Bio-imaging revolution

Keller et al. (2008) Science

Tomer et al. (2012) Nat. Methods

Stephen Young

Zallen lab
Cell tracking & analysis

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

Cadherin-GFP neighbour gain

Cell properties & dynamics
(location, movement, area/volume, best-fit ellipsoid, elongation ratio...)

Cell-cell interface properties & dynamics
(length, orientation, vertex angles, neighbour connectivity...)

Cell tracking & analysis

Ventral view

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

Voronoi tessellated interfaces

Comparison with actual interface length

Focal interface

Cell centroids

Colour scale: Angle Difference from Voronoi Expectation

Interface length deviation (µm)

Time relative to T1 transition (mins)

Non-boundary interfaces

Boundary interfaces
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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

\[\nabla \mathbf{v} = \begin{bmatrix} \frac{\partial u}{\partial x} & \frac{\partial u}{\partial y} \\ \frac{\partial v}{\partial x} & \frac{\partial v}{\partial y} \end{bmatrix} = \text{Tissue strain rate tensor} \hat{\epsilon}_{\text{Tissue}}\]

\[\Omega_{\text{Tissue}}\]

Strain rate:
- expansion
- contraction
- rotation

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

Translation

Dilation

Dilation + translation

Rotation

Rotation + translation

Convergence

Oblique convergence

Pure shear (conv. extension)

Oblique pure shear + translation

Simple shear

Translation velocity (µm/6 mins)

expansion (pp/hr)  contraction (pp/hr)  rotation (radians/hr)
'Discrete’ strain rate methods

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

Using links

Texture tensor

$F \approx \nabla \mathbf{v}$

Using triangles

$<\mathbf{v}^m> \approx \nabla \mathbf{v}$

‘Discrete’ cell behaviours defined by topological changes

- T1 process (rearrangement)
- T2 process (cell loss/gain)
- Cell division

<table>
<thead>
<tr>
<th>Cell centroid</th>
<th>Cell membrane</th>
<th>Cells in contact</th>
<th>Losing contact</th>
<th>Gained contact</th>
</tr>
</thead>
</table>

Kinematic maps

Extension rate
- 0.04 (pp/min)

Convergence rate
- -0.04 (pp/min)

Cell shape change

Cell intercalation

Net strain rates for dividing cell

Cell Shape strain rate tensor
\( \dot{\varepsilon}_{\text{CellShape}} \)

Intercalation strain rate tensor
\( \dot{\varepsilon}_{\text{Intercalation}} \)

Strain rate: \( \leftrightarrow \) expansion \( \rightarrow \) contraction

prophase \( \rightarrow \) anaphase \( \rightarrow \) cytokinesis \( \rightarrow \) relaxation \( \rightarrow \) Cumulative

\[ \text{Cumulative} = \text{prophase} + \text{anaphase} + \text{cytokinesis} + \text{relaxation} = \text{Cumulative} \]
Local impact of cell division

- Domain absorbs cell division
- Domain reflects cell division
- Pre-mitosis
- Post-mitosis

+ Surroundings squash dividing cell (surroundings stiffer than mitosis)
Cell area & Myosin II fluctuations

All cells from example embryo (3LU)
**Drosophila** embryonic morphogenesis

**Gastrulation**

**Germ-band extension**

**Germ-band retraction**

**Dorsal closure**

Tomer et al. (2012) *Nat. Methods*
Amnioserosa dorsal closure

Dorsal view of amnioserosa

stage 13

Membrane (ECad-GFP)

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

**Drosophila dorsal closure trends**

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**

\[ \epsilon_{c,t} = \frac{\text{Area}_{c,t}}{\text{Area0}_{c,t}} - 1 \]

\[ \text{myo}_{c,t} = \frac{\text{Tot.Med.Myos}_{c,t}}{\text{Tot.Med.Myos0}_{c,t}} - 1 \]

Trends retrospective
Rates calculated over 10sec time window

**Minima trends**
Neighbour accommodation: patterns at intermediate scale
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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
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 motors
Actin filaments
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

Kymograph

cortical circumference

time
## Analysis of Epithelial Morphogenesis

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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
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Drosophila embryonic morphogenesis

Gastrulation
Germ-band extension
Germ-band retraction
Dorsal closure

Germ-band extension

Coordinating space & time in WT and mutant embryos

*Drosophila* germ-band tracked data sets

<table>
<thead>
<tr>
<th>embryo A</th>
<th>embryo B</th>
<th>embryo C</th>
<th>embryo D</th>
<th>embryo E</th>
<th>Total cell hours (sampled 30s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>Align embryos by imposing new coordinate system</td>
<td></td>
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<tr>
<td></td>
<td>• x-axis: distance from AP feature (e.g. cephalic furrow)</td>
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<tr>
<td></td>
<td>• y-axis: distance from embryonic mid-line</td>
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<tr>
<td></td>
<td>• time: relative to stage (e.g. start of germ-band elongation)</td>
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<td></td>
</tr>
<tr>
<td>Kruppel</td>
<td><img src="image" alt="Kruppel embryo A" /></td>
<td><img src="image" alt="Kruppel embryo B" /></td>
<td><img src="image" alt="Kruppel embryo C" /></td>
<td><img src="image" alt="Kruppel embryo D" /></td>
<td><img src="image" alt="Kruppel embryo E" /></td>
</tr>
<tr>
<td>Twist</td>
<td><img src="image" alt="Twist embryo A" /></td>
<td><img src="image" alt="Twist embryo B" /></td>
<td><img src="image" alt="Twist embryo C" /></td>
<td><img src="image" alt="Twist embryo D" /></td>
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</tr>
</tbody>
</table>

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

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

- 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

Germ-band extension
- Bénédicte Sanson
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- Tara Finnegan
- Nathan Hervieux
- Thomas Sharrock

Biophysics
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- Alex Fletcher

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- Nora Schultz
- Stephen Young
- Alexandre Kabla
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