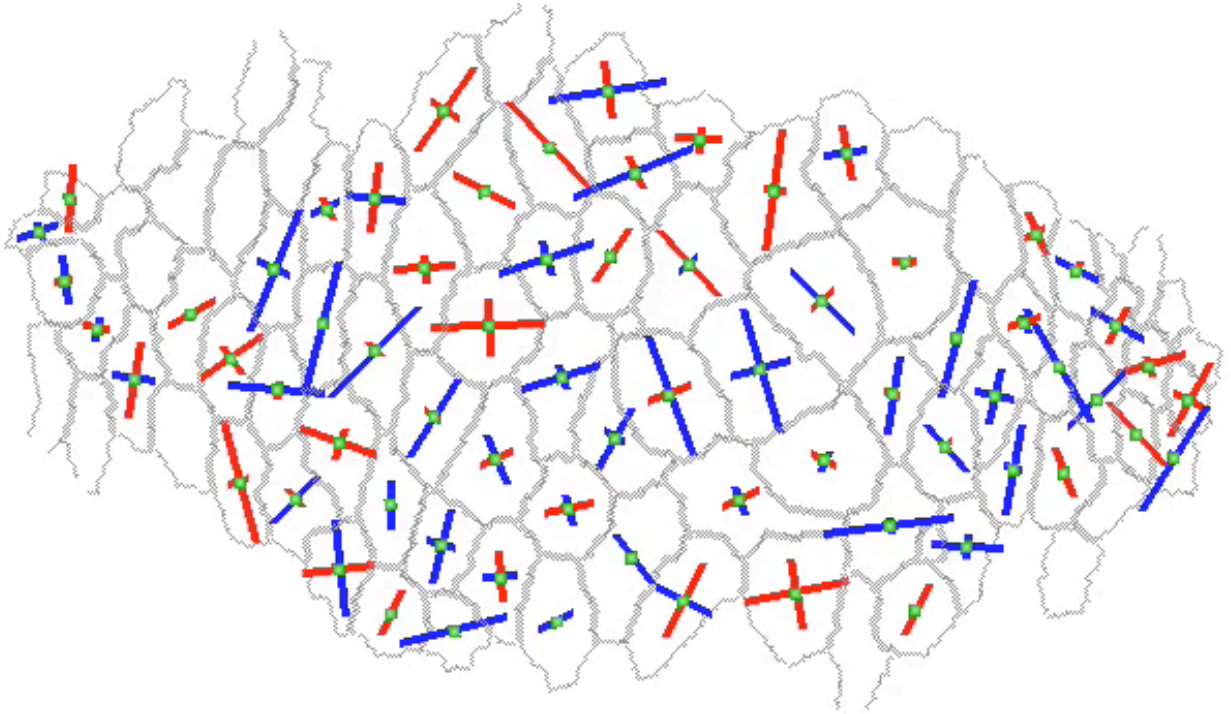
$$myo_{c,t} = \frac{Tot.Med.Myo._{c,t}}{Tot.Med.Myo.0_{c,t}} - 1$$

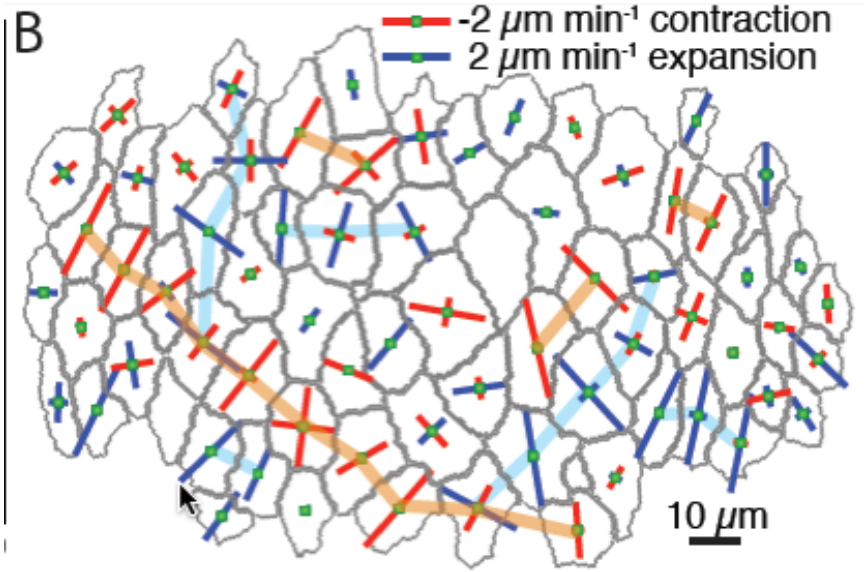
Trends retrospective
Rates calculated over 10sec time window

Minima trends



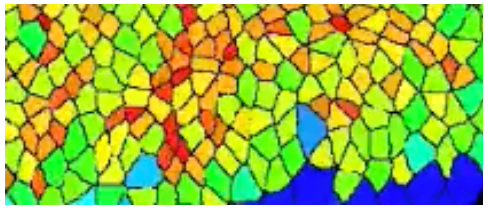
Neighbour accommodation: patterns at intermediate scale



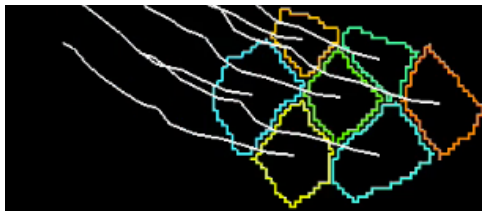


Analysis of Epithelial Morphogenesis

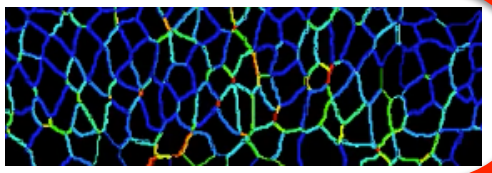
1 Static measures
- Cell shapes
- Expected cell shapes
- Cell centroid arrangement
- Cell-cell interface shapes
- Number of neighbours



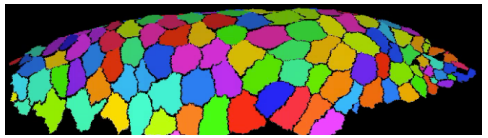
2 Dynamic measures
- Cell-cell interface dynamics
- Small domain deformations
- Impact of cell division
- Measuring fluctuations



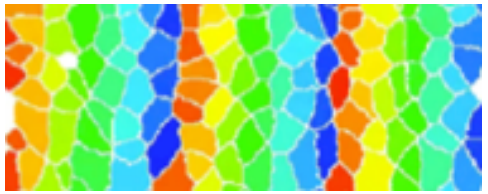
3 Fluorescence intensity
- Quantification of sub-cellular fluorescence intensity
- Medial dynamics
- Polarity of junctional proteins



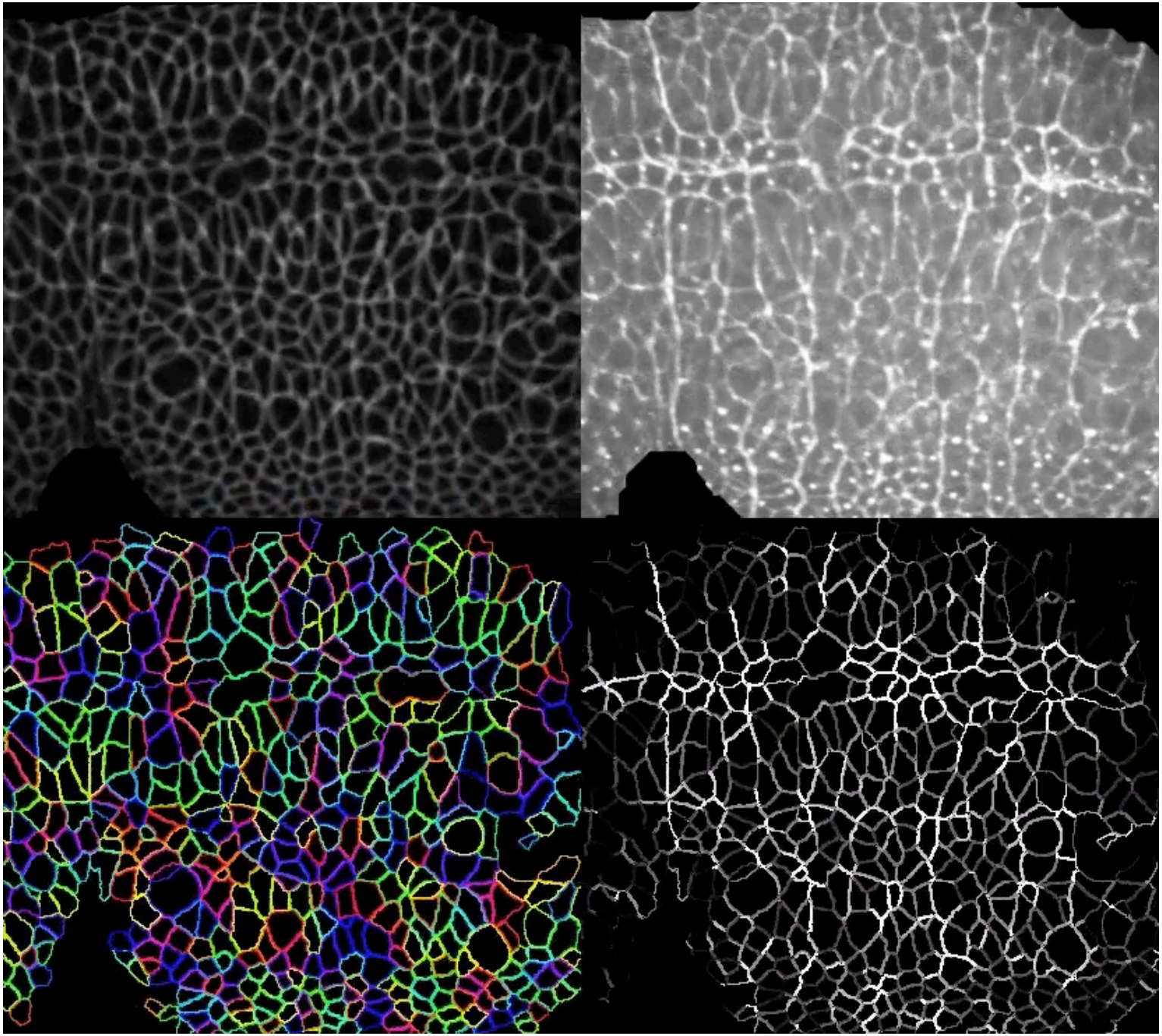
4 Towards 3D
- Epithelial tilt & curvature
- Combining tracking of apical & basal layers



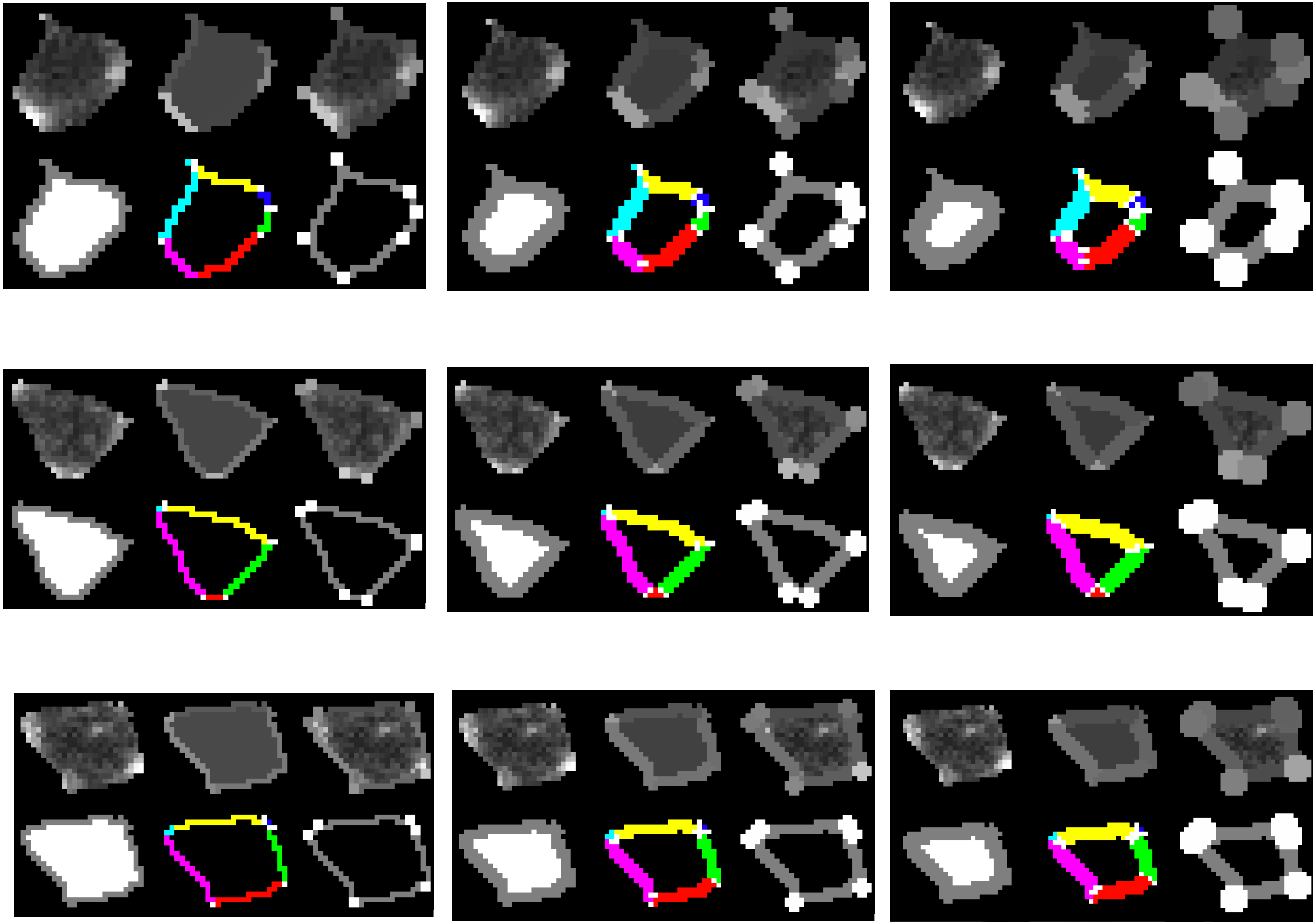
5 Time & space coordinate systems
- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries



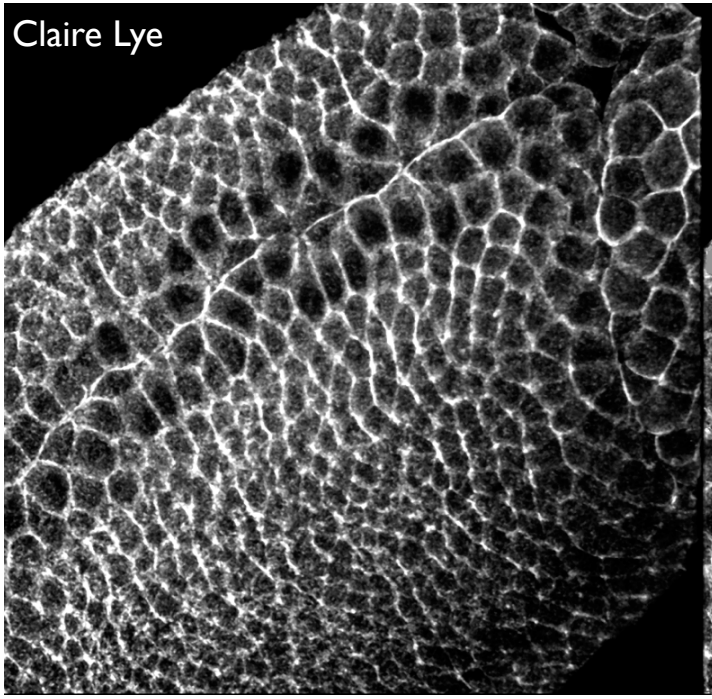
Cell-cell interface Myosin II fluorescence



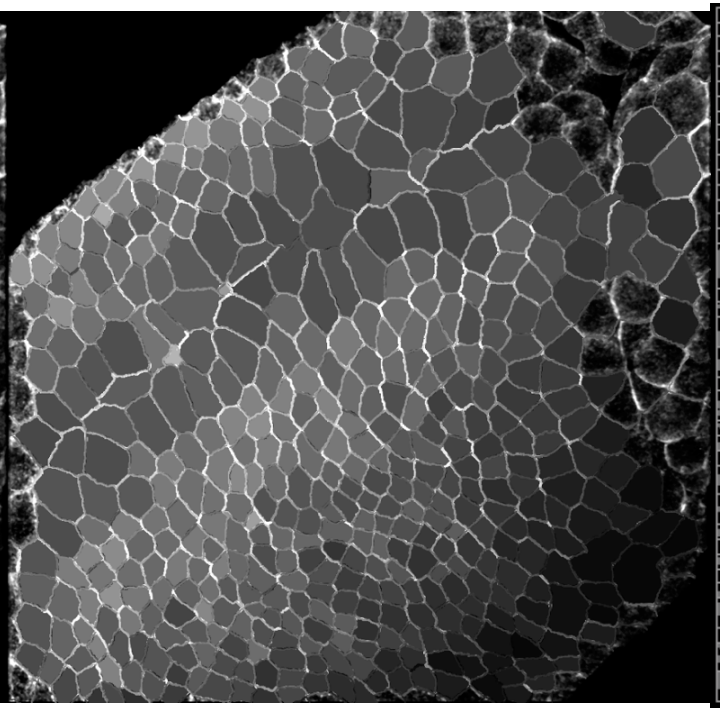
Cell fluorescence quantification



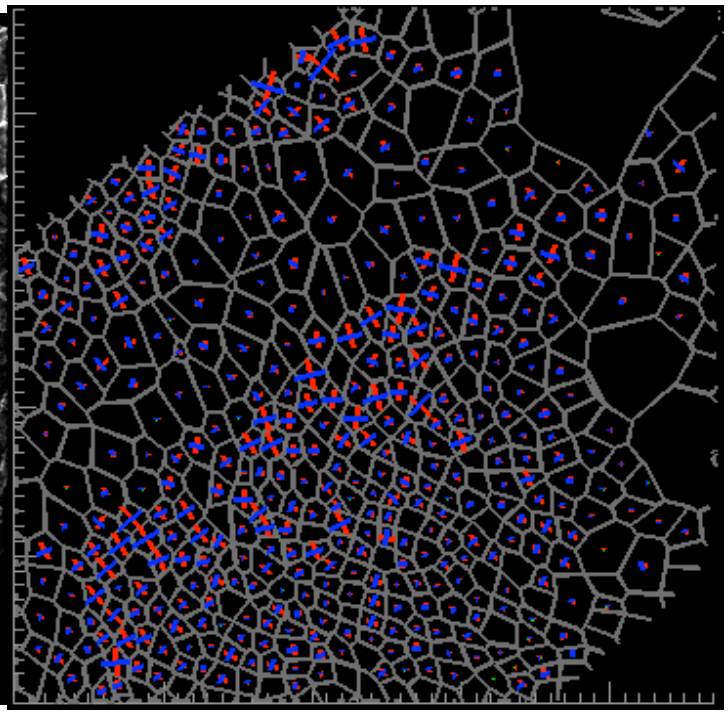
Fluorescence (Planar-)Polarity



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

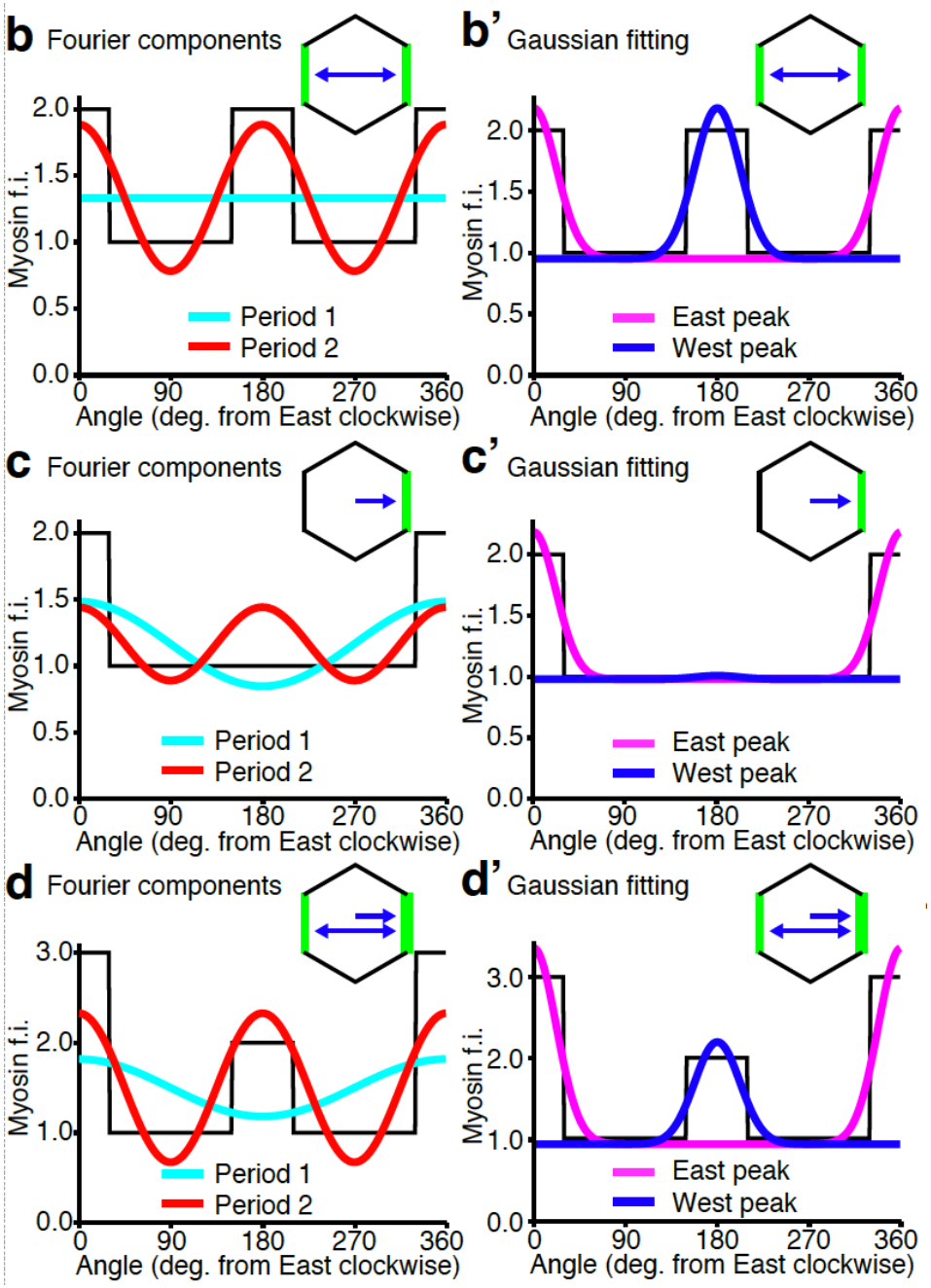


Mean fluorescence has been calculated across cell apices and for each cell-cell interface. Mean intensity for each part of each cell is drawn here.

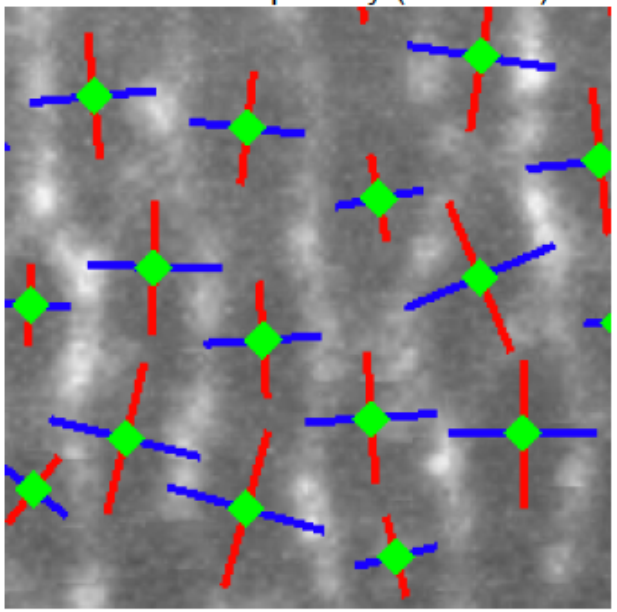


The relative fluorescence intensity of each of a cell's interfaces is compared. Any polarity in the pattern is captured and drawn here as a cross, with red in the orientation of least intensity.

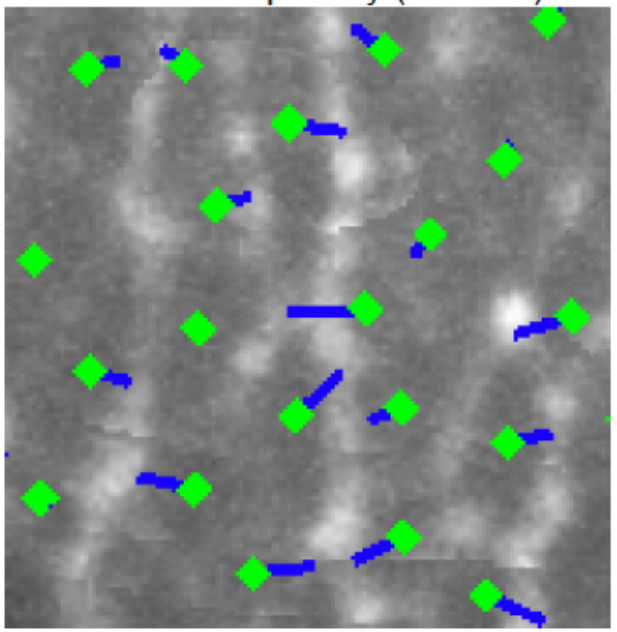
Apical cell myosin polarity



Bidirectional polarity (8.5 mins)

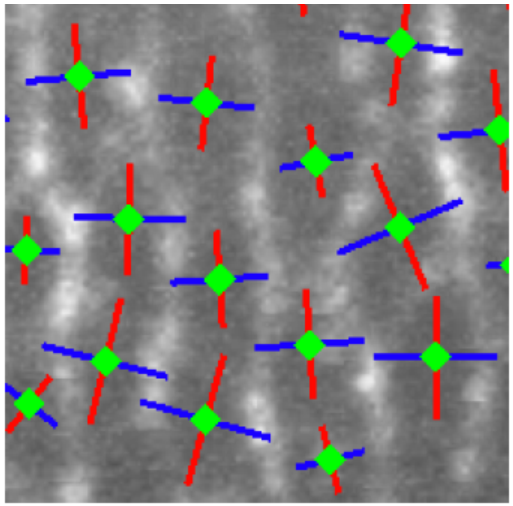


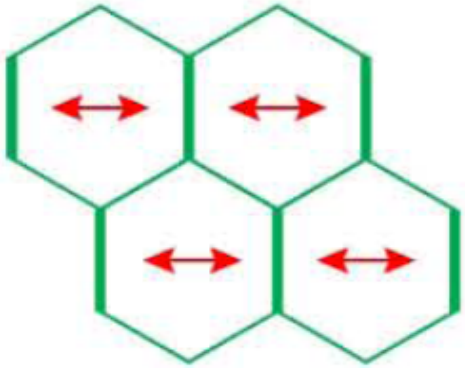
Unidirectional polarity (39 mins)



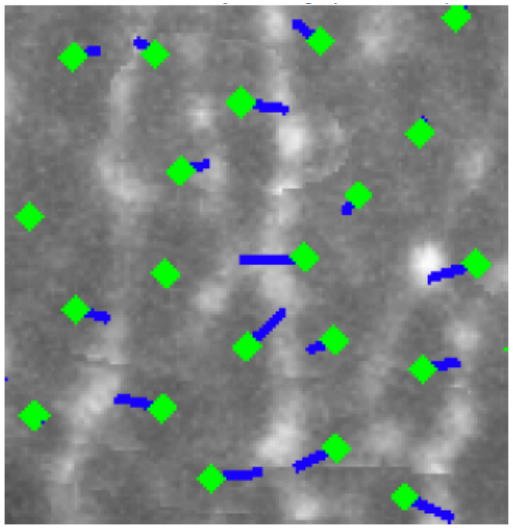
Myosin polarity patterns across AP axis

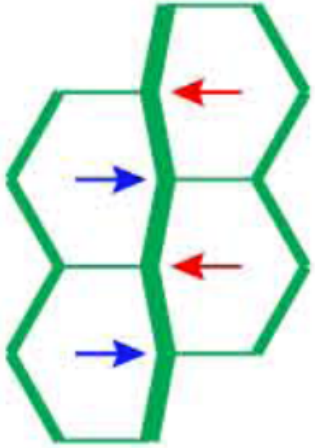
Bidirectional polarity

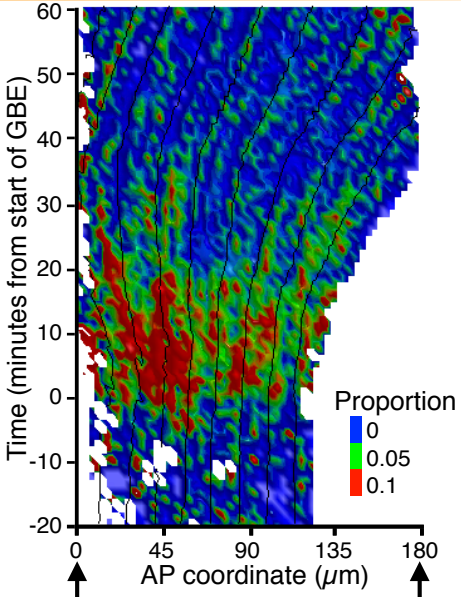


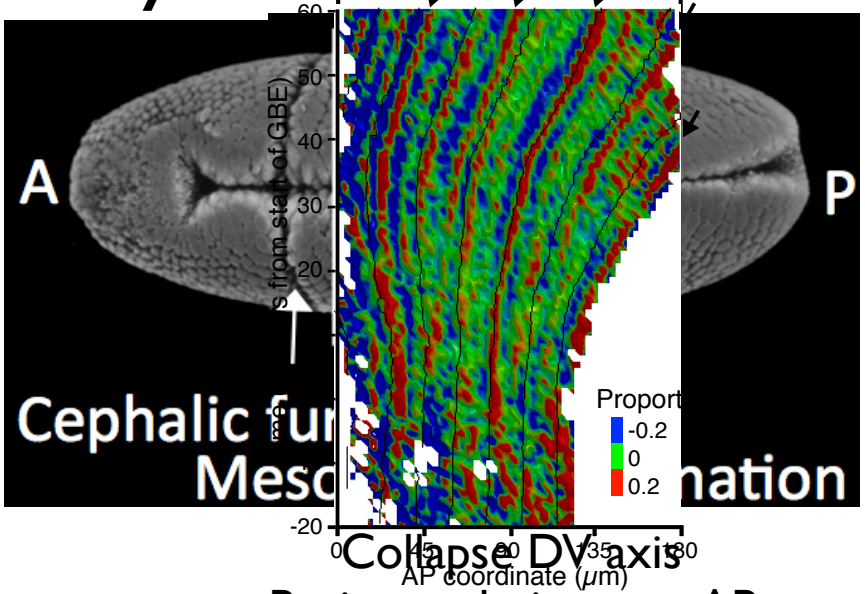


Unidirectional polarity







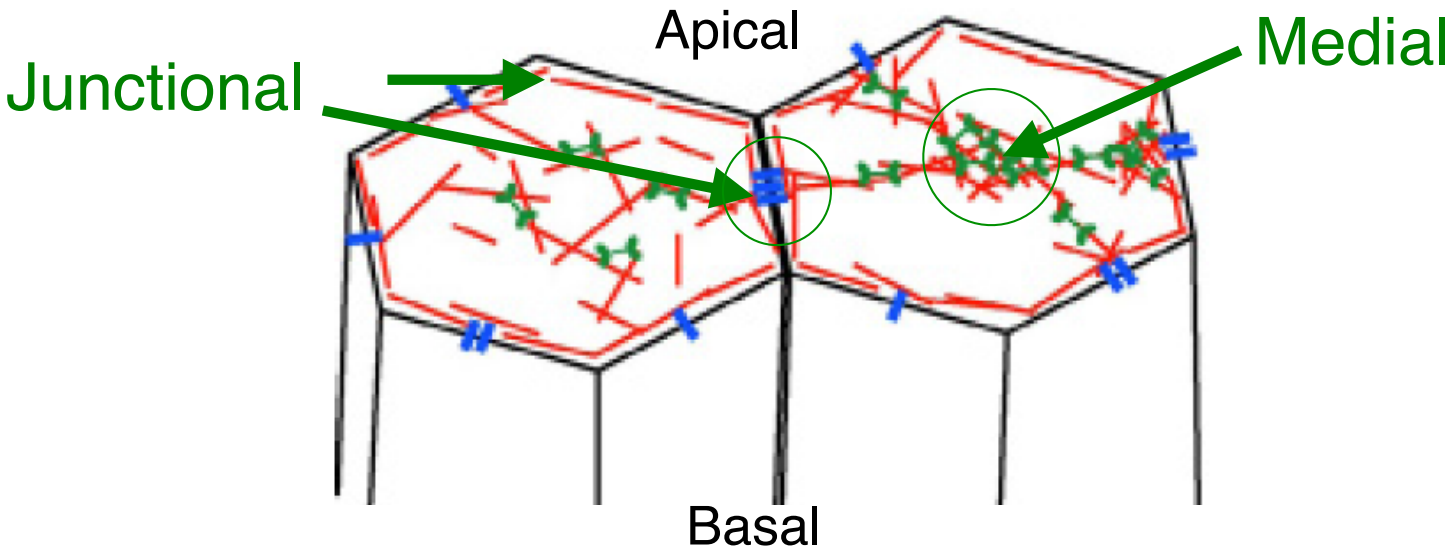


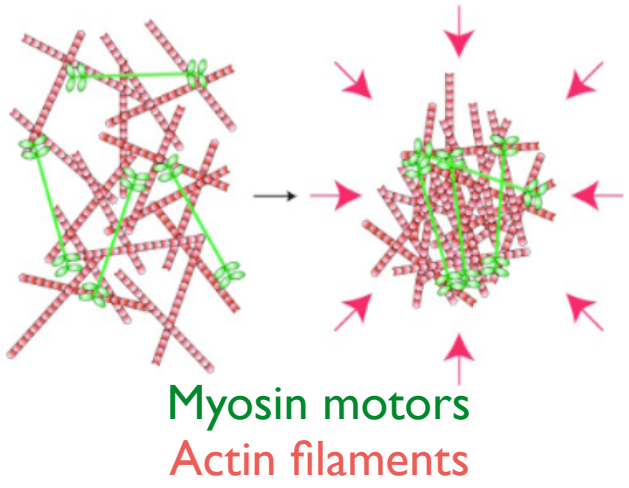
Collapse DV axis

Project polarity onto AP

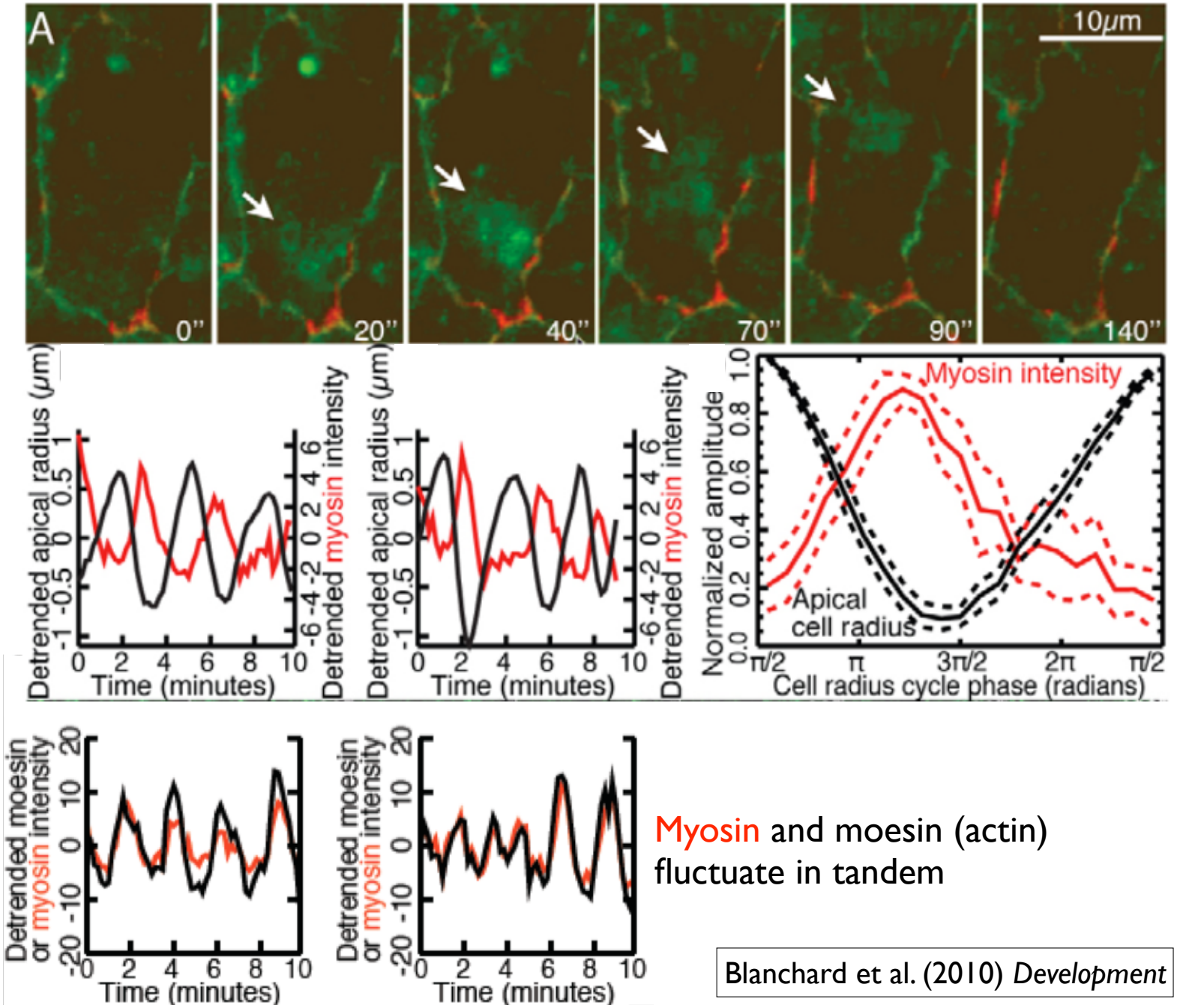
Single embryos. To find stereotypical behaviour want **standardised AP axis coordinate system.**

Actomyosin drives apical cell contractility





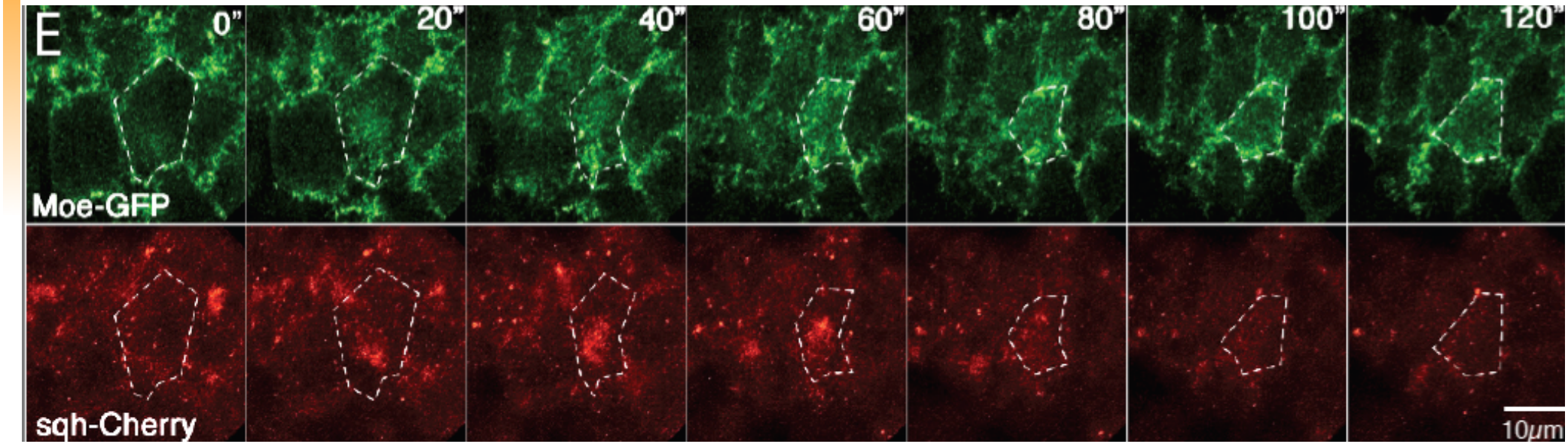
Myosin II fluctuations



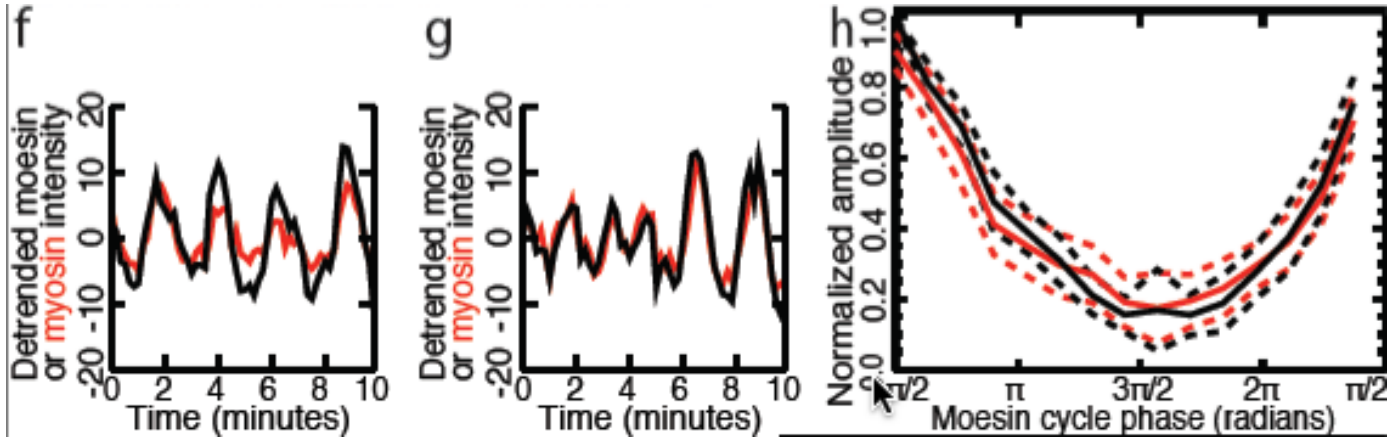
Myosin and moesin (actin) fluctuate in tandem

Blanchard et al. (2010) *Development*

Actin and myosin dynamics in cell fluctuations

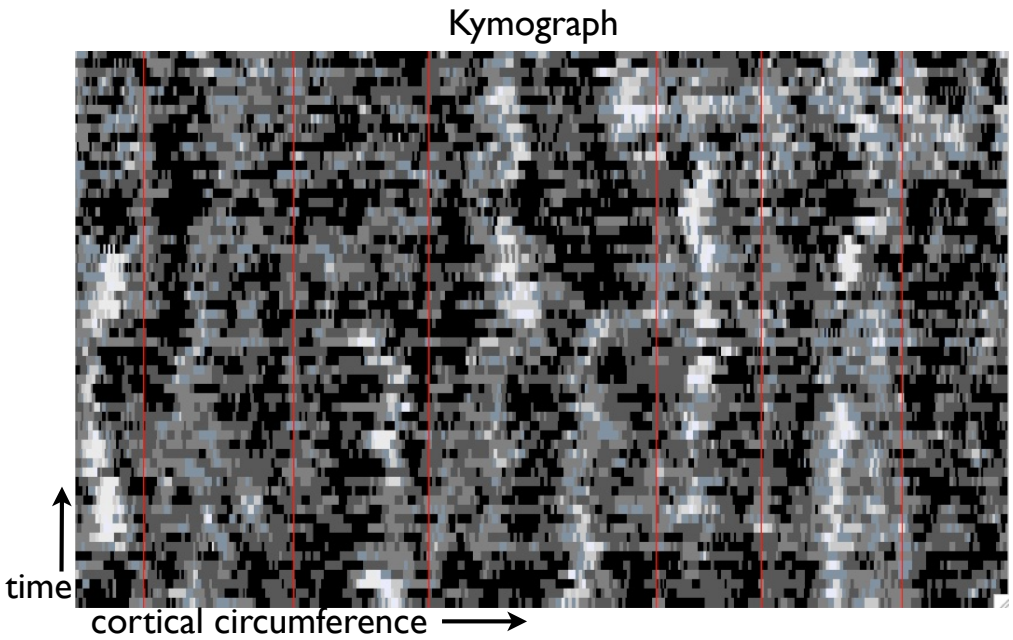


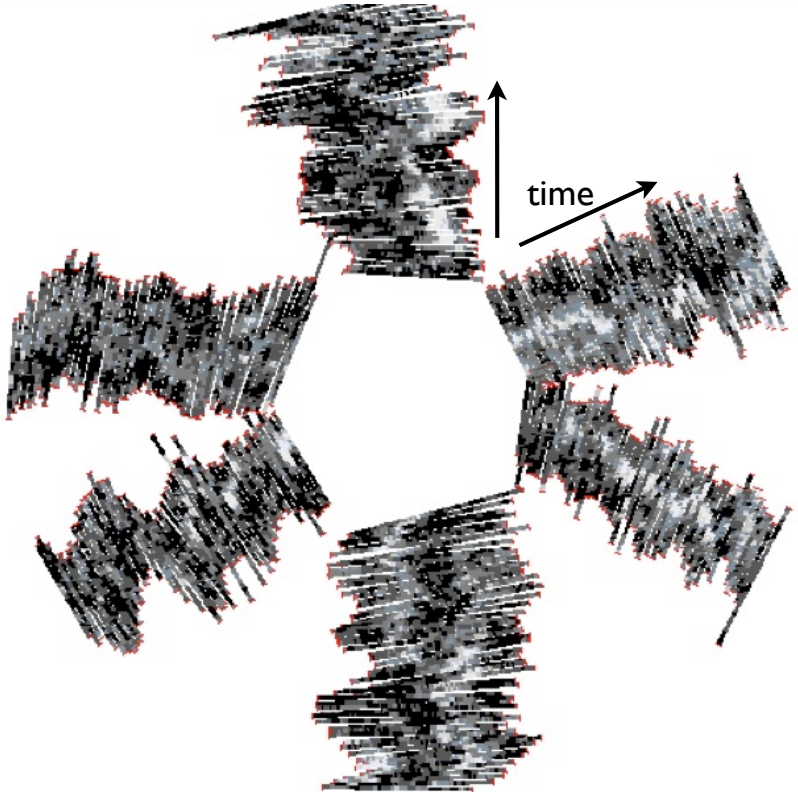
Myosin and moesin (actin) fluctuate in tandem



Protein Fluorescence Intensity

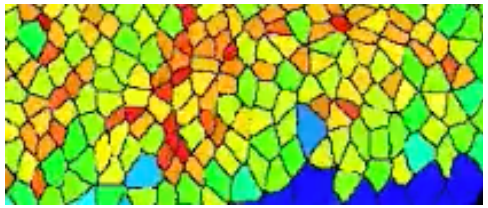
Cortical ring fluorescence intensity



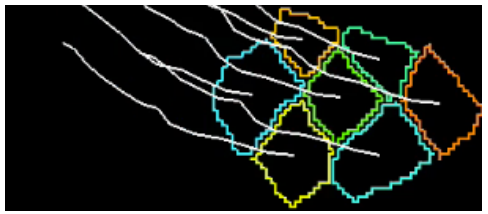


Analysis of Epithelial Morphogenesis

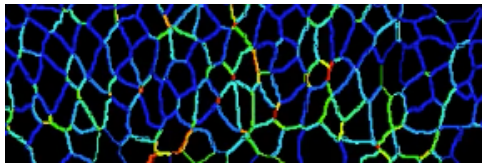
1 Static measures
- Cell shapes
- Expected cell shapes
- Cell centroid arrangement
- Cell-cell interface shapes
- Number of neighbours



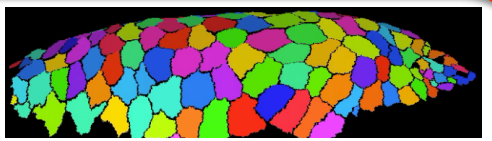
2 Dynamic measures
- Cell-cell interface dynamics
- Small domain deformations
- Impact of cell division
- Measuring fluctuations



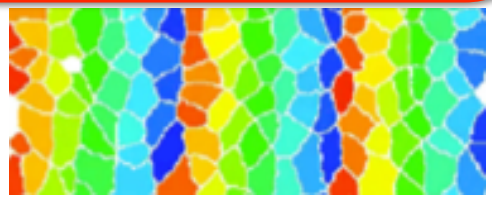
3 Fluorescence intensity
- Quantification of sub-cellular fluorescence intensity
- Medial dynamics
- Polarity of junctional proteins



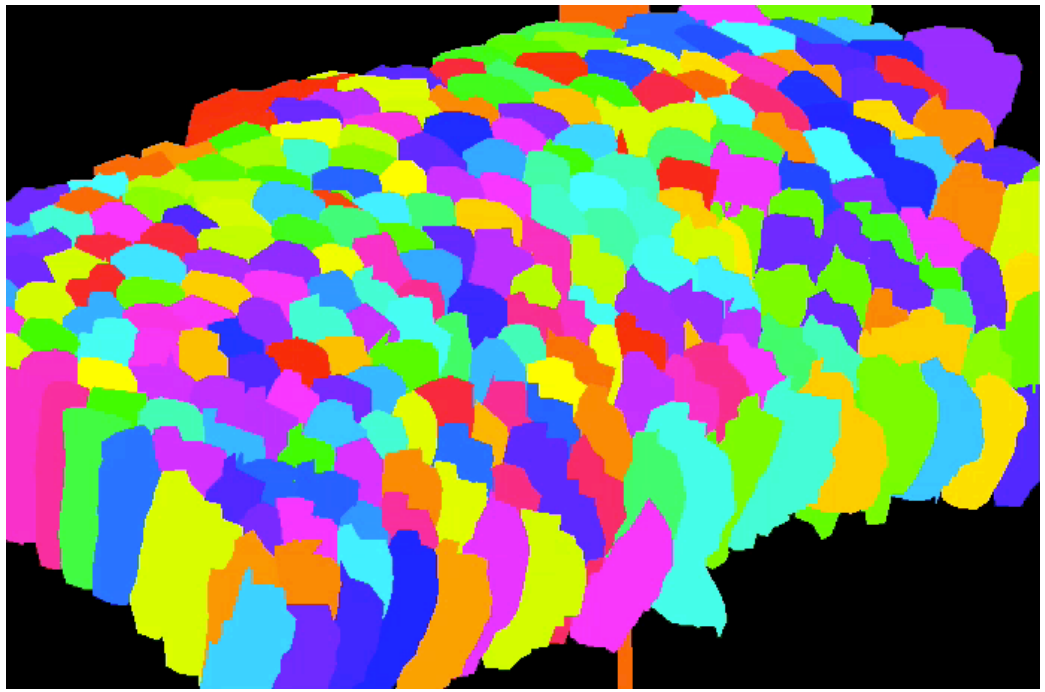
4 Towards 3D
- Epithelial tilt & curvature
- Combining tracking of apical & basal layers

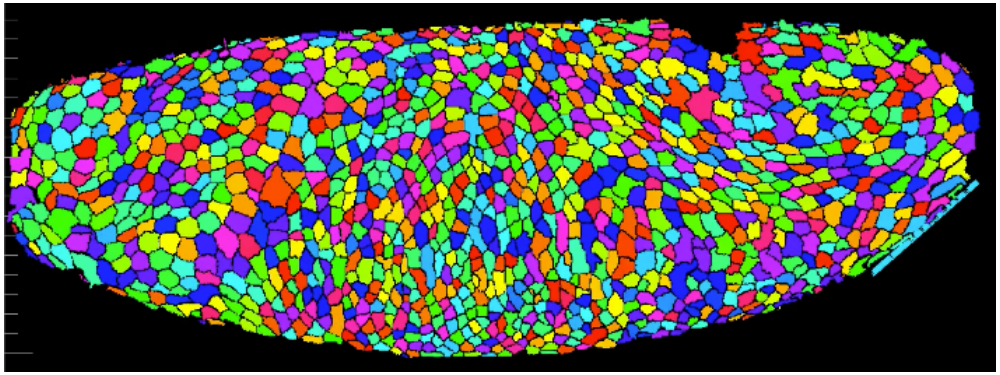


5 Time & space coordinate systems
- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries

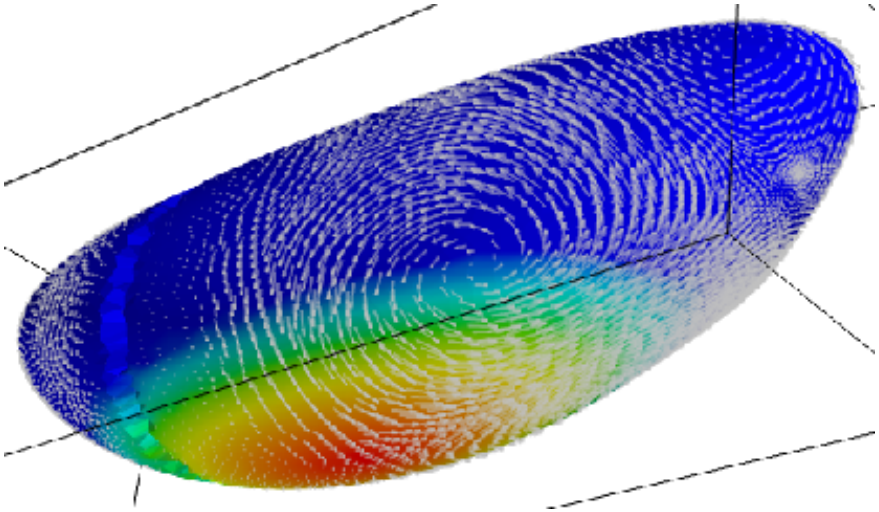


3D and *in toto* tracking & modelling

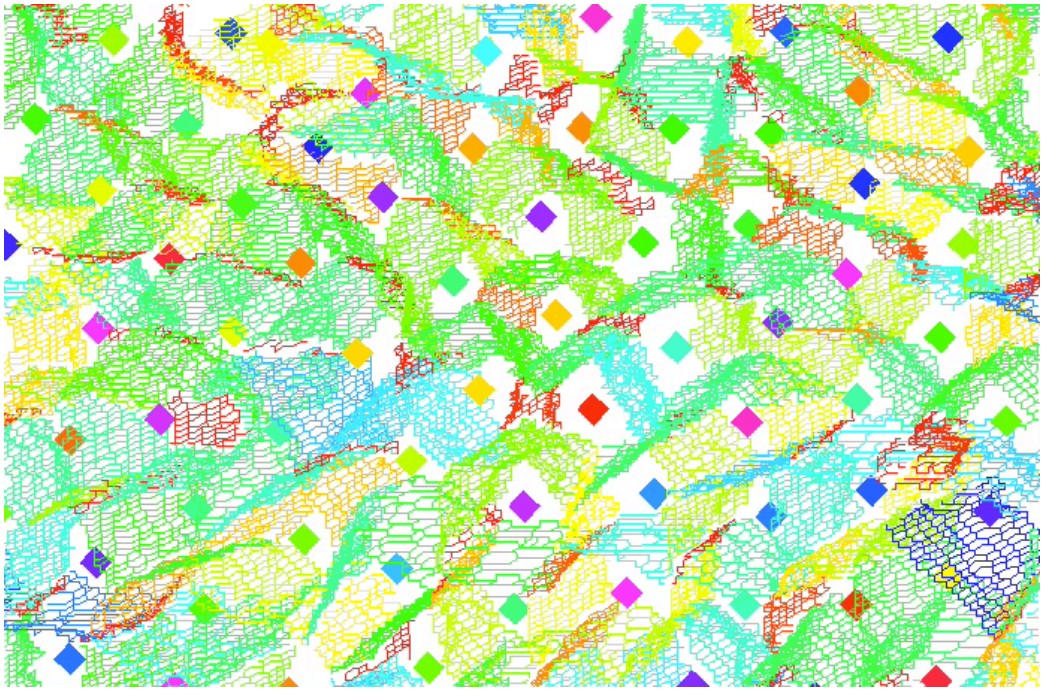




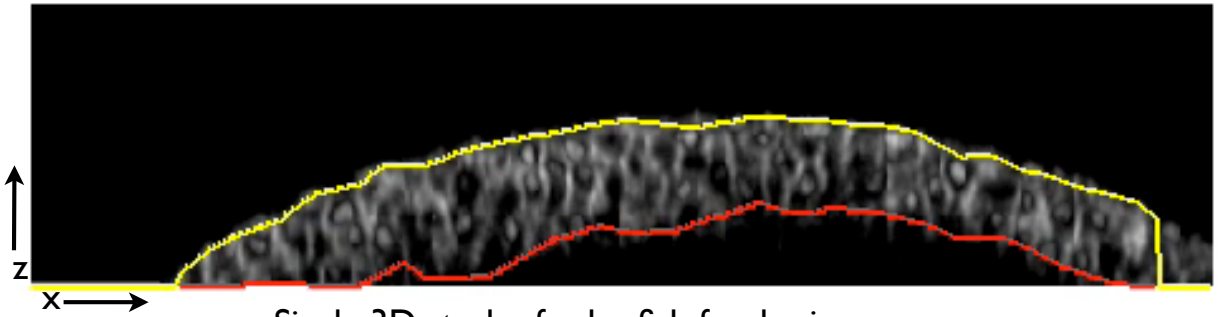
Claire Lye



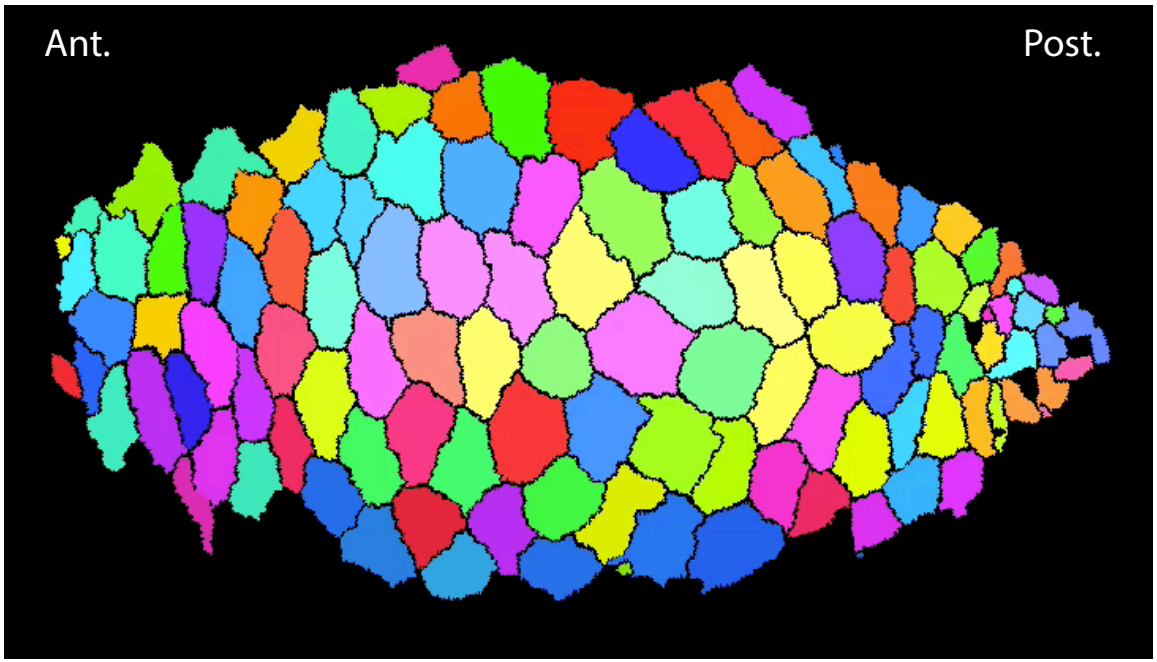
Jocelyn Etienne, Grenoble



Analysis on quasi-2D surfaces

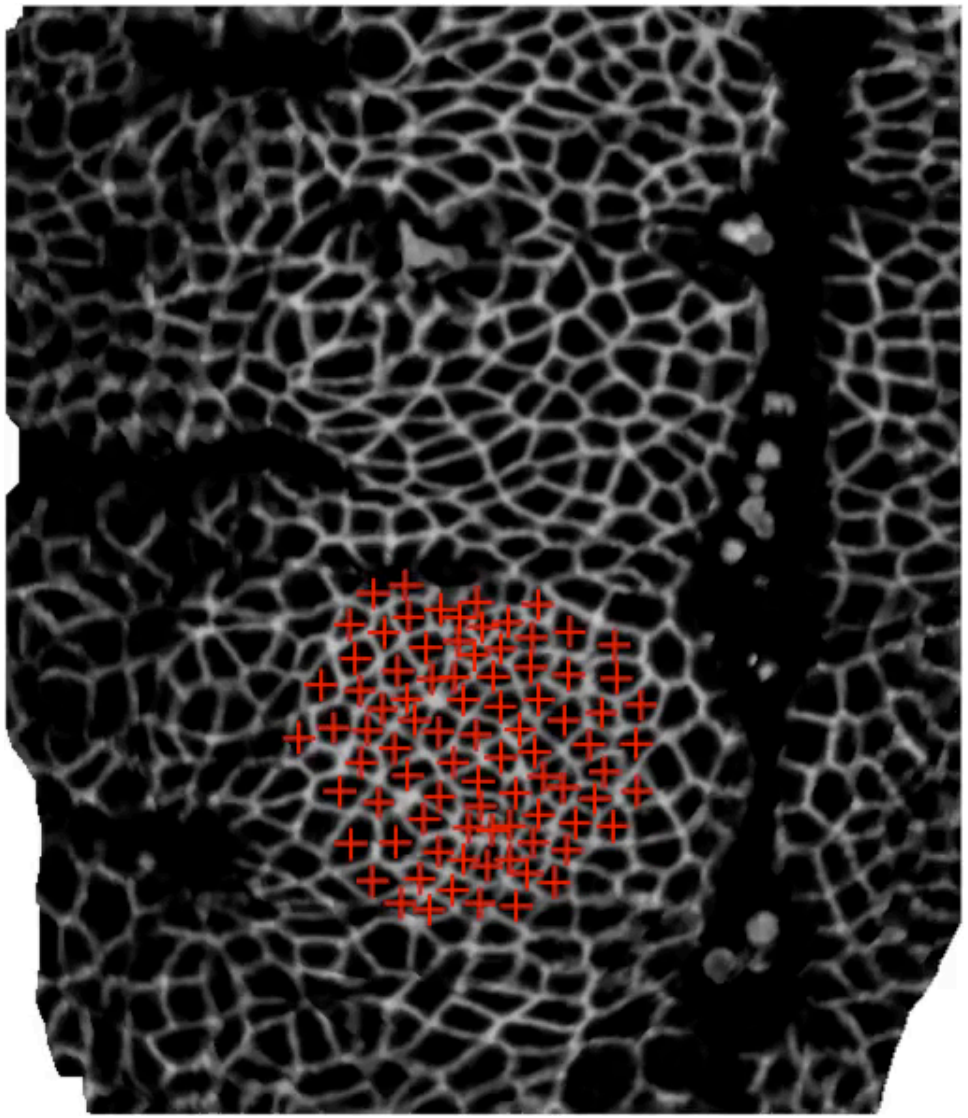


Single 3D stack of zebrafish forebrain



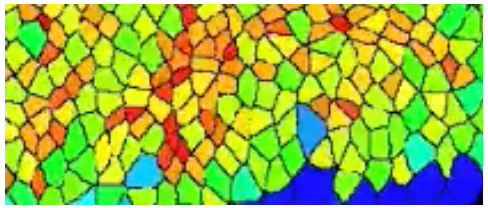
Drosophila amnioserosa tissue profile

2-layer tracking of salivary placode

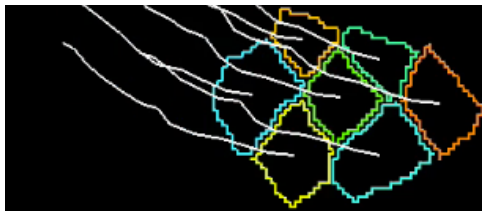


Analysis of Epithelial Morphogenesis

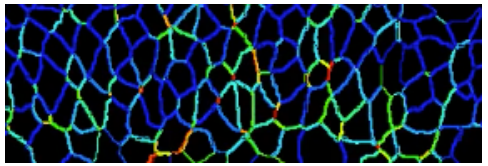
1 Static measures
- Cell shapes
- Expected cell shapes
- Cell centroid arrangement
- Cell-cell interface shapes
- Number of neighbours



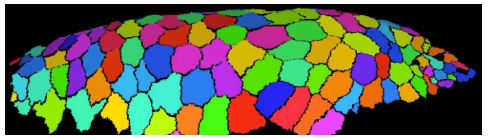
2 Dynamic measures
- Cell-cell interface dynamics
- Small domain deformations
- Impact of cell division
- Measuring fluctuations



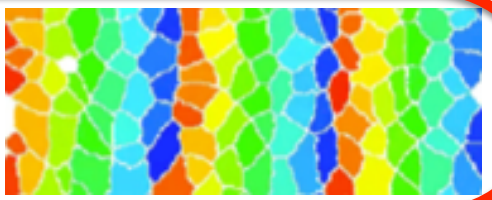
3 Fluorescence intensity
- Quantification of sub-cellular fluorescence intensity
- Medial dynamics
- Polarity of junctional proteins



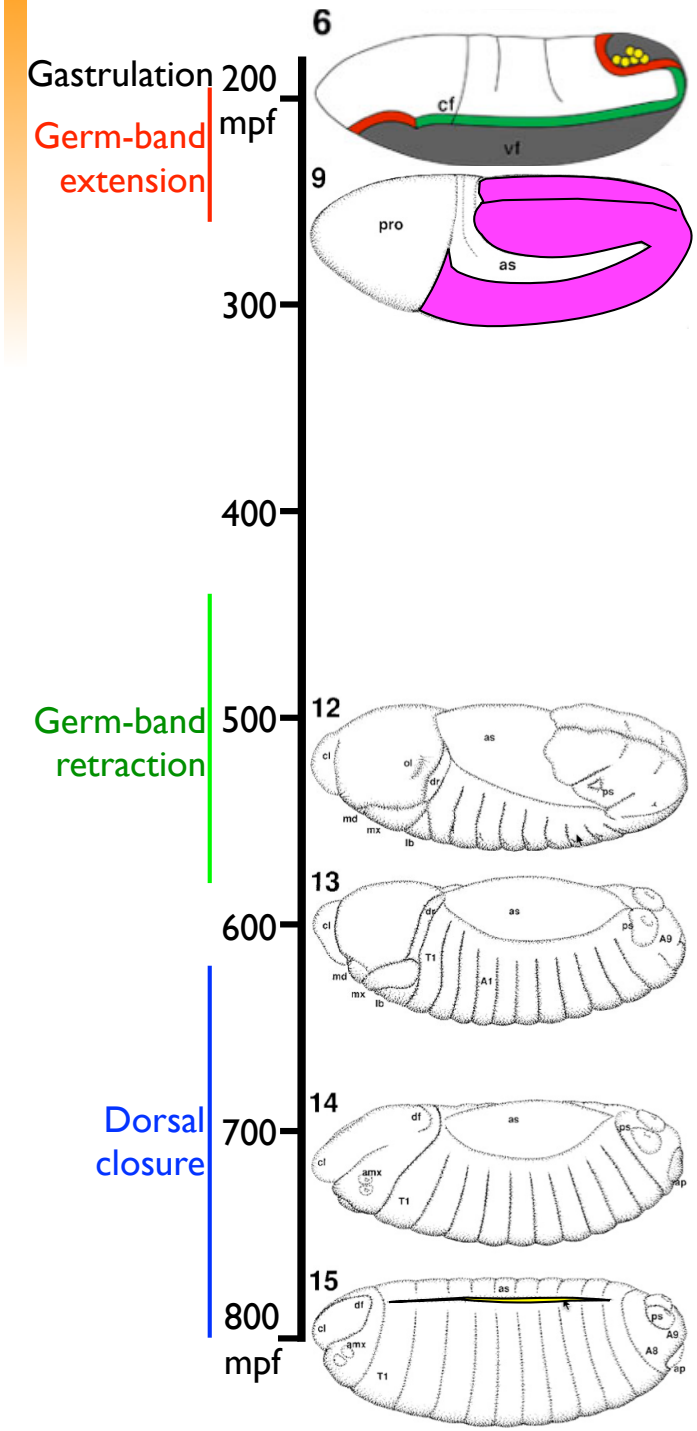
4 Towards 3D
- Epithelial tilt & curvature
- Combining tracking of apical & basal layers



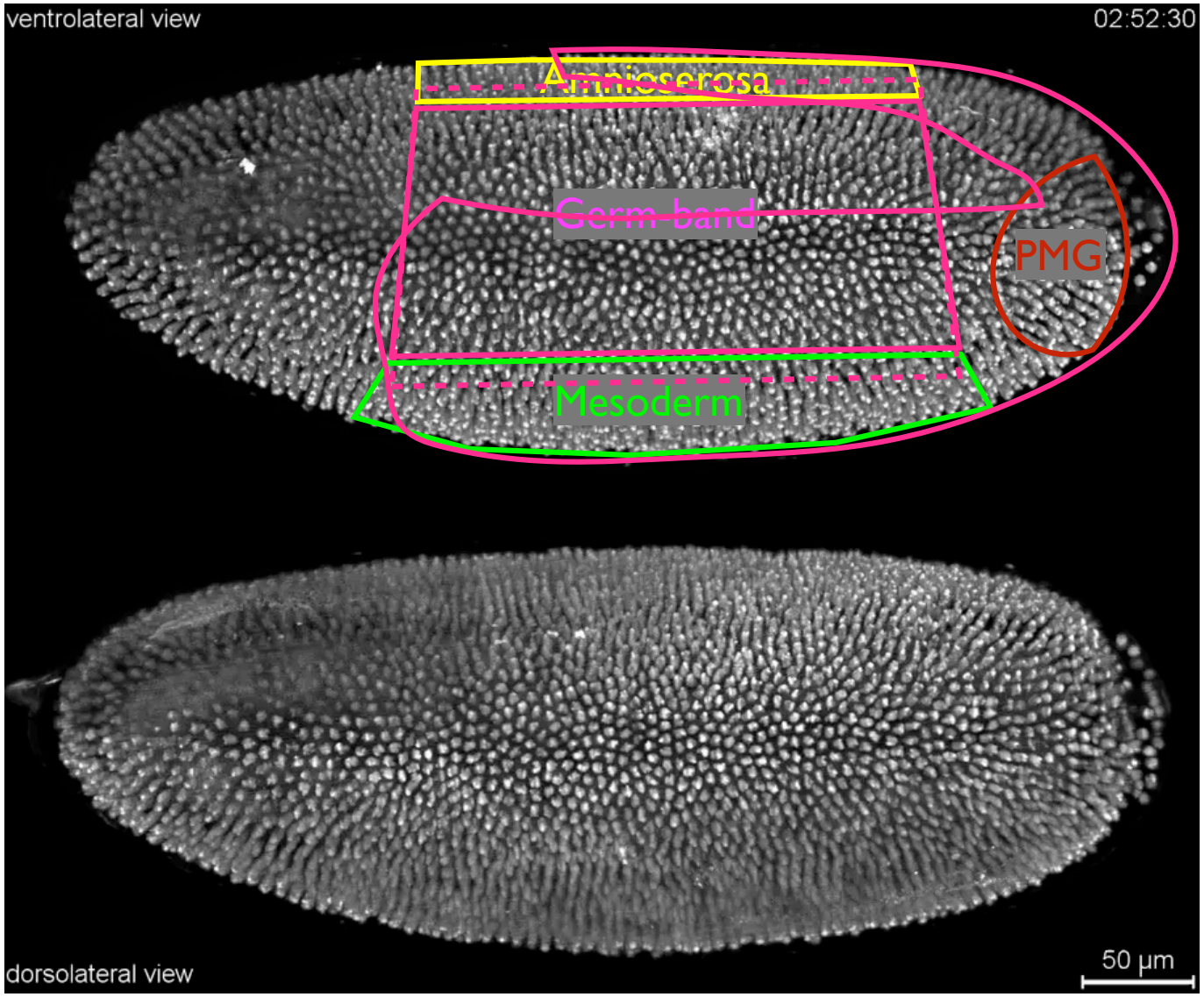
5 Time & space coordinate systems
- Embryonic or radial axes
- Synchronising time between WT embryos, and with mutants
- Compartments and boundaries



Drosophila embryonic morphogenesis



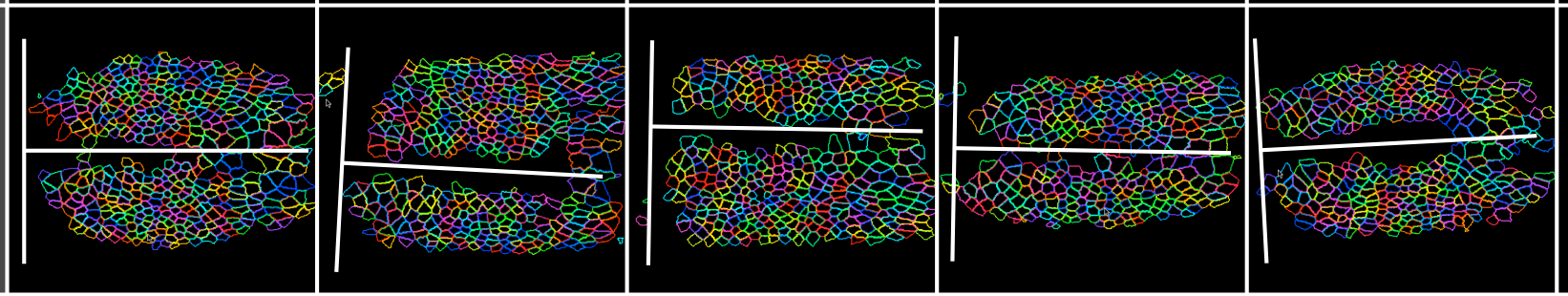
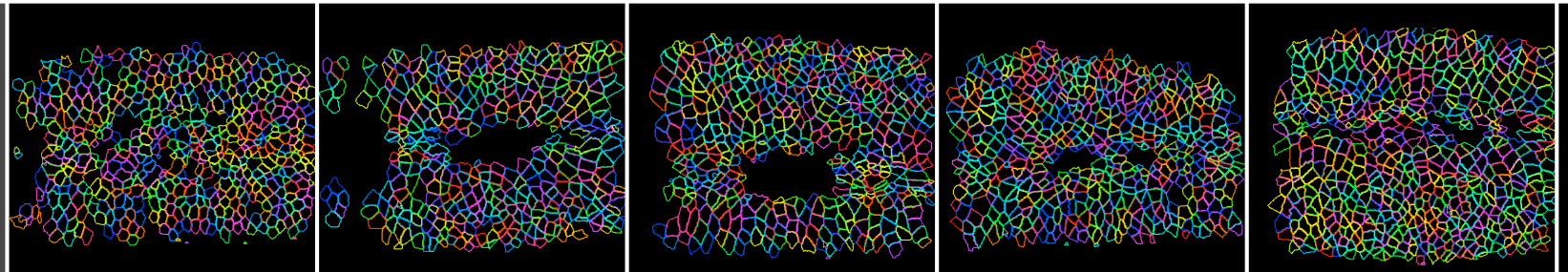
Germ-band extension



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

Coordinating space & time in WT and mutant embryos

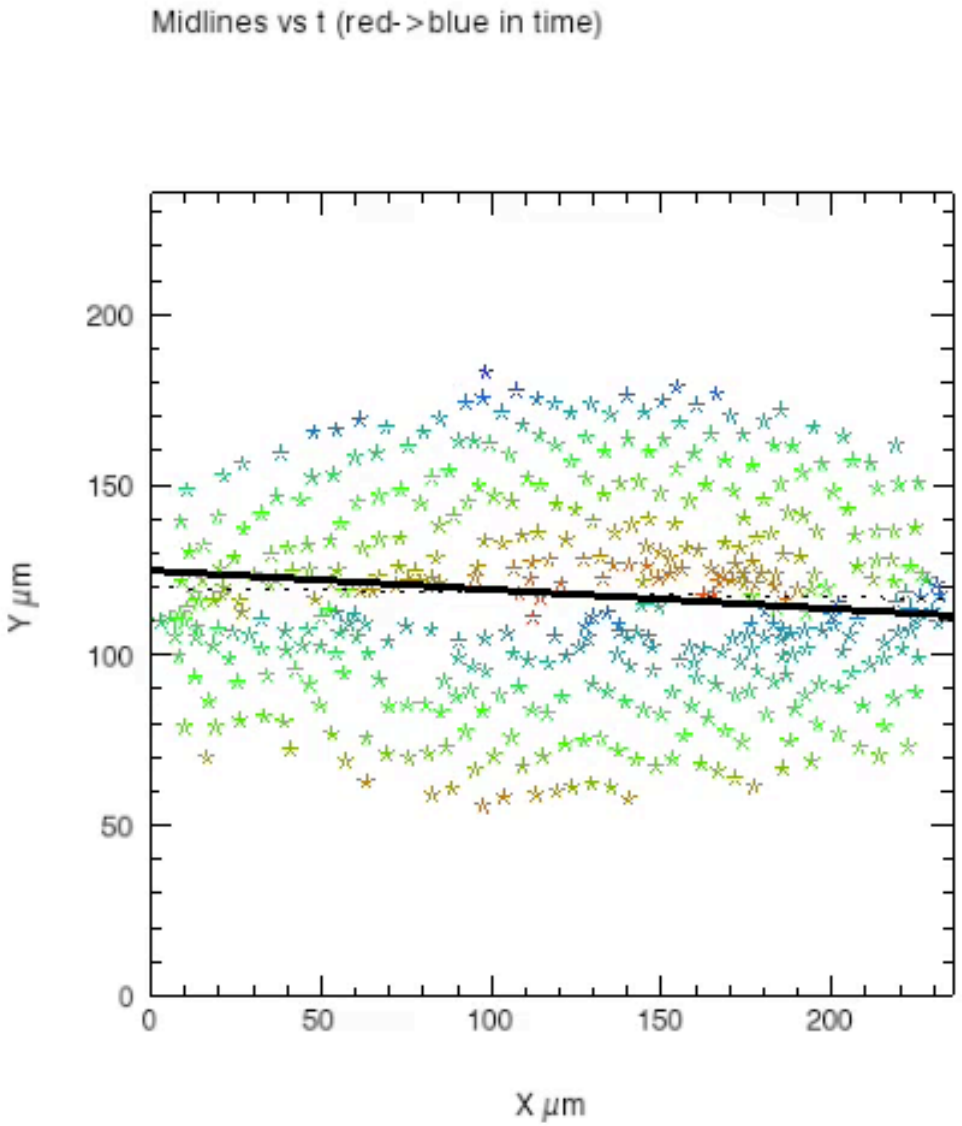
Drosophila germ-band tracked data sets

	embryo A	embryo B	embryo C	embryo D	embryo E	Total cell **hours** (sampled 30s)
wild type	**Align embryos by imposing new coordinate system** • **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

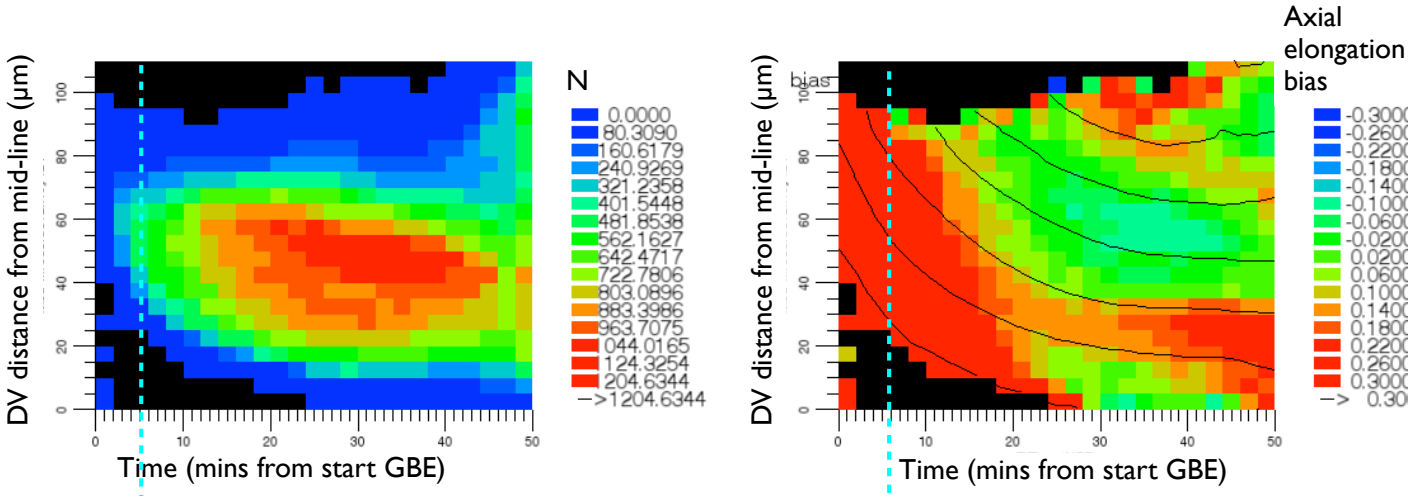
with Lucy Butler & Benedicte Sanson

Moving embryonic axes

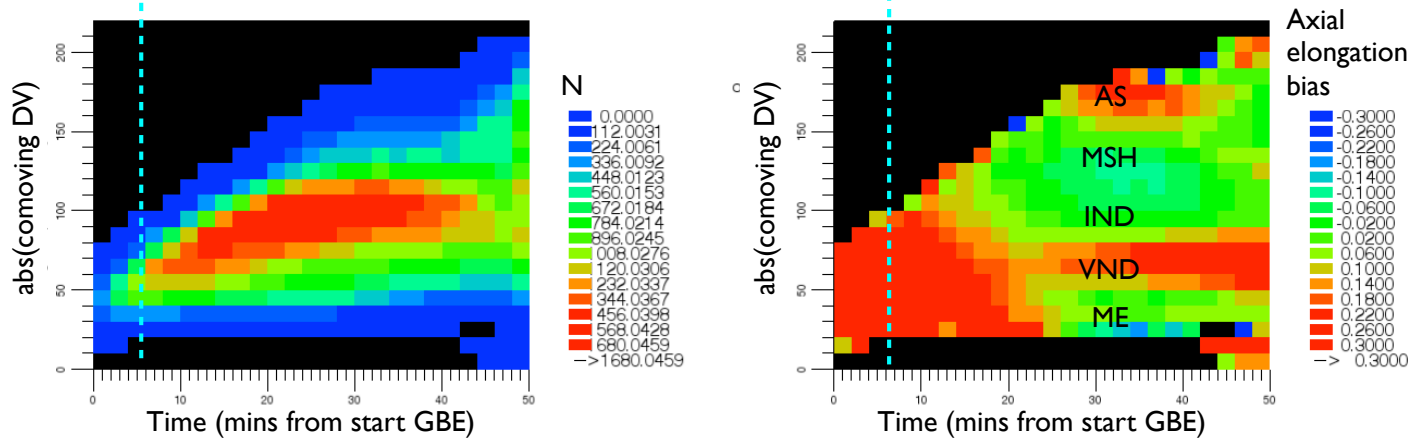


Fixed and comoving coordinate systems

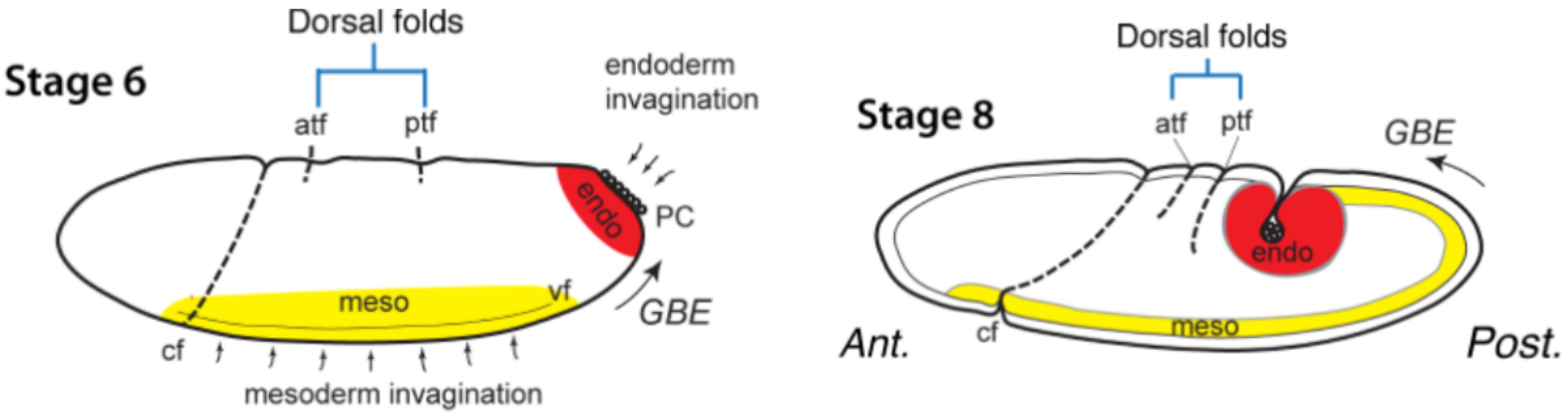
Fixed coordinate system, µm from ventral midline



Comoving coordinate system, 'painted' on at 5 minutes

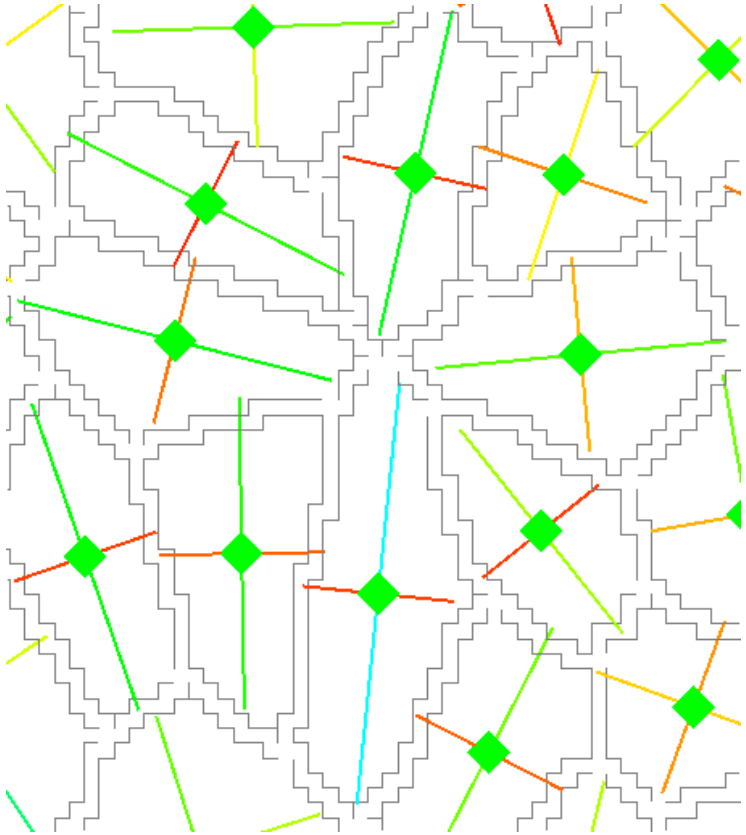


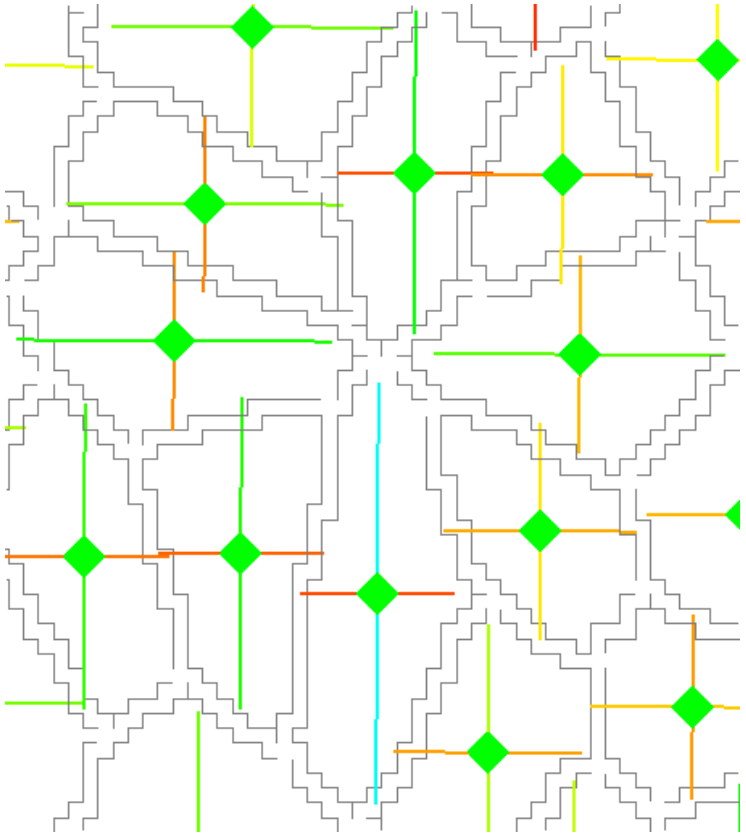
Posterior mid-gut pulls the germ-band



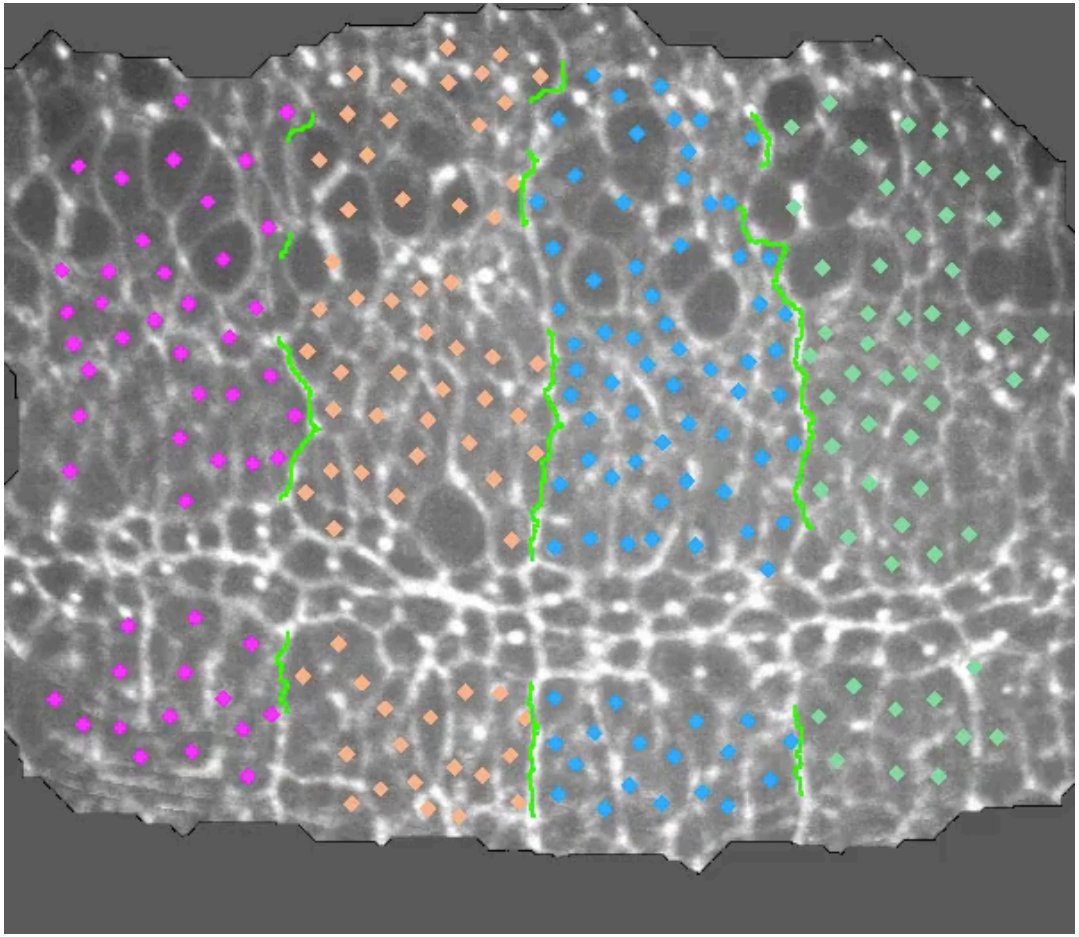
Butler et al., (2009) *Nat. Cell Biol.*; Lye et al., (2015) *PLOS Biology;* see also Collinet et al., (2015) *Nat. Cell Biol.*

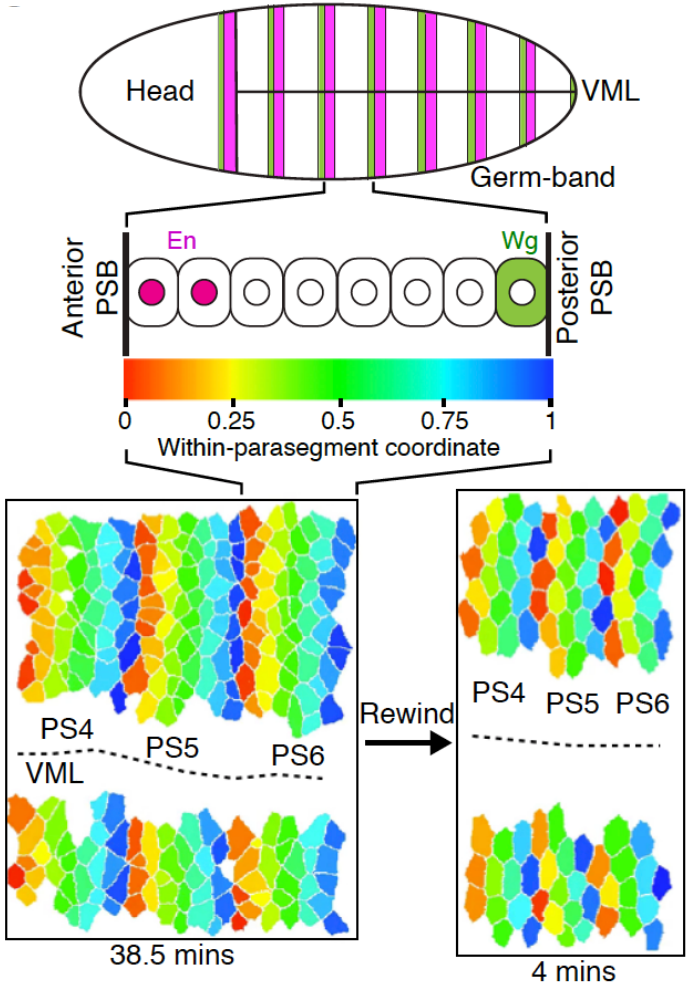
Project 2D or vectorial quantities onto defined axes





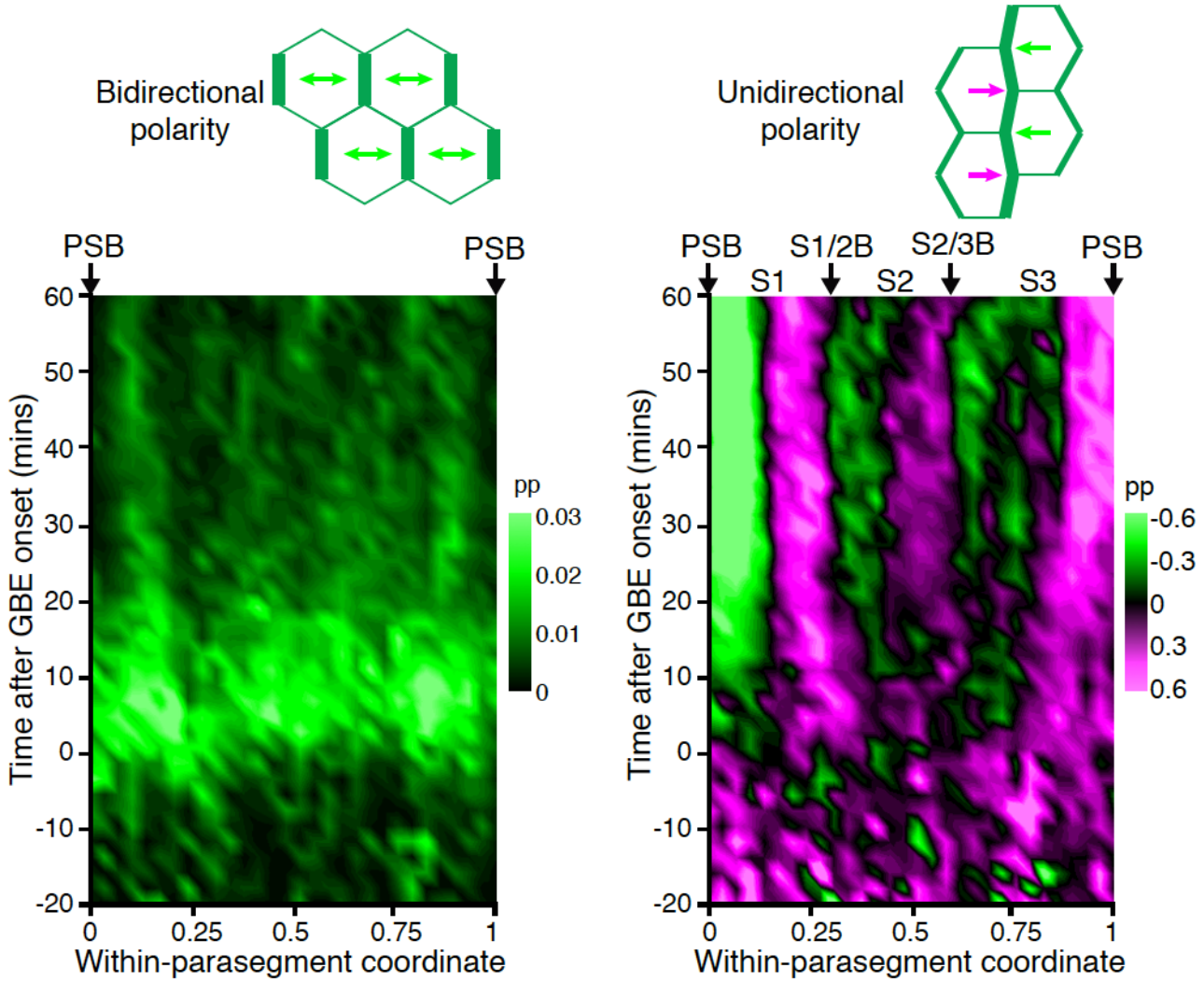
Assigning within-parasegment coordinate system





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

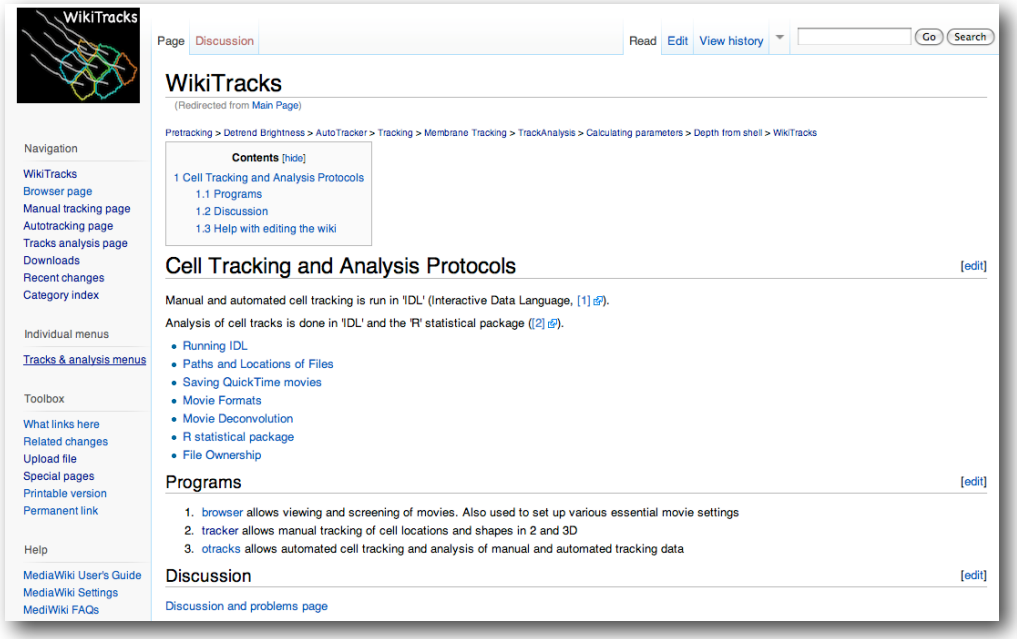
Within-parasegment patterns

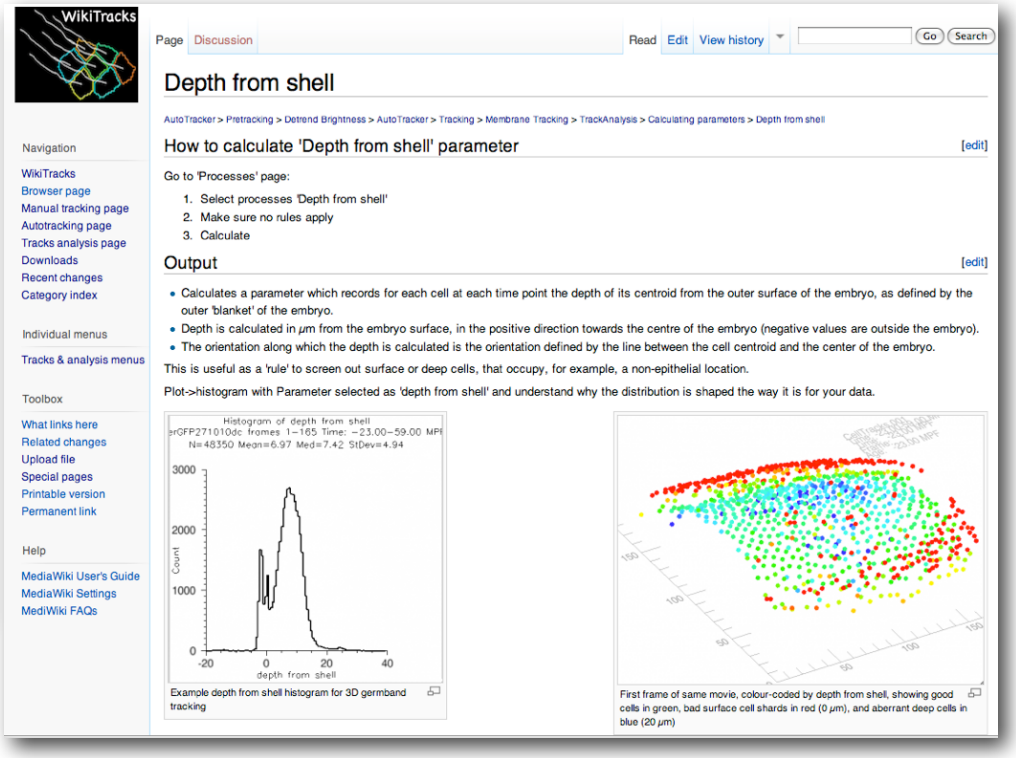


6 embryos, 3-4 parasegments each

Stereotypical within-parasegmental 'cable' locations

WikiTracks





Example pages from WikiTracks web-site

<u>wiki-based resource for sharing tracking and analysis methods</u>

- 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

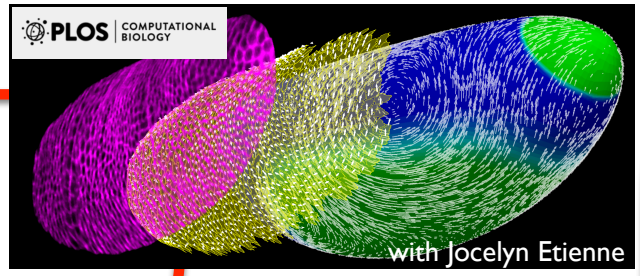
CBM Severo Ochoa – Centro de Biología Molecular

- Nicole Gorfinkiel
- Julia Duque

Biophysics

Université Grenoble Alpes

- Jocelyn Etienne



Germ-band extension

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

Vertex-based modelling

- Alex Fletcher

The University Of Sheffield.

Salivary placode

- Katja Röper
- Alex Booth
- Yara Sanchez-Corrales

Zebrafish

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

BBSRC bioscience for the future

wellcome