Dynamics of dorsal closure during *Drosophila* morphogenesis

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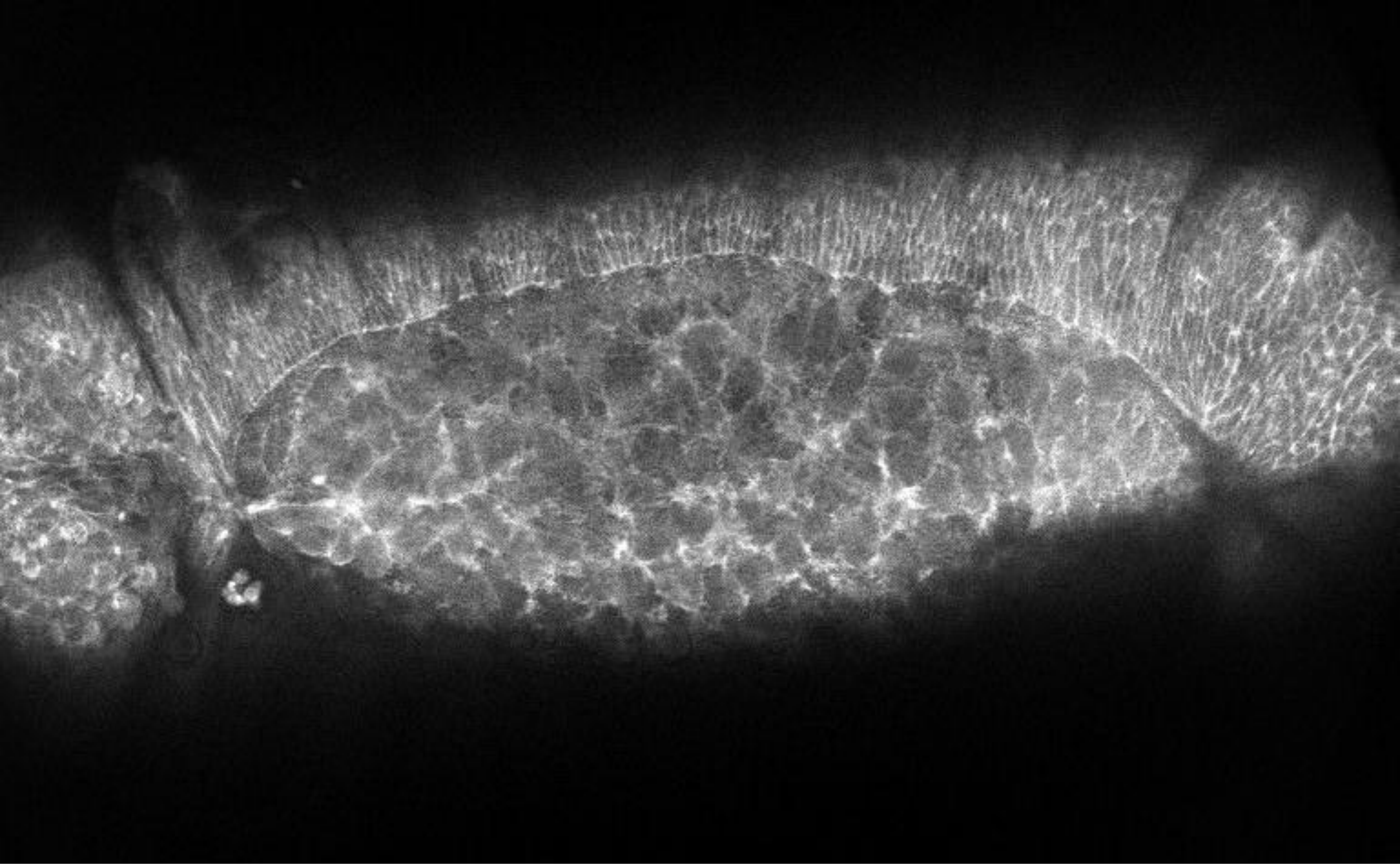
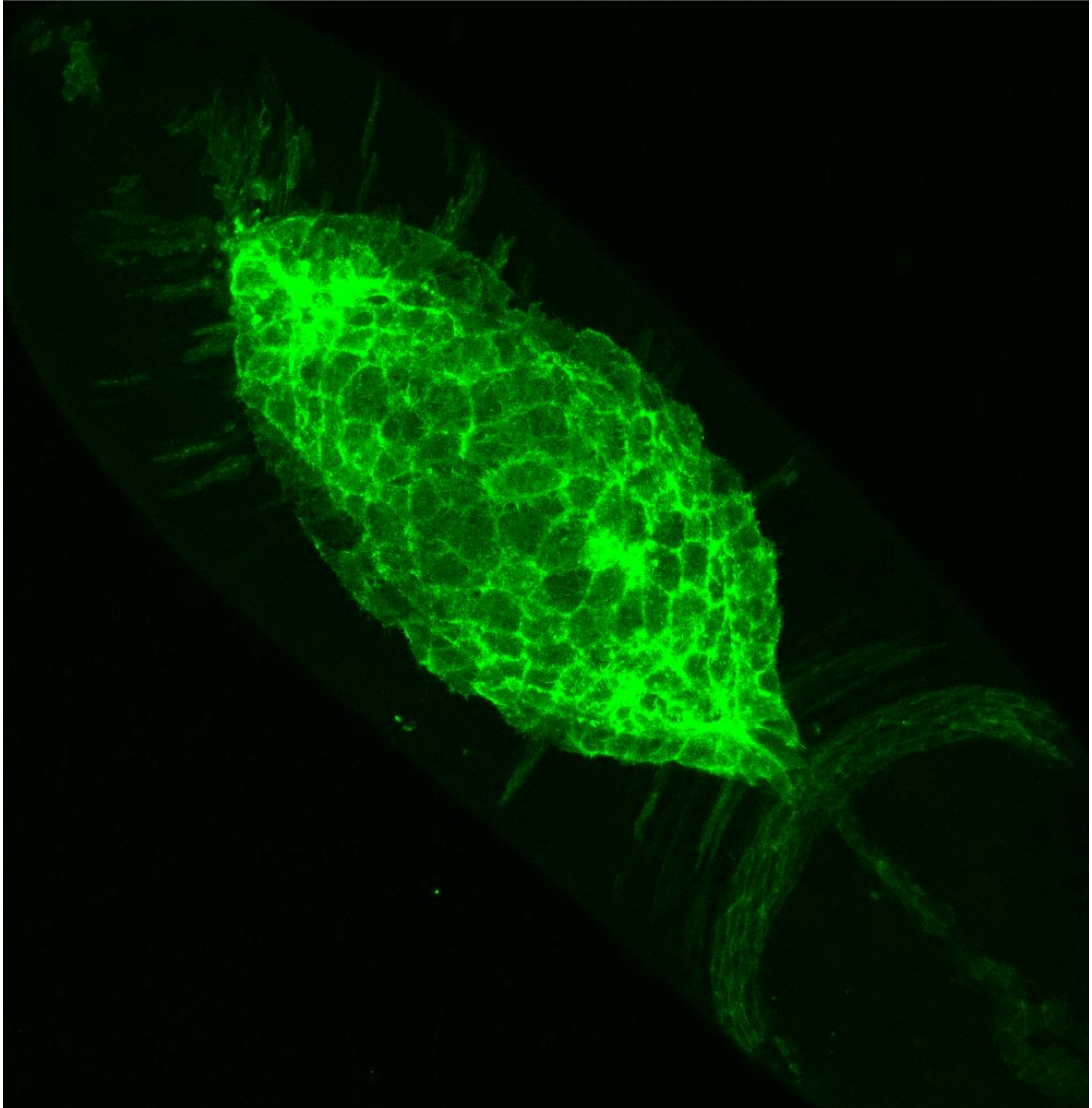
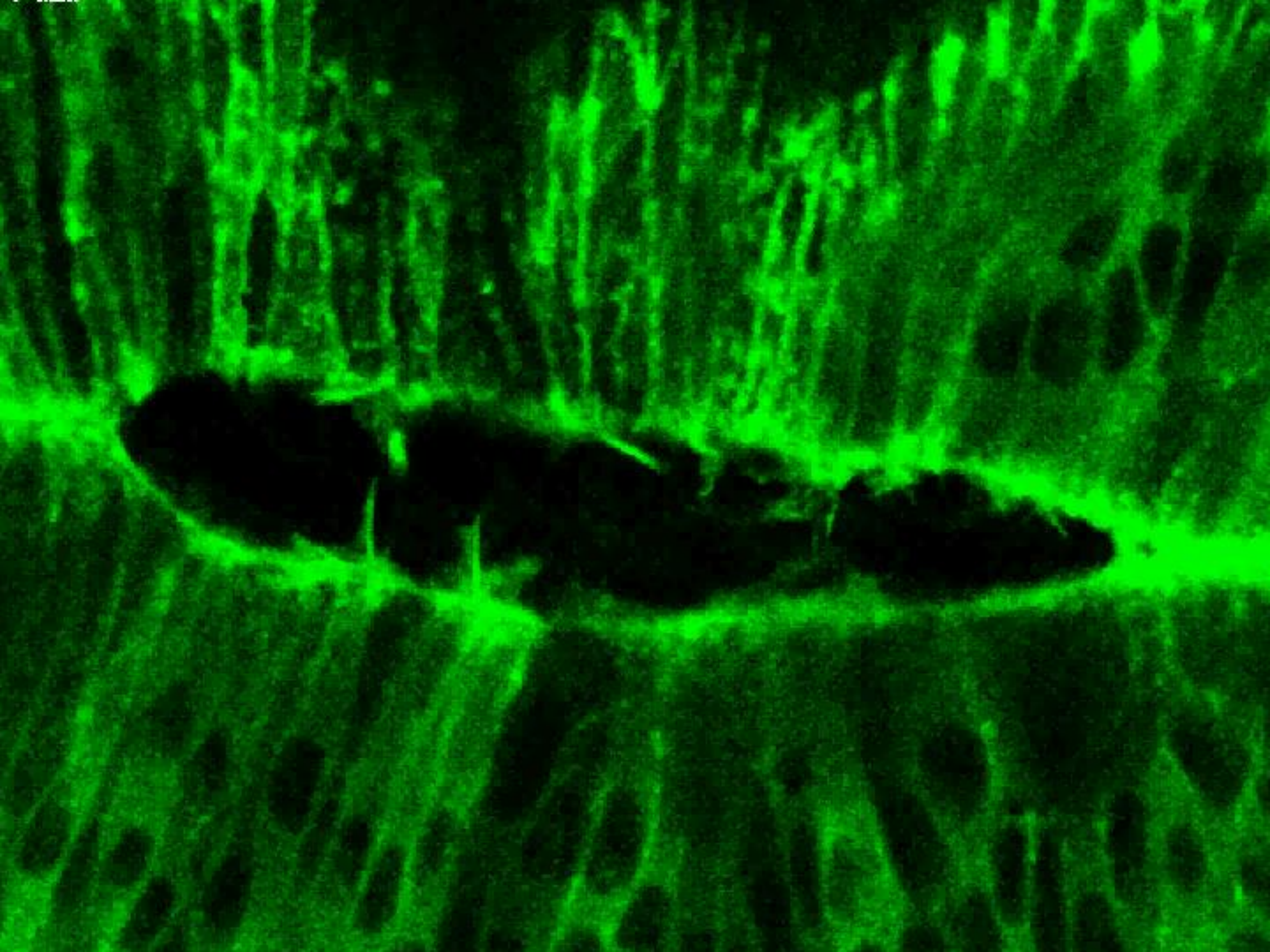


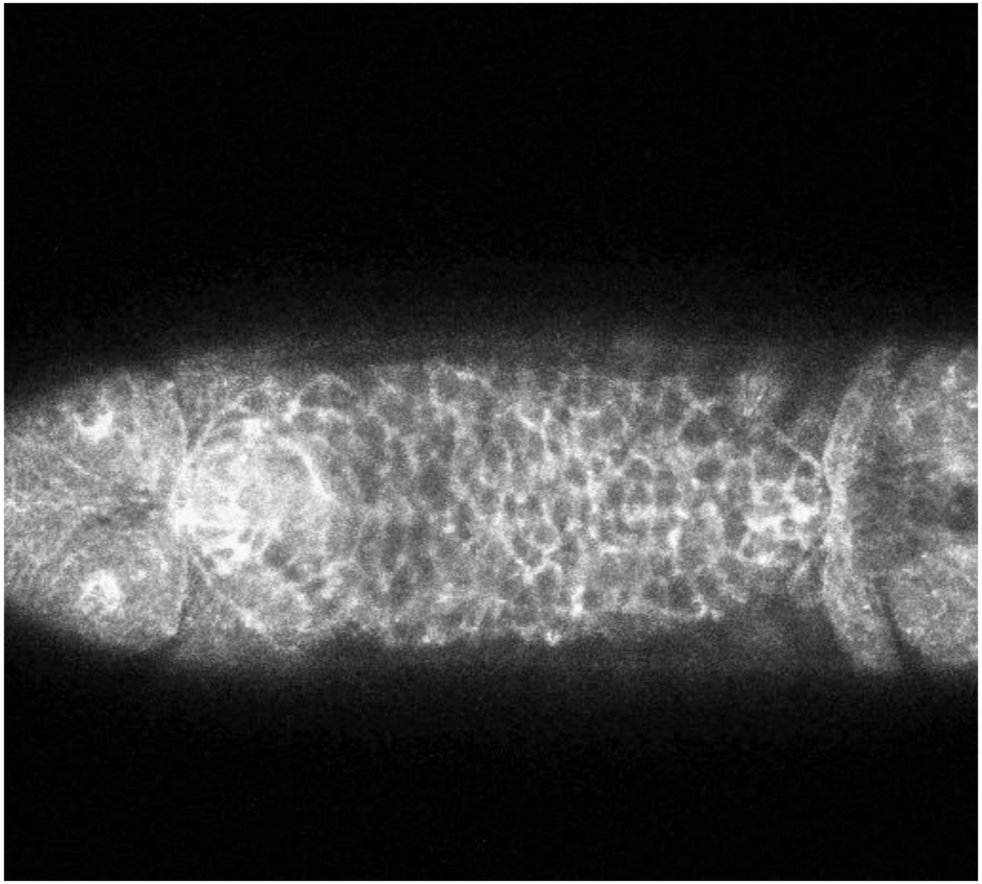
Dorsal closure commences ~10-10.5 hours after egg laying and completes in ~2.5-3 hours.

QT movie of 99b08.mov





Failure during dorsal closure stage in myospheroid mutants



Key Results

1. Ingression (and oscillations) of amnioserosa cells
2. Three classes of cell ingression in the amnioserosa

Adam Sokolow, Ph.D. Dissertation, “Biophysical investigation of cell oscillations and cell ingression in tissue dynamics” 2011

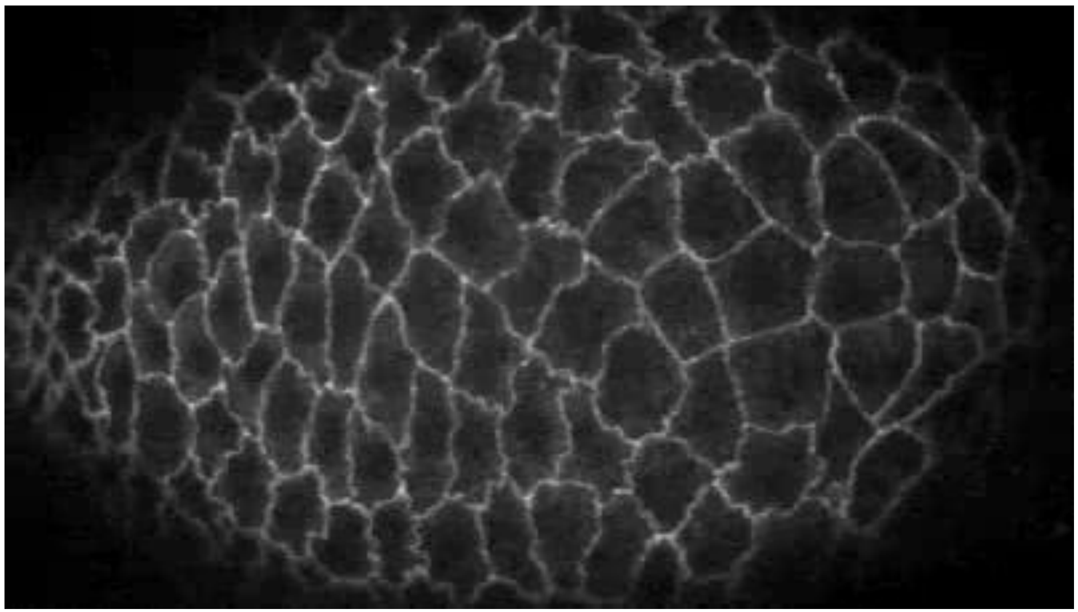
“Cell Ingression and apical shape oscillations during dorsal closure in *Drosophila*,” Adam Sokolow, Yusuke Toyama, Dan Kiehart, and Glenn Edwards (in revision)

3. Regulating and upregulating tissue dynamics by cell ingression

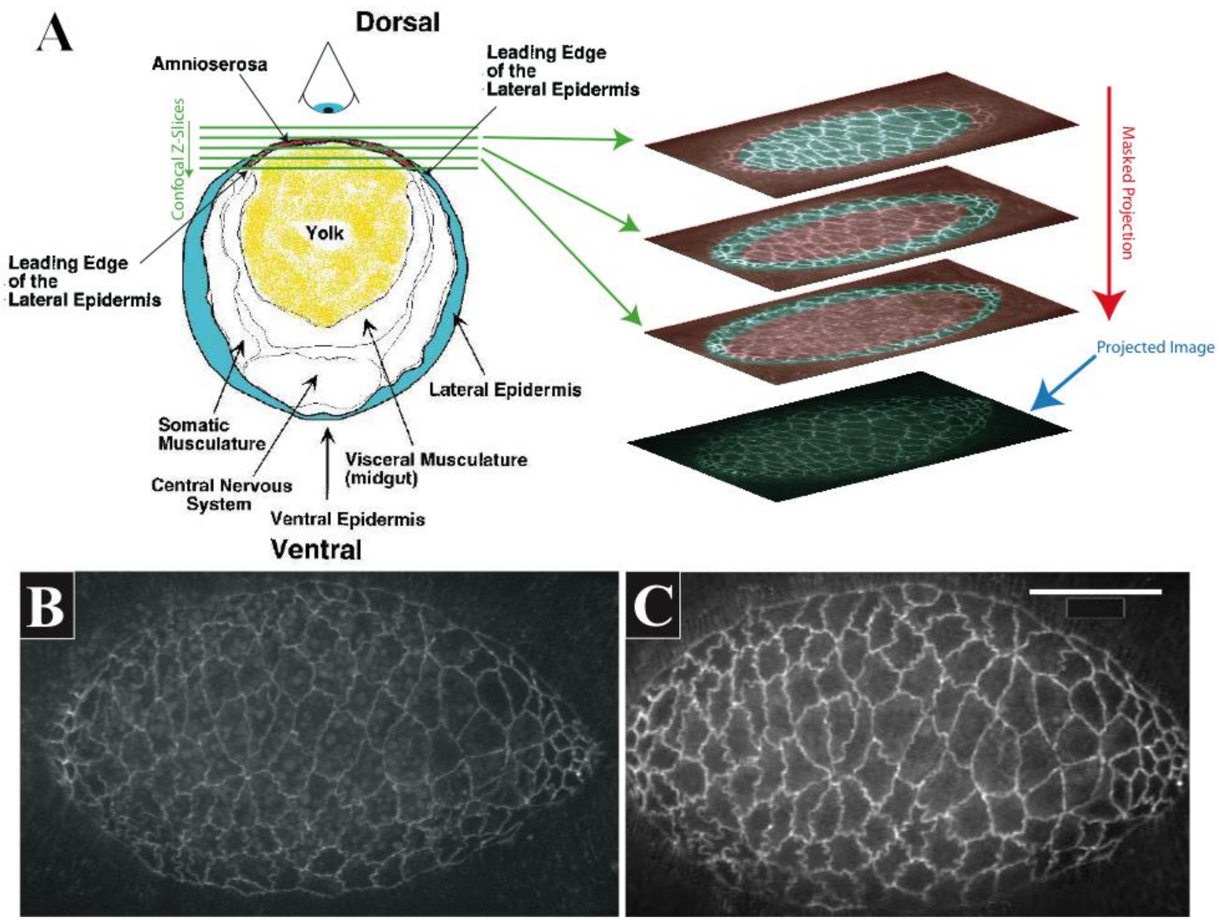
Science 300, 145 (2003); *Cell Biology: A Laboratory Handbook, 3rd edition*, edited by J.E. Celis (editor), Chapter 9, pages 87 (2006); Biophysical Journal 92, 2583(2007); Physical Biology 5, 015004 (2008) ; Human Frontier Science Program Journal 2, 220 (2008); Science 321, 1683 (2008)

Ingression and oscillations of amnioserosa cells

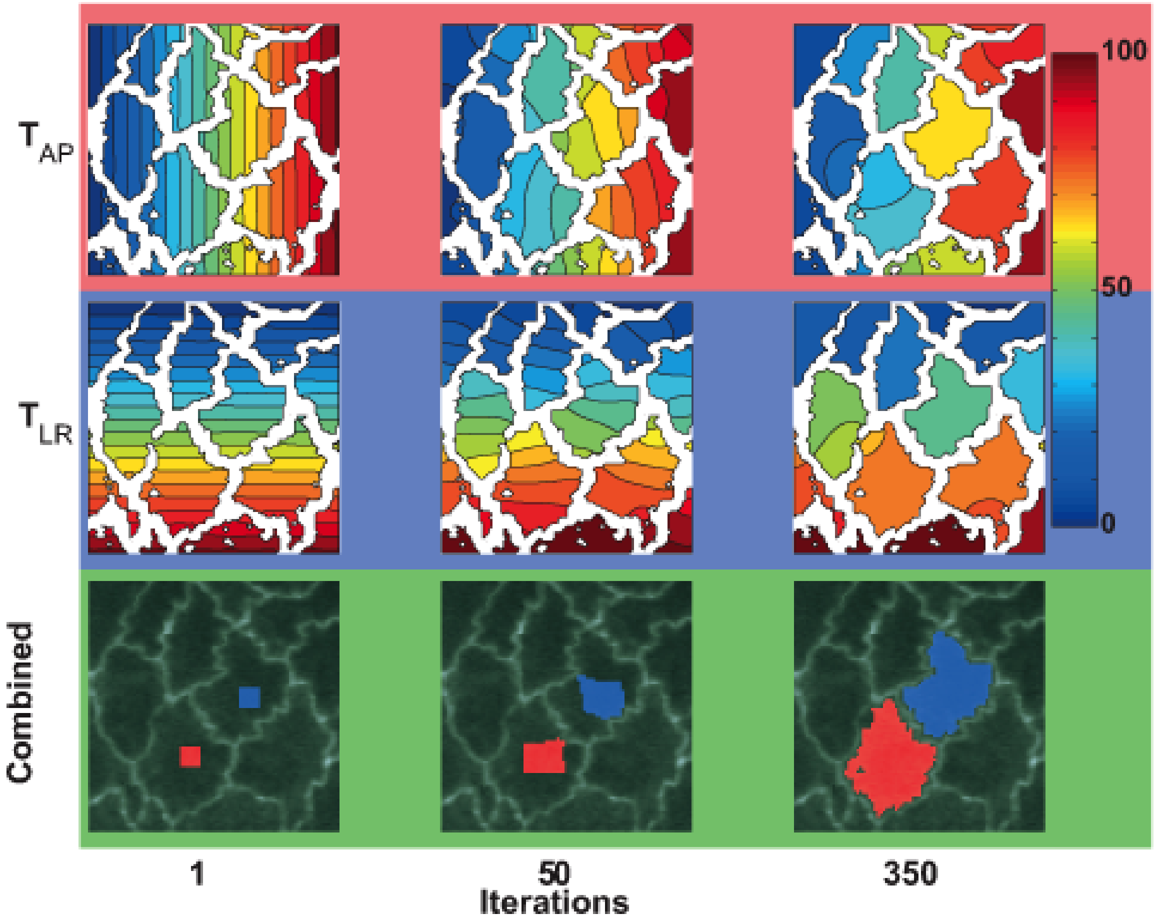
Time series of confocal images of dorsal closure (DE-cadherin)



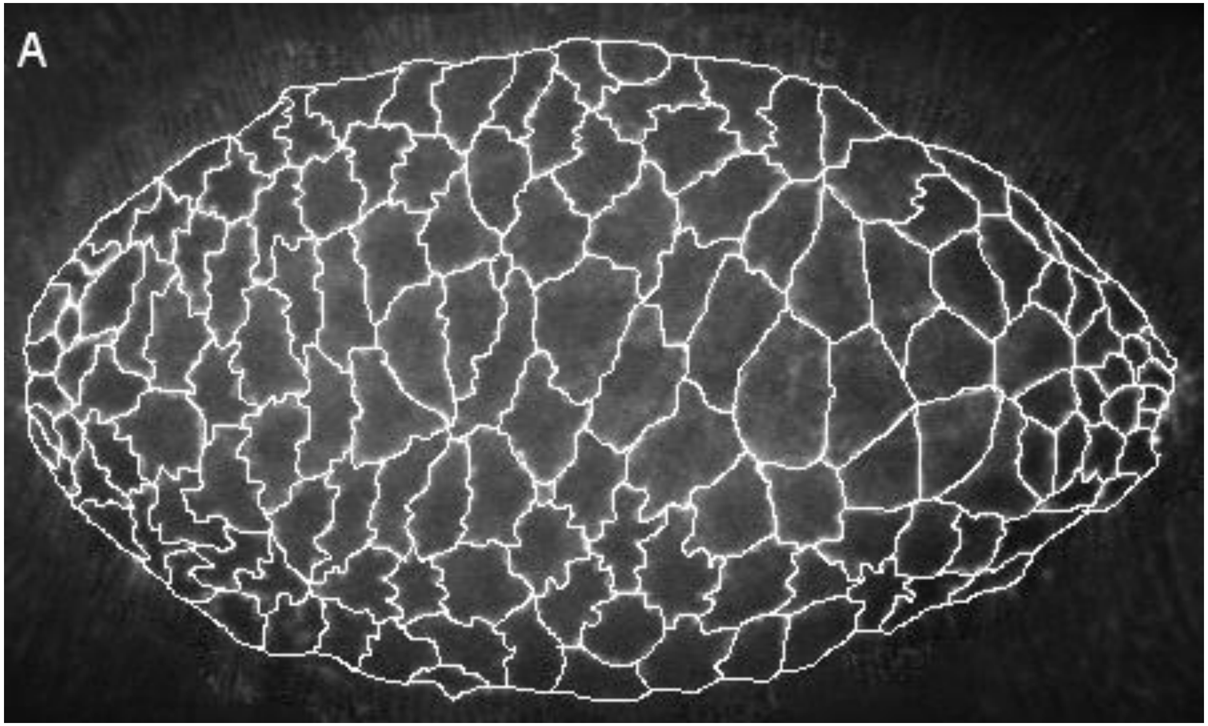
Mask projection method



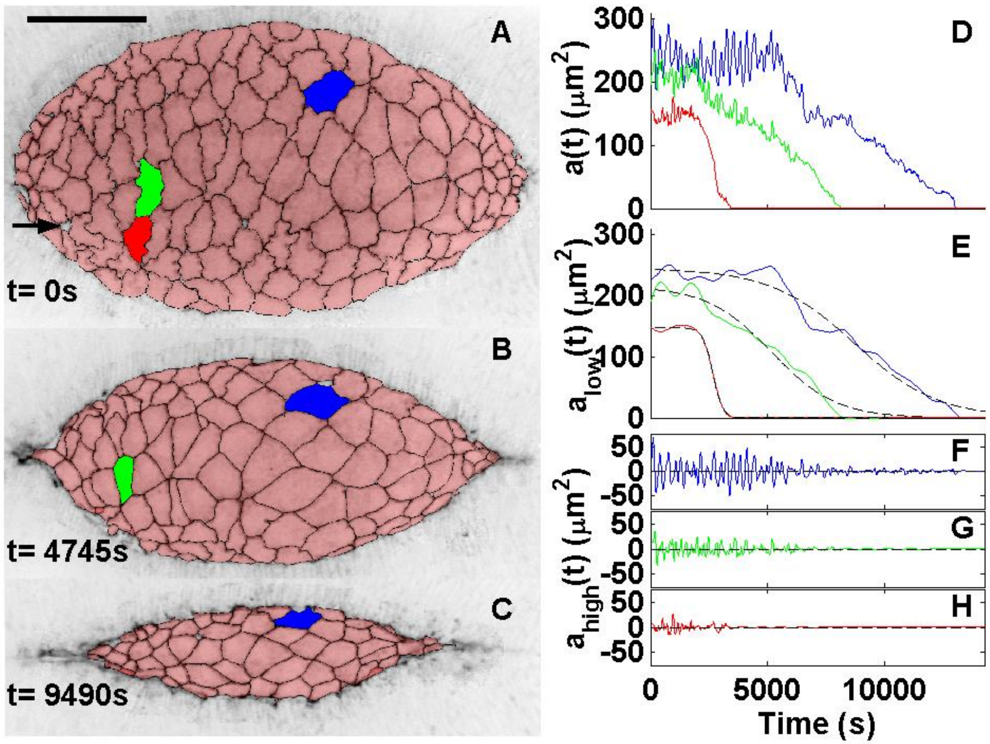
Segmentation algorithm: seed segmentation



Time series of segmented confocal images of dorsal closure (DE-cadherin)



Ingression and oscillation of amnioserosa cells



Signal analysis

$$a_i(t) = a_{low,i}(t) + a_{high,i}(t)$$

$$I_i(t) = (\alpha_i/2)\,[1 - \tanh(\varepsilon_i t - \tau_i)]$$

Biophysical modeling

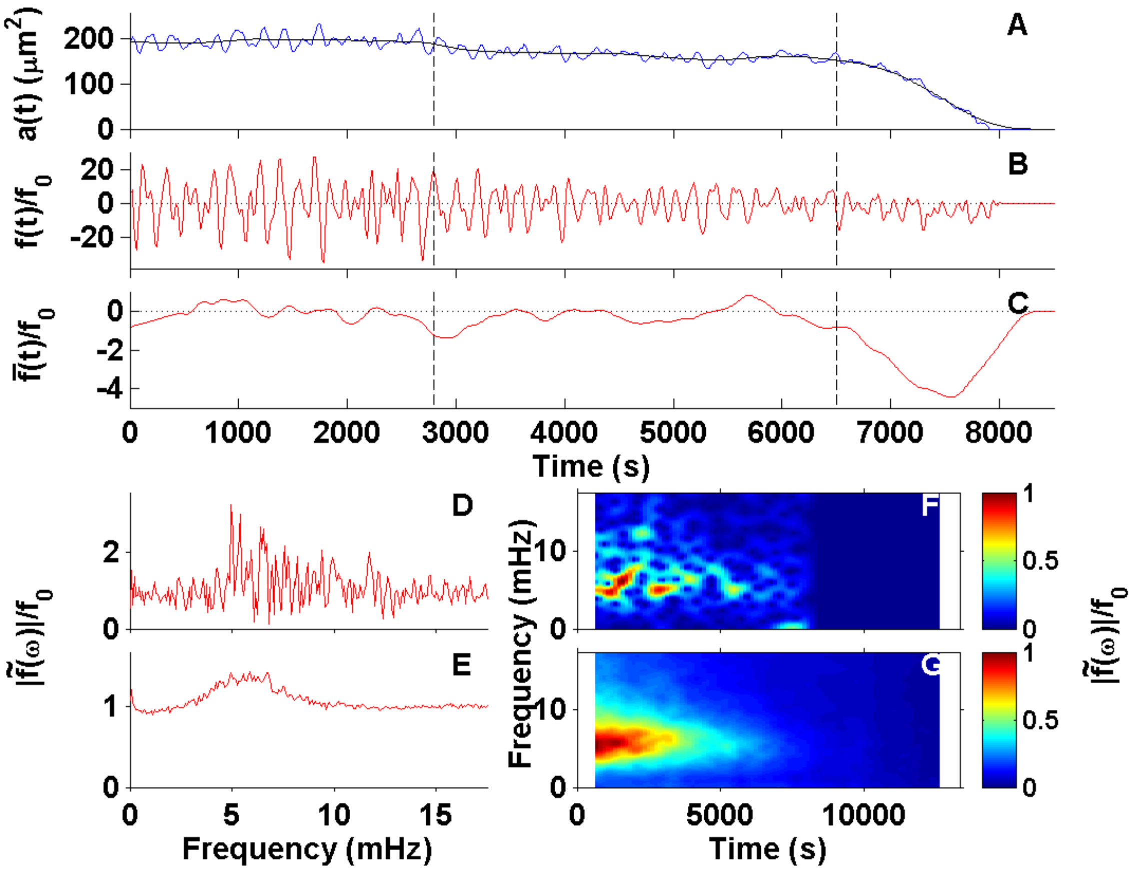
$b\, da_i(t)/dt = f_i(t) - k_{eff,i} a_i(t)$

$f_i(t)/f_{o,i} = (b/f_{o,i})\, [da_i(t)/dt + (k_{eff,i}/b)\, a_i(t)]$

Taking the Fourier transform, solving for $A_i(\omega)$:

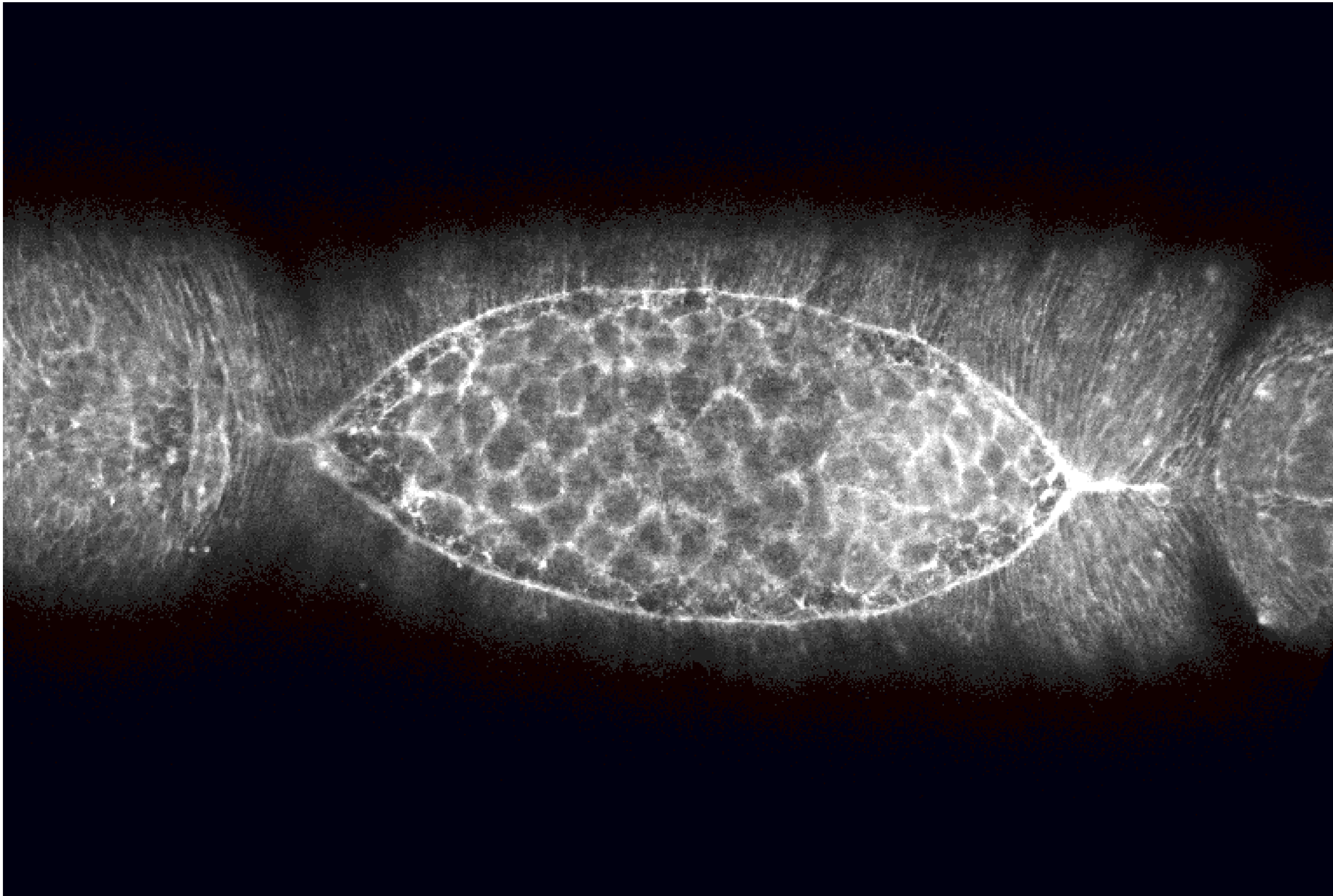
$A_i(\omega) = \{(b\omega[1 + (k_{eff,i}/b\omega)^2]\}^{-1}\, F_i(\omega)$

Determination of a spatially averaged, net force in a relatively fast closing cell

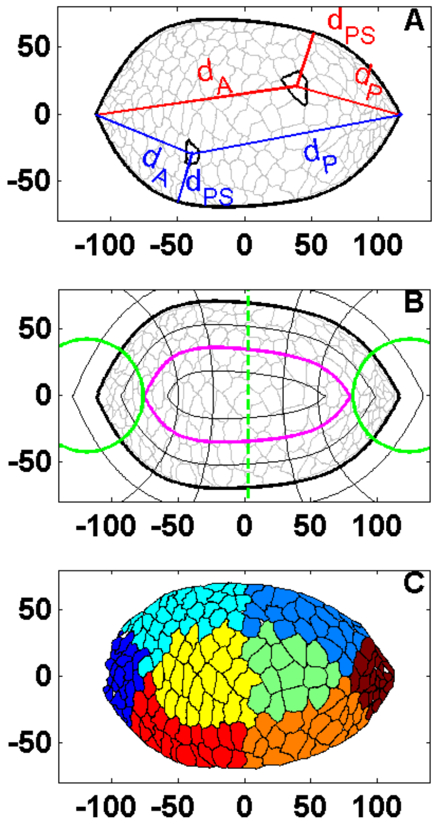


Three classes of cell ingression for amnioserosa cells

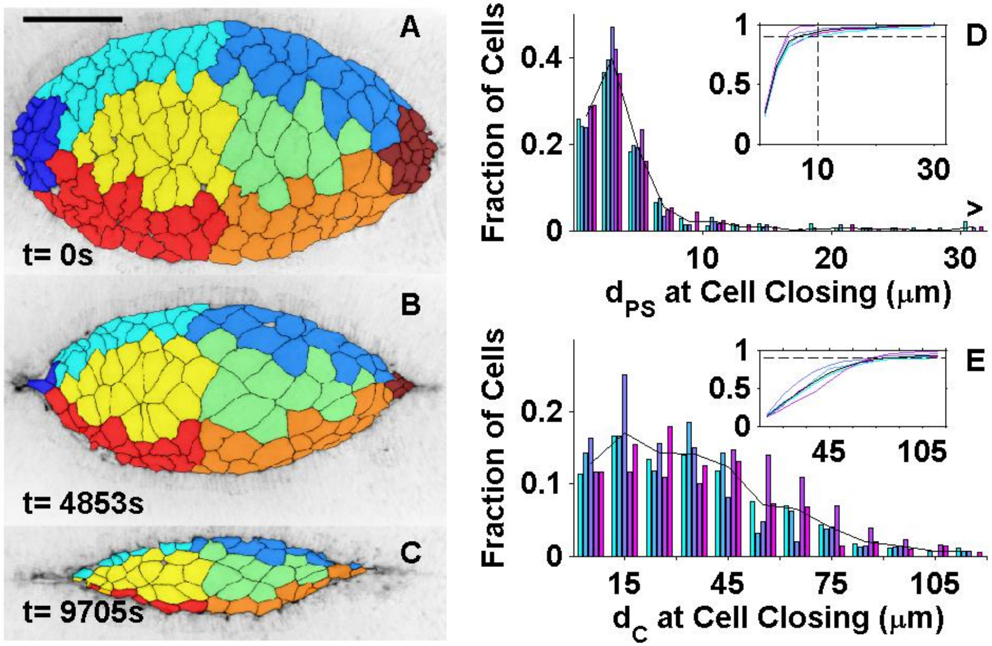
Dorsal Closure



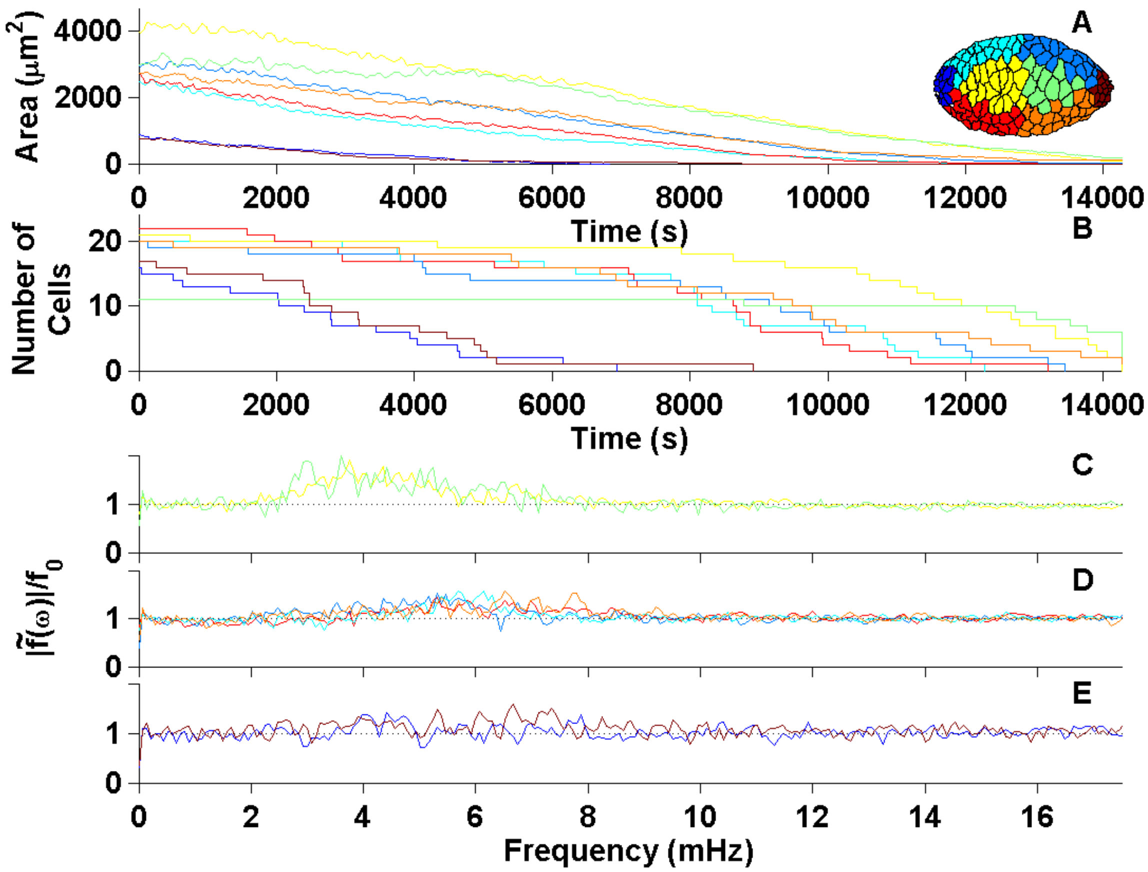
Regionalization Geometry



Location of cell ingression

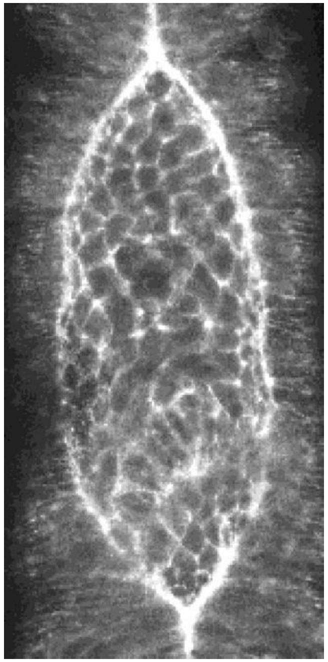


Regional analysis of closure dynamics



Regulation of dorsal closure and ingression

Segmenting the two leading edges
Active Contours (Snakes)



Raw Image

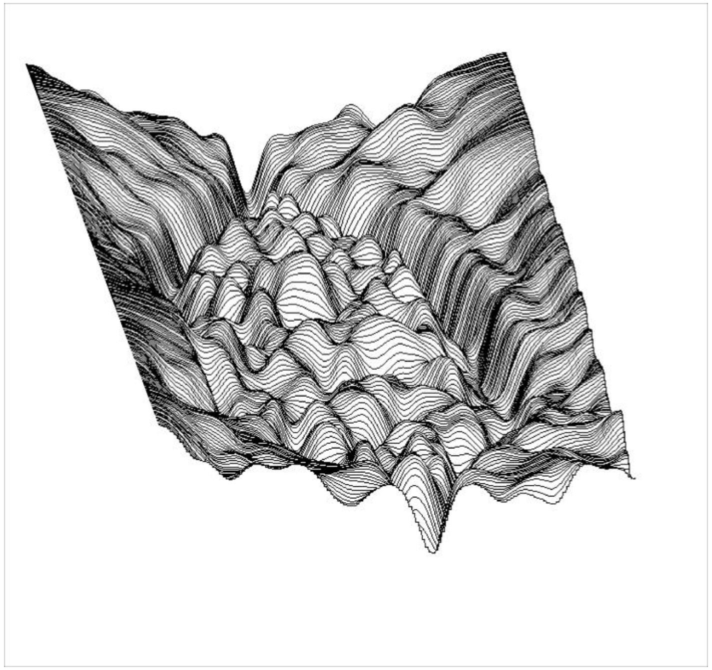
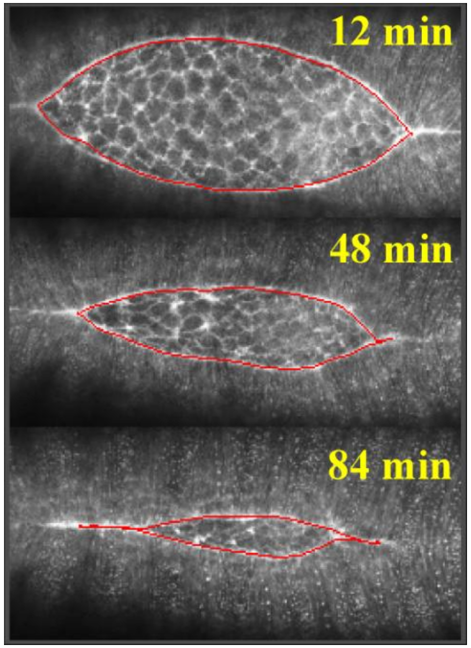
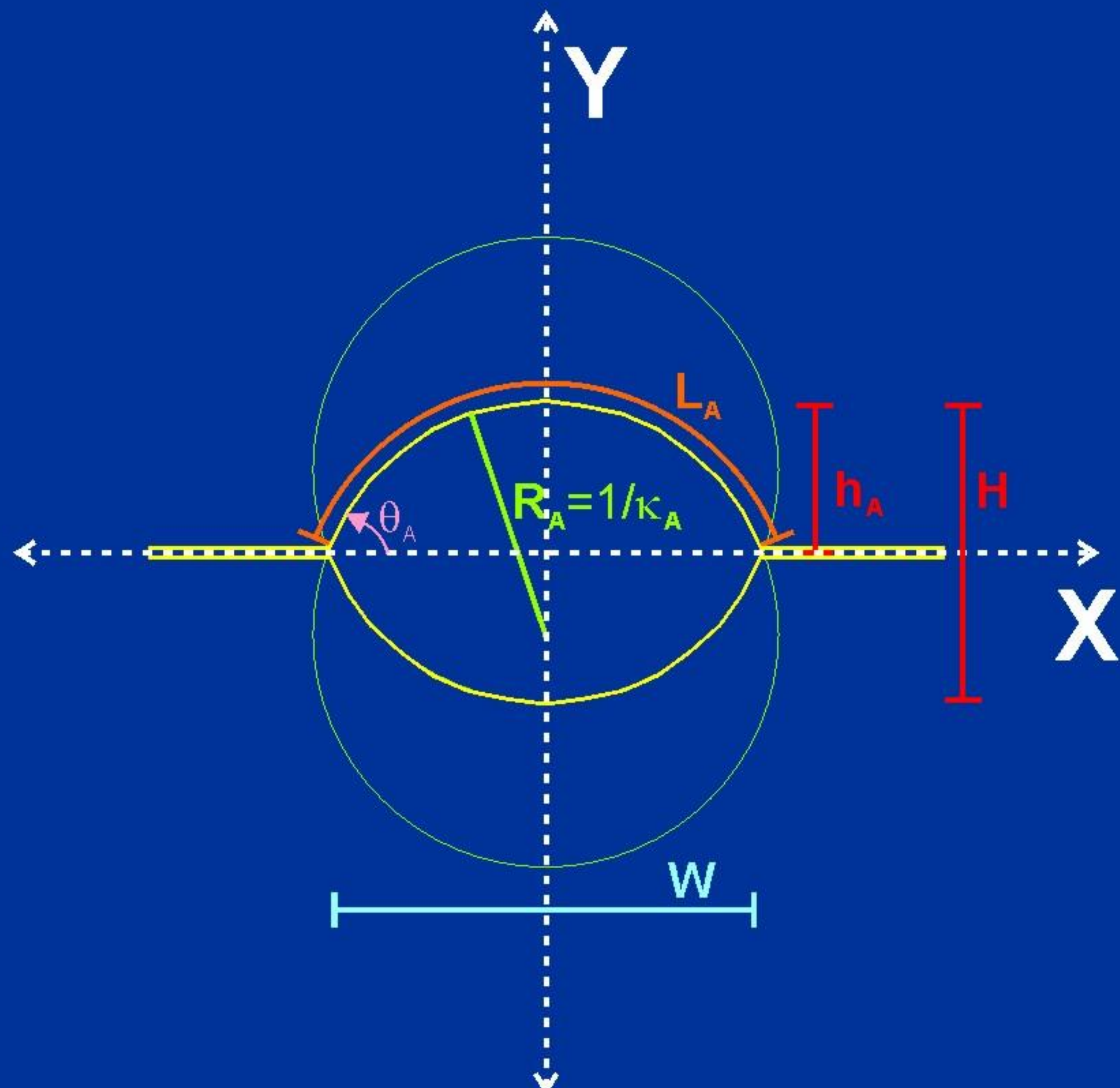
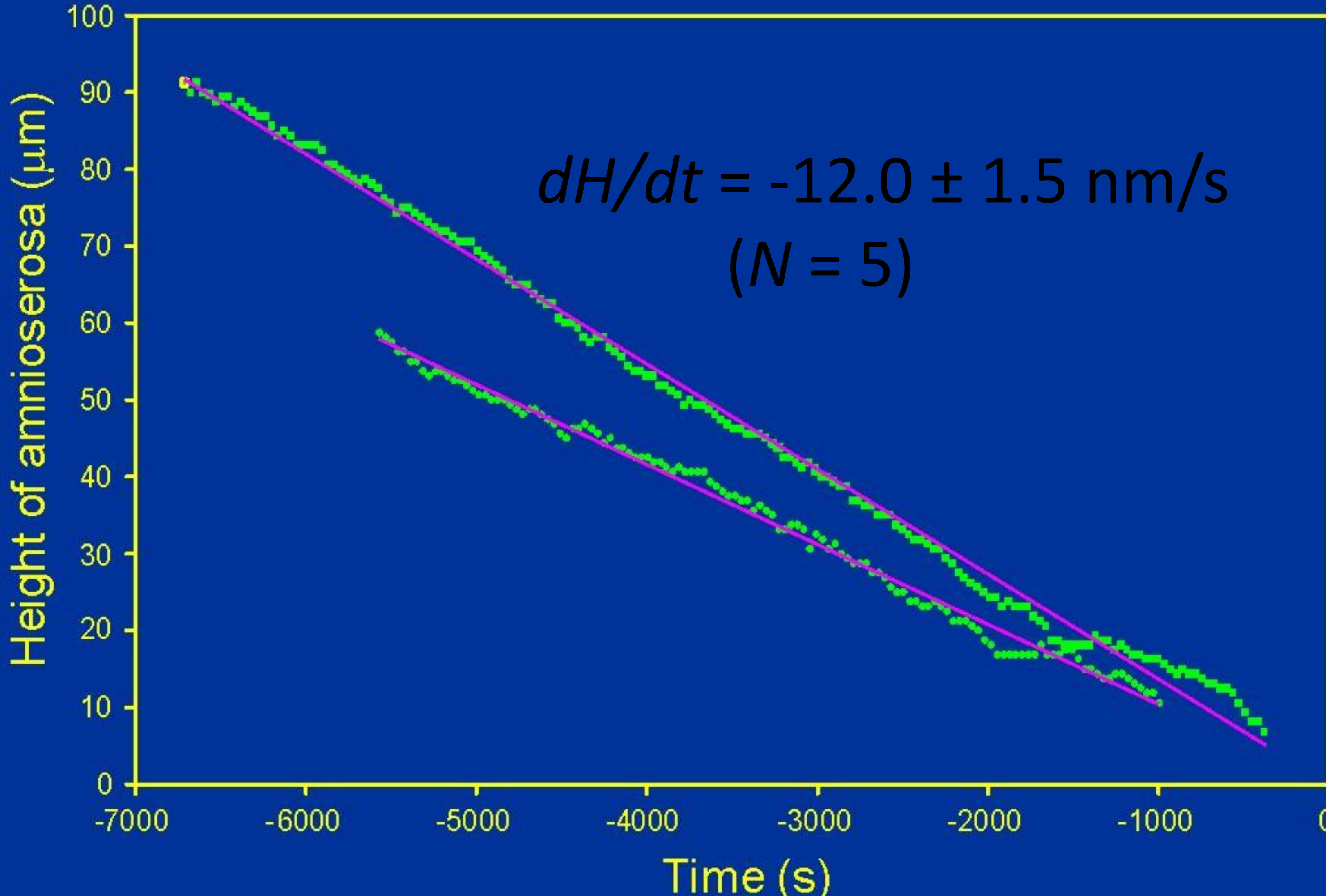


Image as an Inverse Intensity Landscape

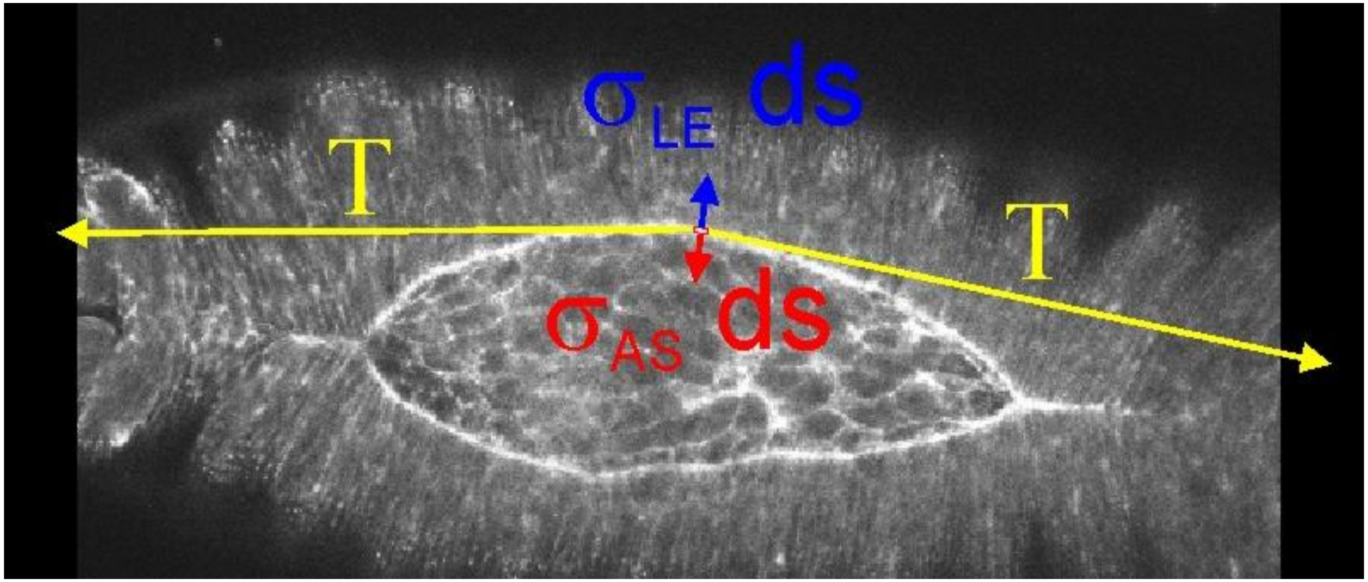


Images with Overlaid Snakes


Y
X
L_A
h_A
H
$R_A=1/\kappa_A$
θ_A
W



$dH/dt = -12.0 \pm 1.5$ nm/s
($N = 5$)
Height of amnioserosa (μm)
Time (s)
100
90
80
70
60
50
40
30
20
10
0
-7000
-6000
-5000
-4000
-3000
-2000
-1000
0

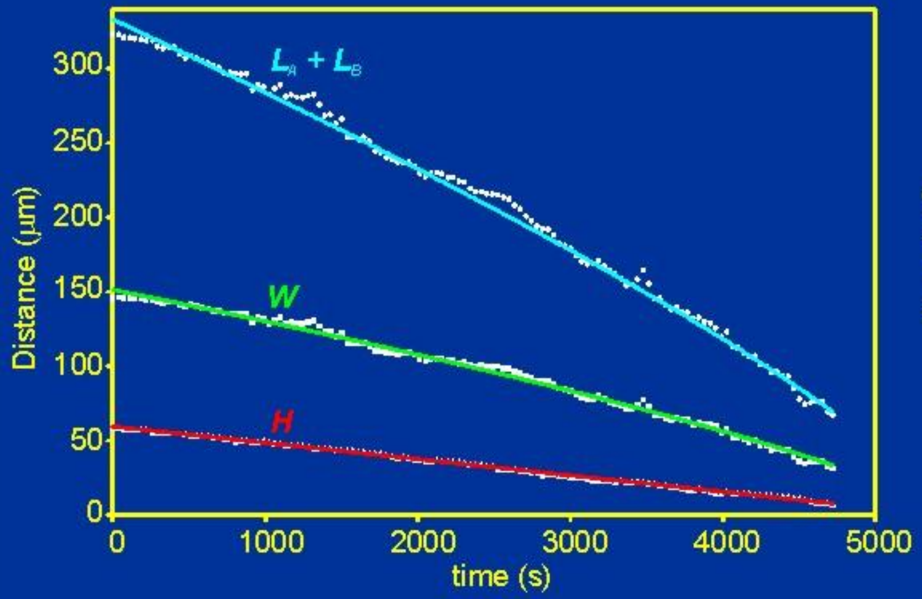


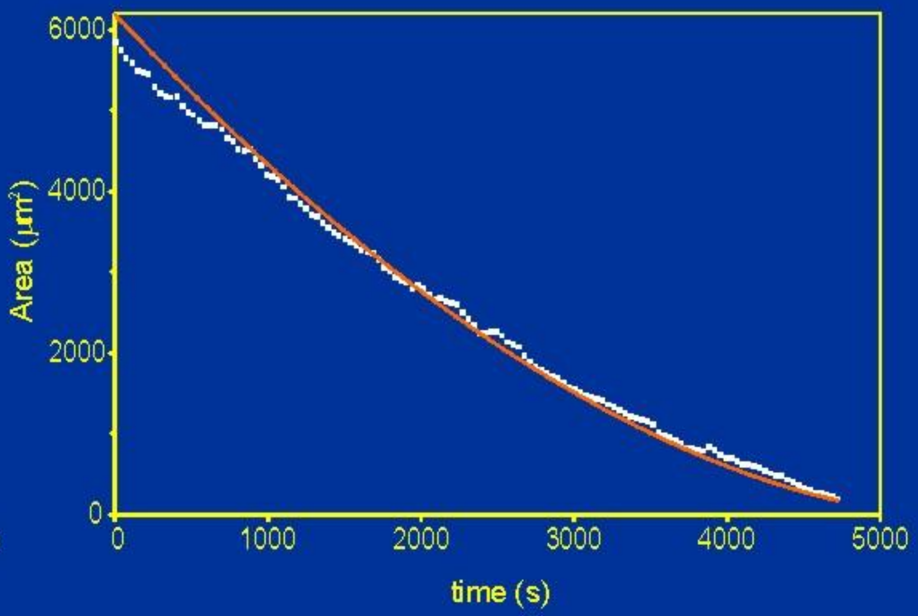
$$\frac{dW}{dt} = \frac{-k_z}{\tan(\theta_A/2) + \tan(\theta_B/2)} = \frac{-k_z W}{2H}$$

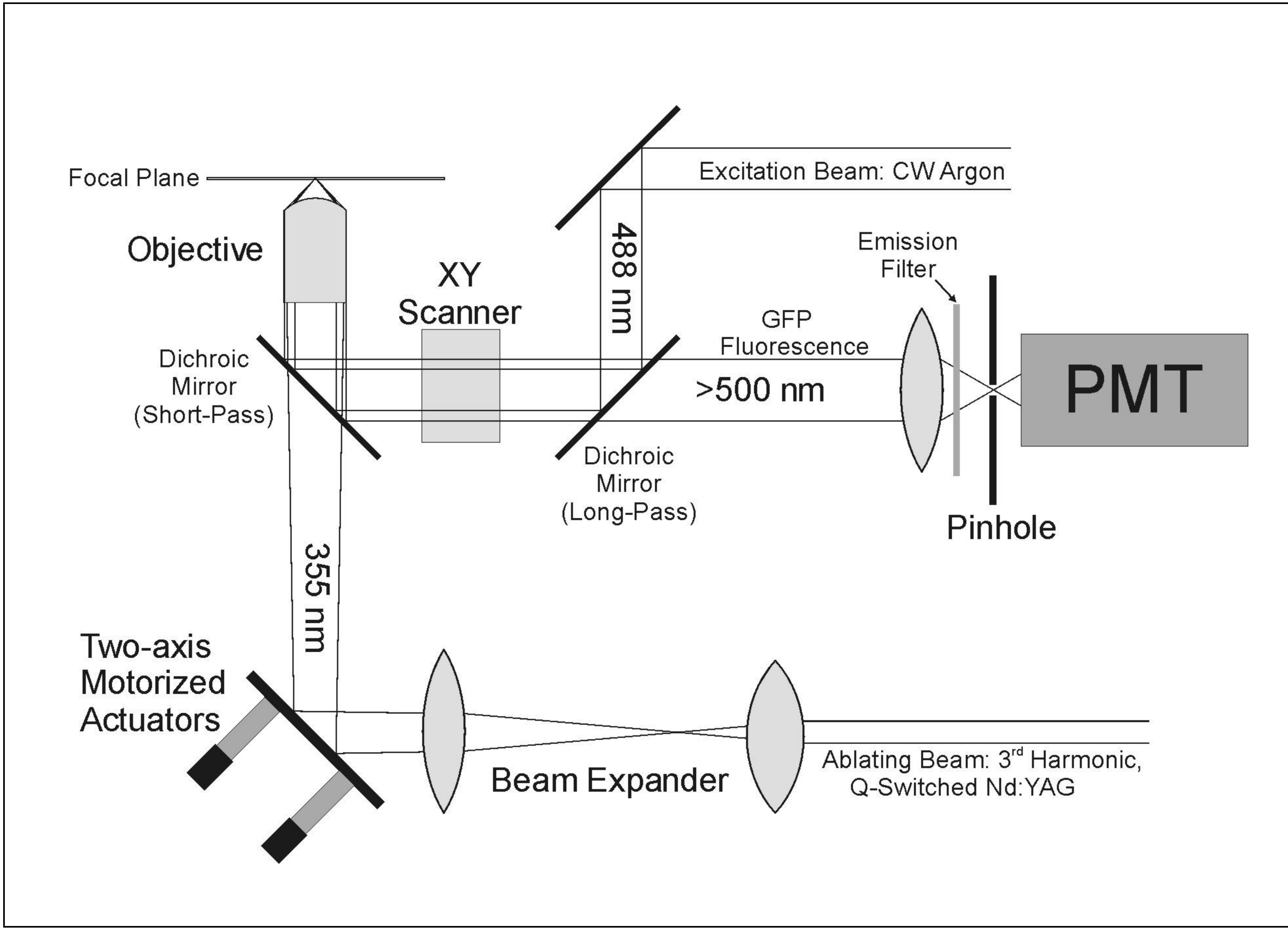
$$h_{A/B}(t) = h_{A/B}(0) - \mathrm{v}_{A/B}\, t$$

$$W(t) = W(0)\left(\frac{h_A(0) + h_B(0) - Vt}{h_A(0) + h_B(0)}\right)^{k_z/2V}$$

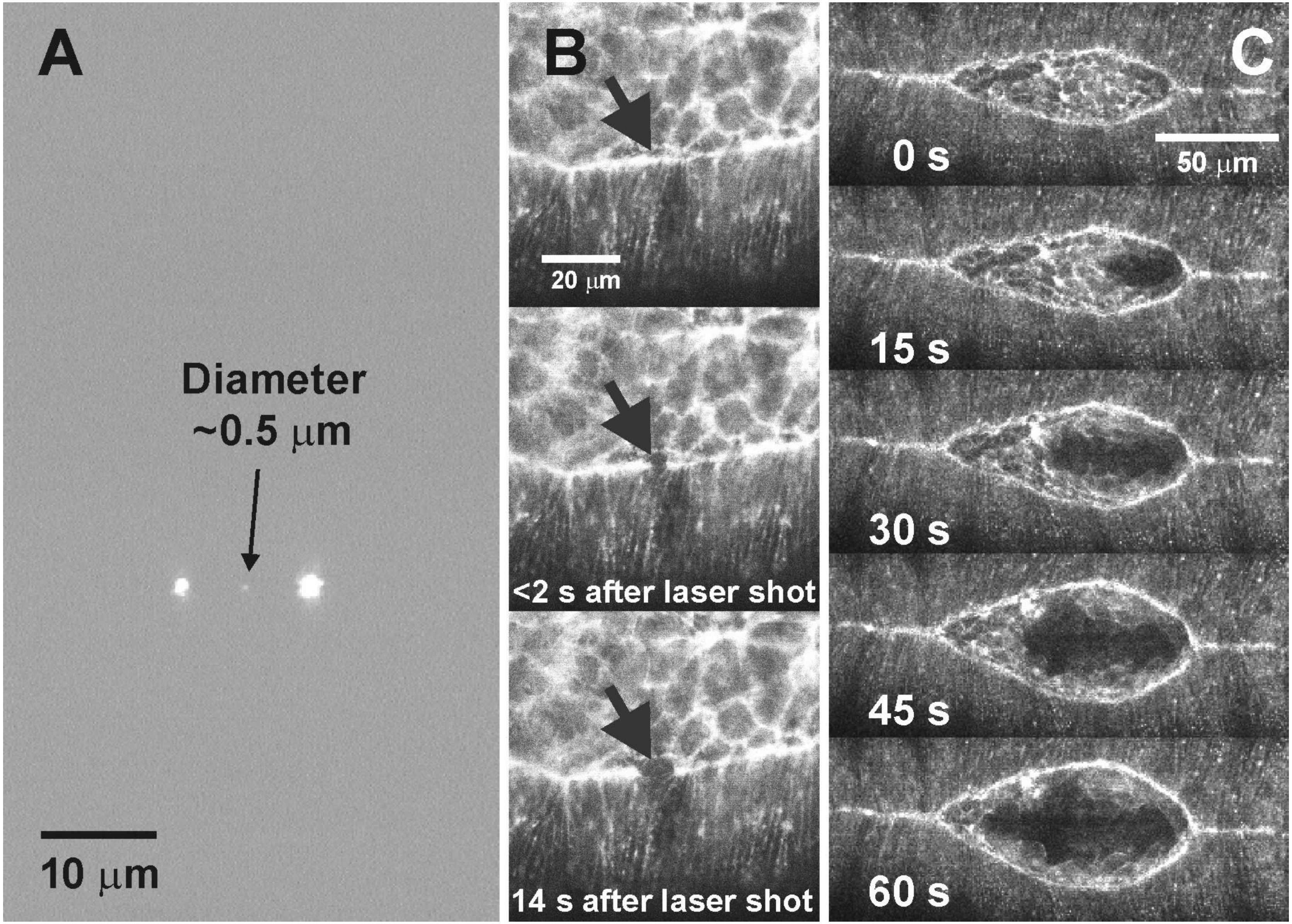
Quantitative model of wild-type dorsal closure based on (bio)physical reasoning



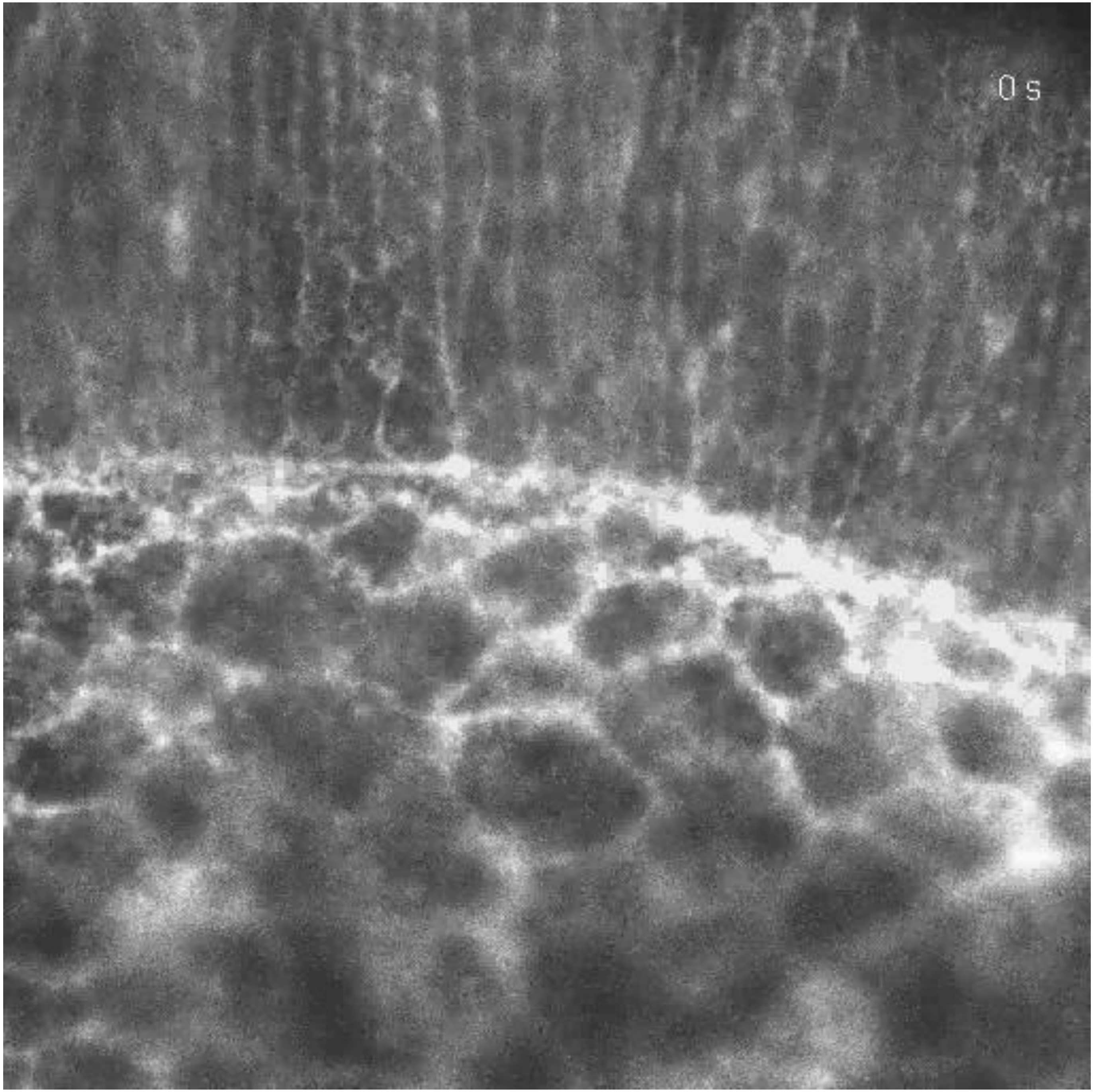


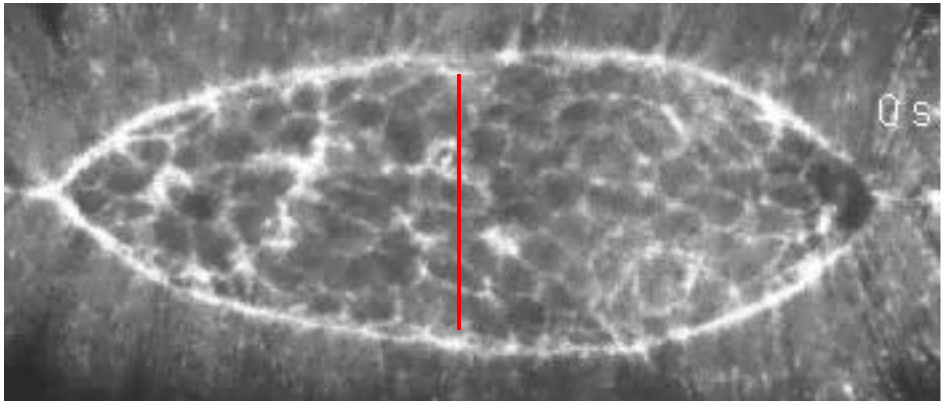


Focal Plane
Objective
XY
Scanner
Excitation Beam: CW Argon
488 nm
Emission
Filter
GFP
Fluorescence
Dichroic
Mirror
(Short-Pass)
>500 nm
PMT
Dichroic
Mirror
(Long-Pass)
Pinhole
355 nm
Two-axis
Motorized
Actuators
Beam Expander
Ablating Beam: 3rd Harmonic,
Q-Switched Nd:YAG


A
Diameter
~0.5 μm
10 μm
B
20 μm
<2 s after laser shot
14 s after laser shot
C
50 μm
0 s
15 s
30 s
45 s
60 s

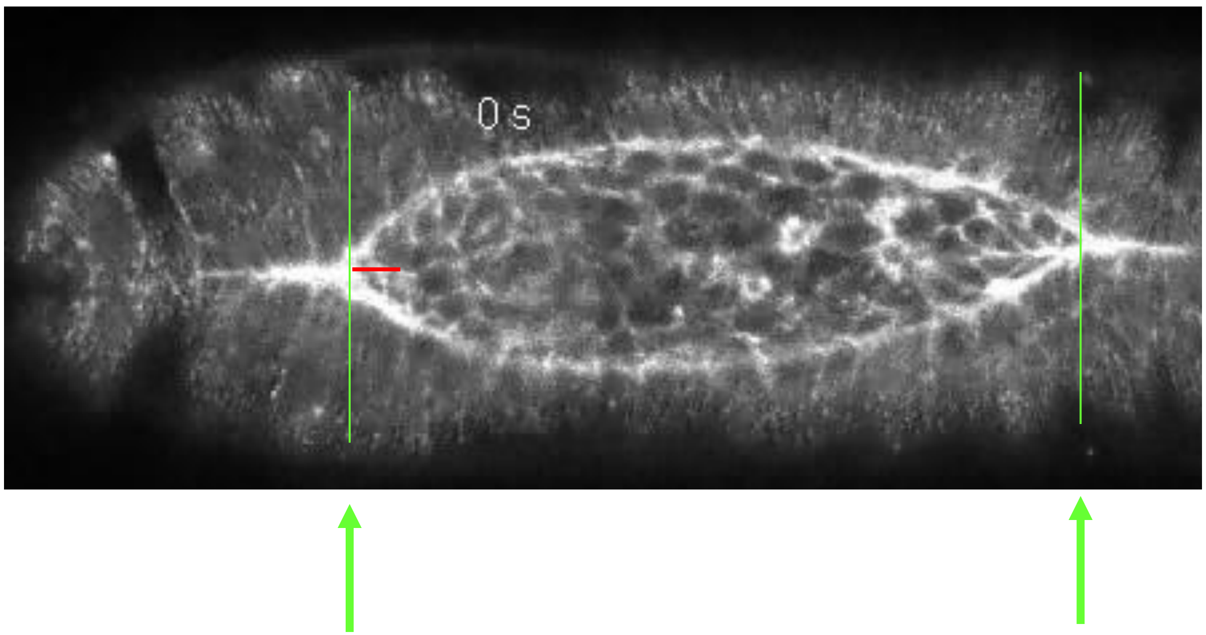
Laser perturbation indicates tissue is under tension and capable of "wound healing."



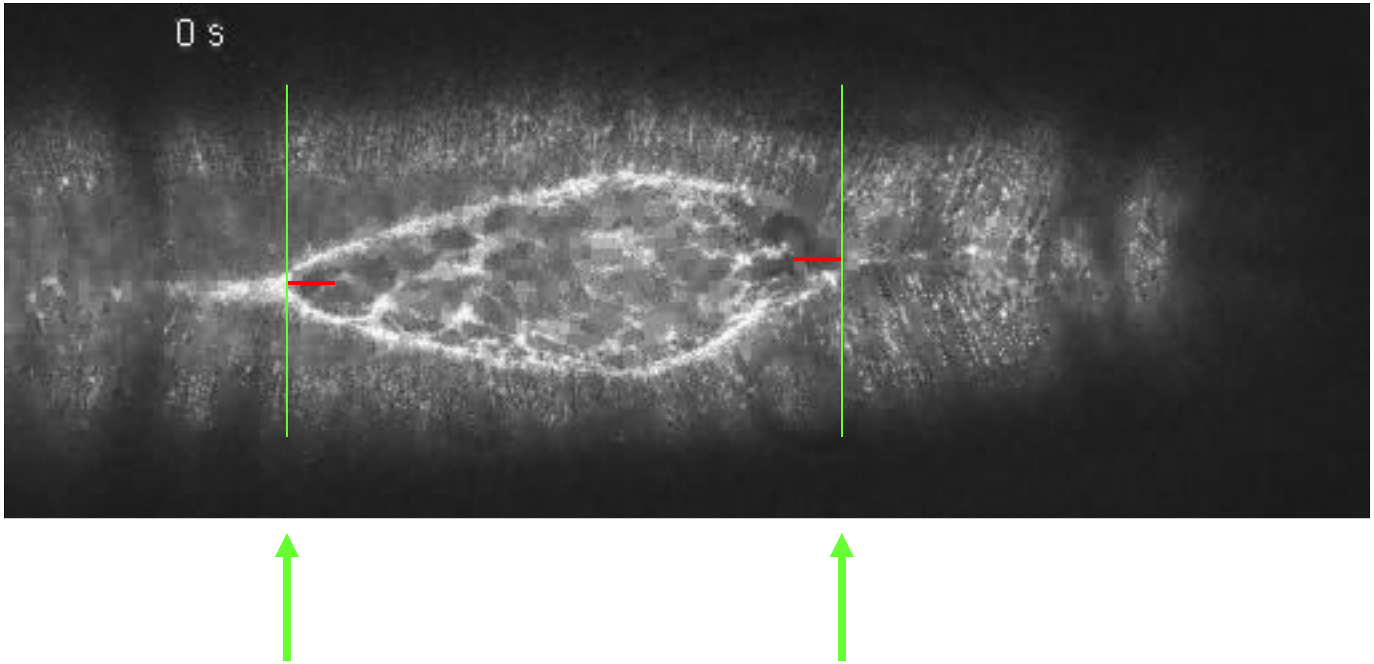


Laser perturbation to the amnioserosa also exhibits a wound healing response

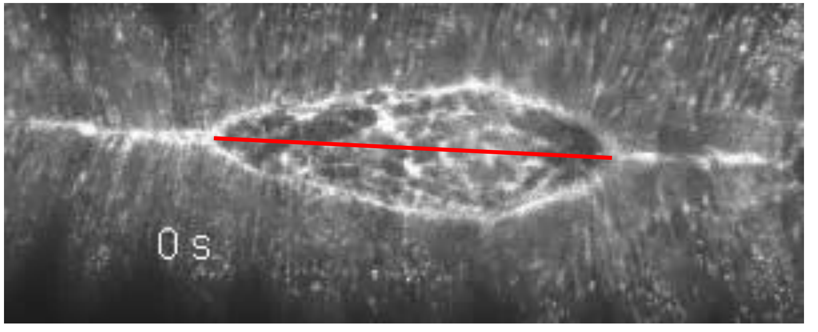
If we repeatedly cut a small incision near one end of the amnioserosa, . . .



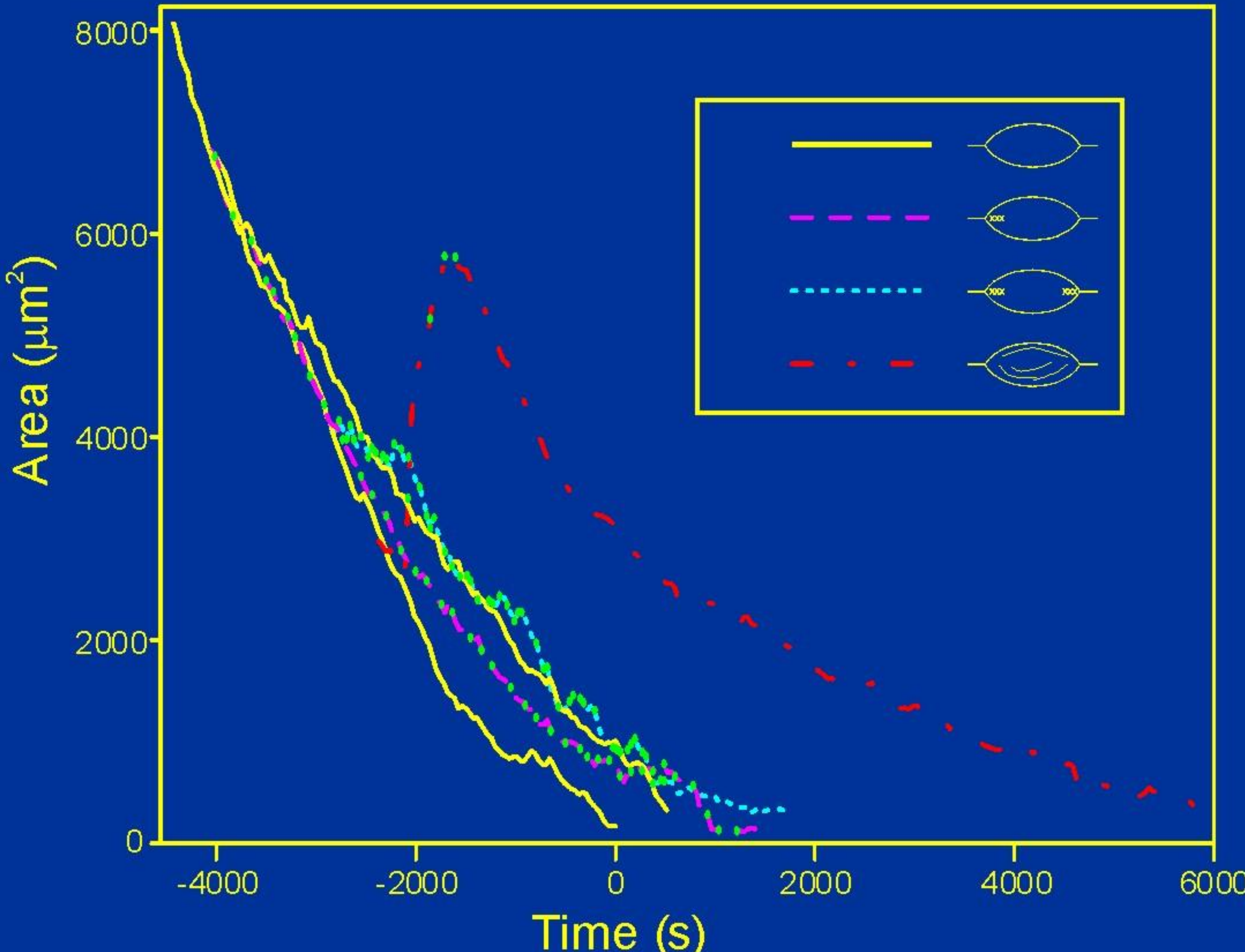
If we repeatedly cut small incisions near both ends of the amnioserosa, . . .



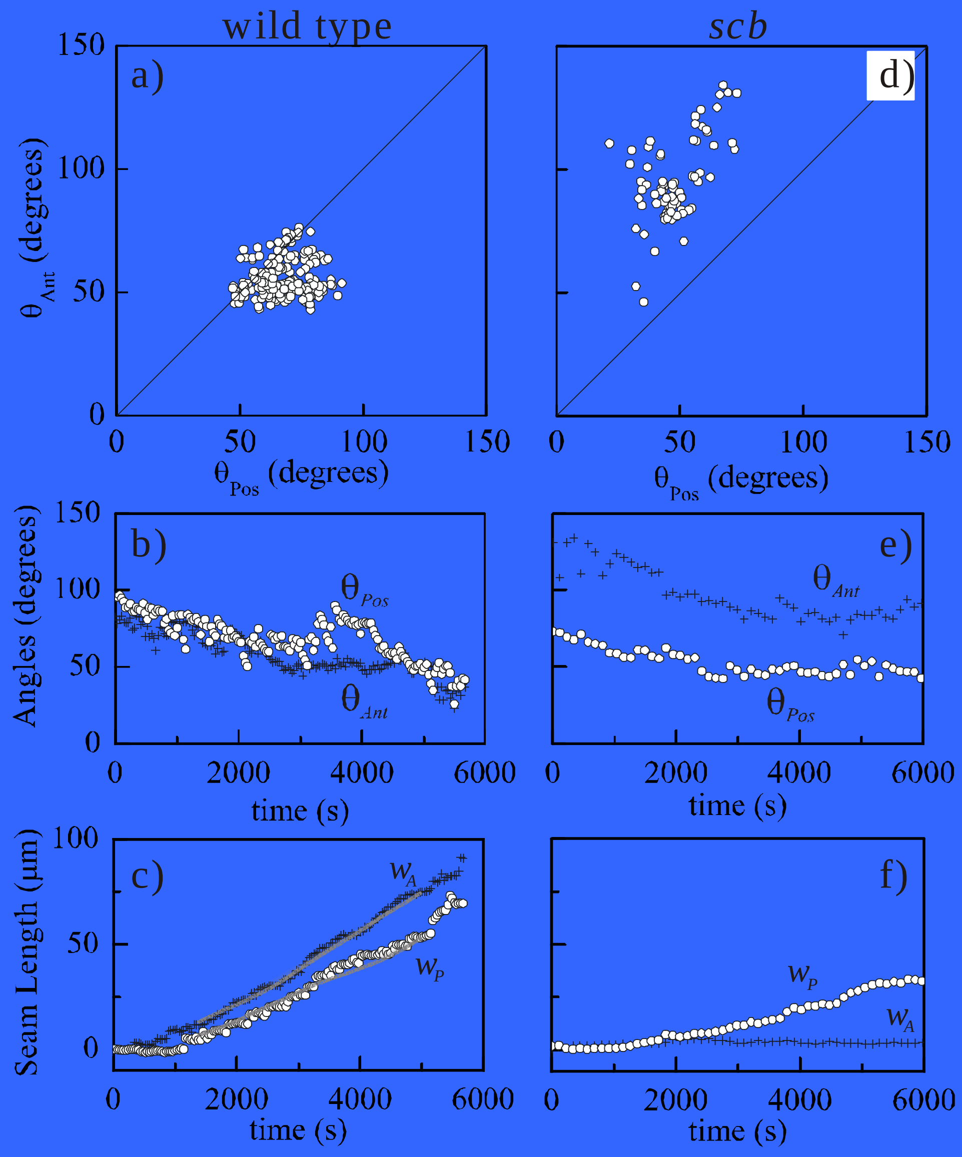
After a canthus to canthus cut, . . .



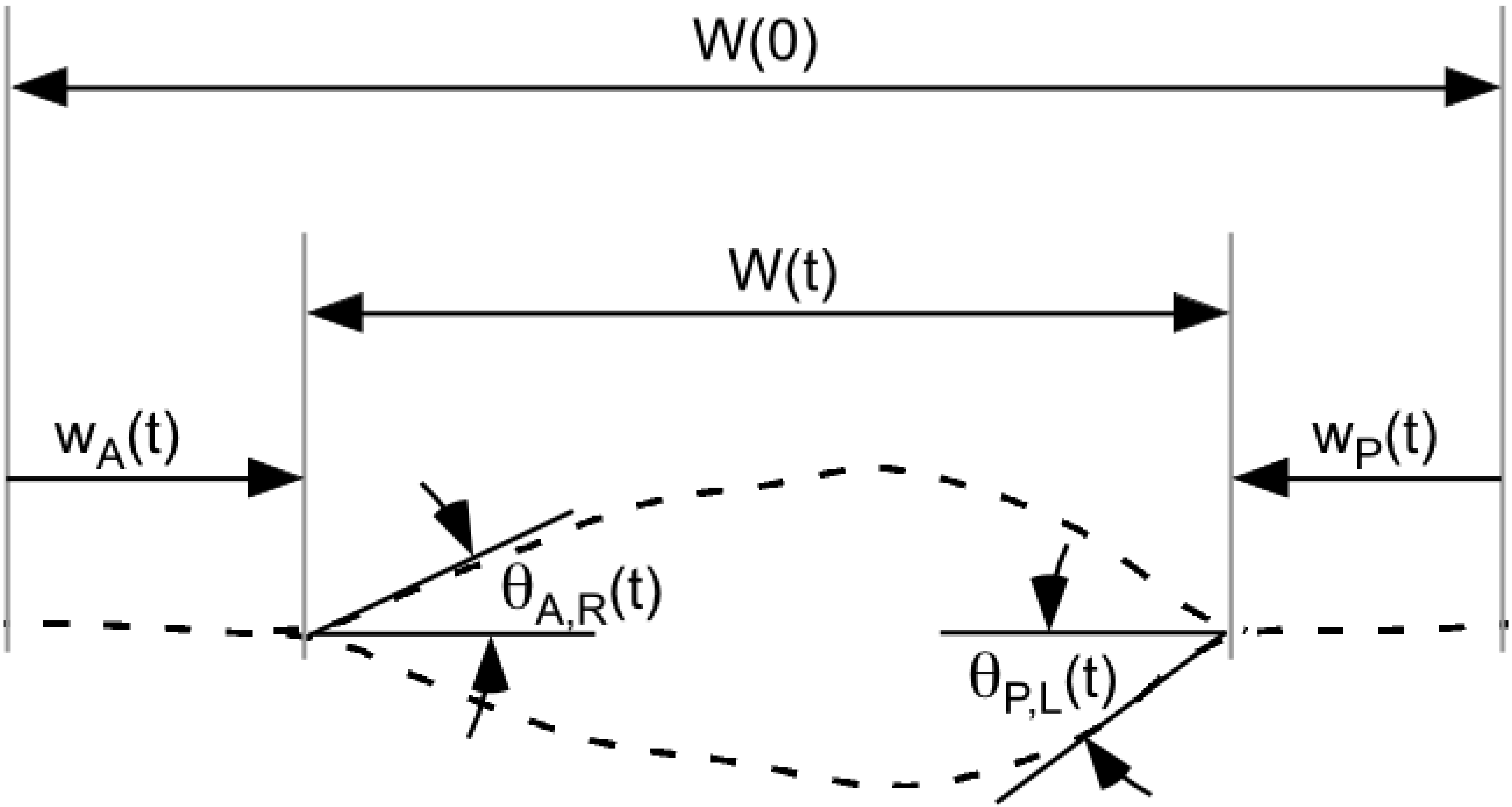
the system relaxes to a new geometry and continues to close.



8000
6000
4000
2000
0
Area (μm²)
-4000
-2000
0
2000
4000
6000
Time (s)
xxx
xxx
xxx



wild type
scb
a)
d)
b)
e)
c)
f)
θAnt (degrees)
θPos (degrees)
Angles (degrees)
Seam Length (μm)
time (s)
θPos
θAnt
wA
wP



W(0)
W(t)
$w_A(t)$
$w_P(t)$
$\theta_{A,R}(t)$
$\theta_{P,L}(t)$

Explicitly treat zipping at each canthus

$$\frac{dw_{A/P}}{dt} = \frac{k_{z,A/P}}{\tan(\theta_i) + \tan(\theta_{ii})} = \frac{k_{z,A/P} W}{2H}$$

$$k_{z,A/P} = \frac{w_{A/P}(t)}{\sum_{0}^{t} \frac{\Delta t}{\tan(\theta_i) + \tan(\theta_{ii})}}$$

Upregulation of zipping rate constants

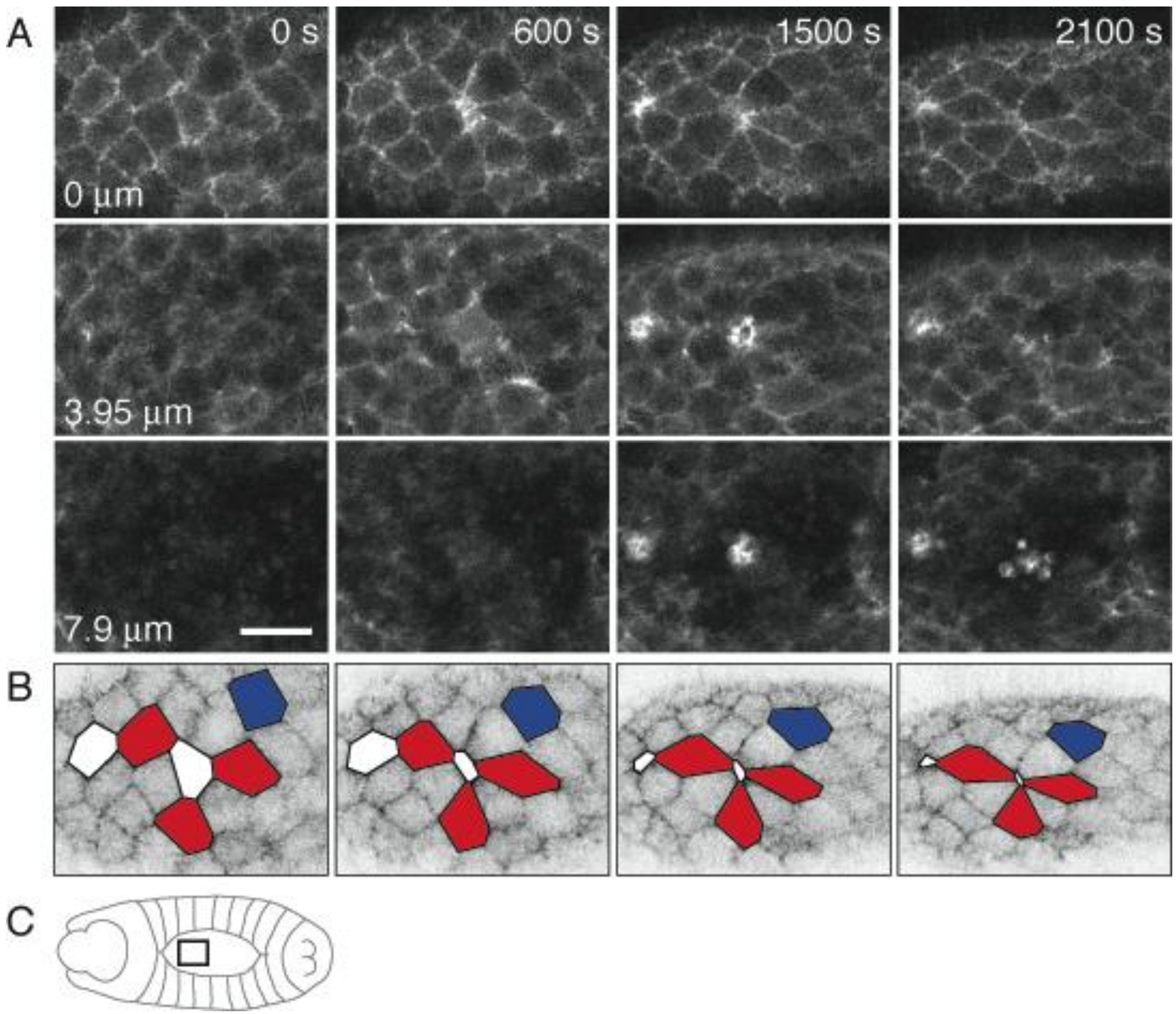
Table 2. Spatial and dynamic asymmetries.

	n	$\Delta x_c/\Delta t$ (nm/s)	$k_{z,A}$ (nm/s)	$k_{z,P}$ (nm/s)
Native Closure	7	1.9 ± 0.4	15.3 ± 0.7	10.6 ± 1.7
Nick on Posterior	8	7.5 ± 0.6	17.5 ± 0.9	blocked
Nick on Anterior	6	-9.0 ± 0.7	blocked	15.8 ± 1.0

As far as we can tell, native dorsal closure essentially is L-R symmetric.

But there are distinct A-P asymmetries, including canthus angles, zipping rates, and zipping rate constants.

Apoptosis:
A-P asymmetries, force production, and upregulation


A
0 s
600 s
1500 s
2100 s
0 µm
3.95 µm
7.9 µm
B
C

Genetically alter the rate of apoptosis

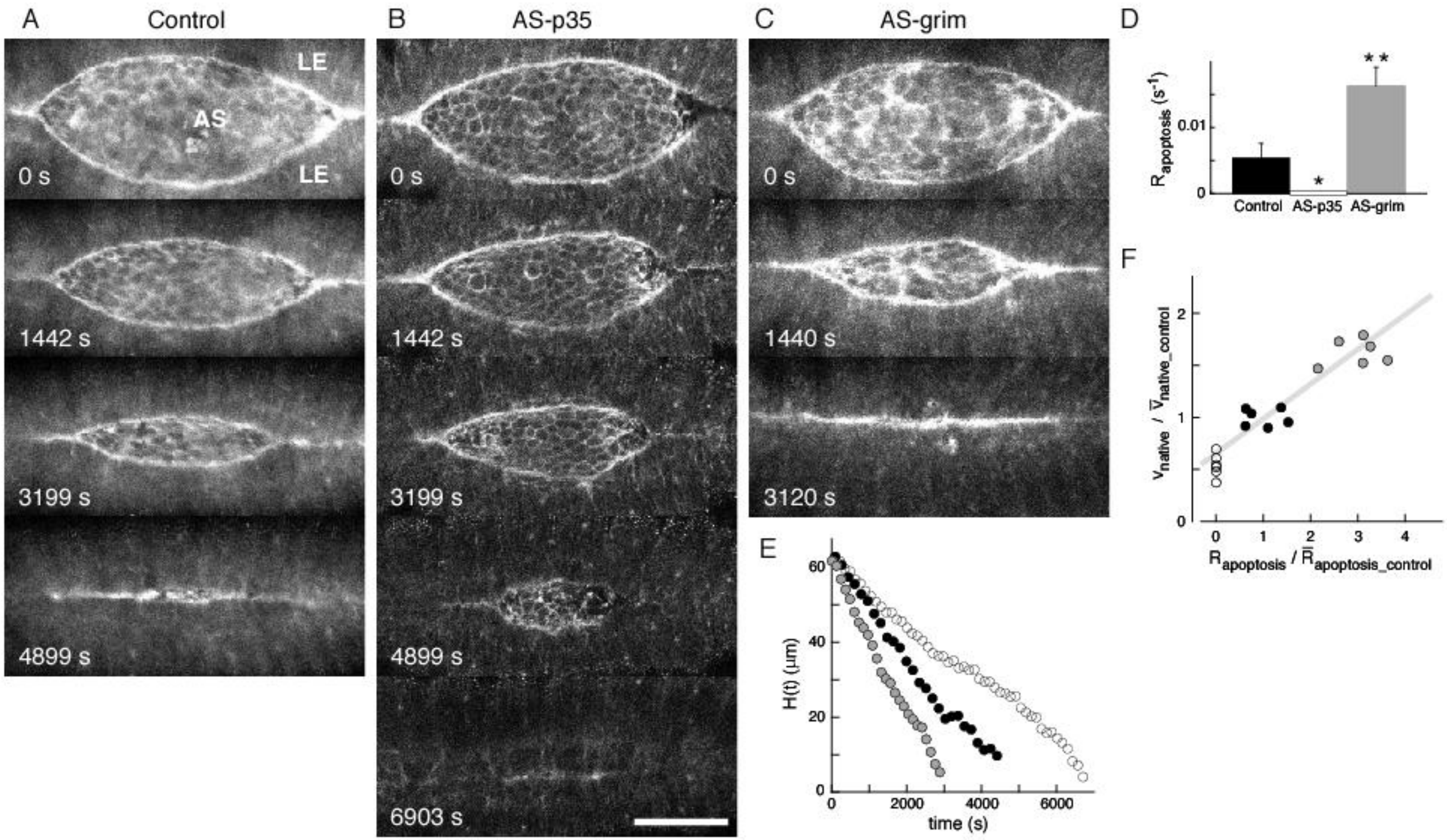
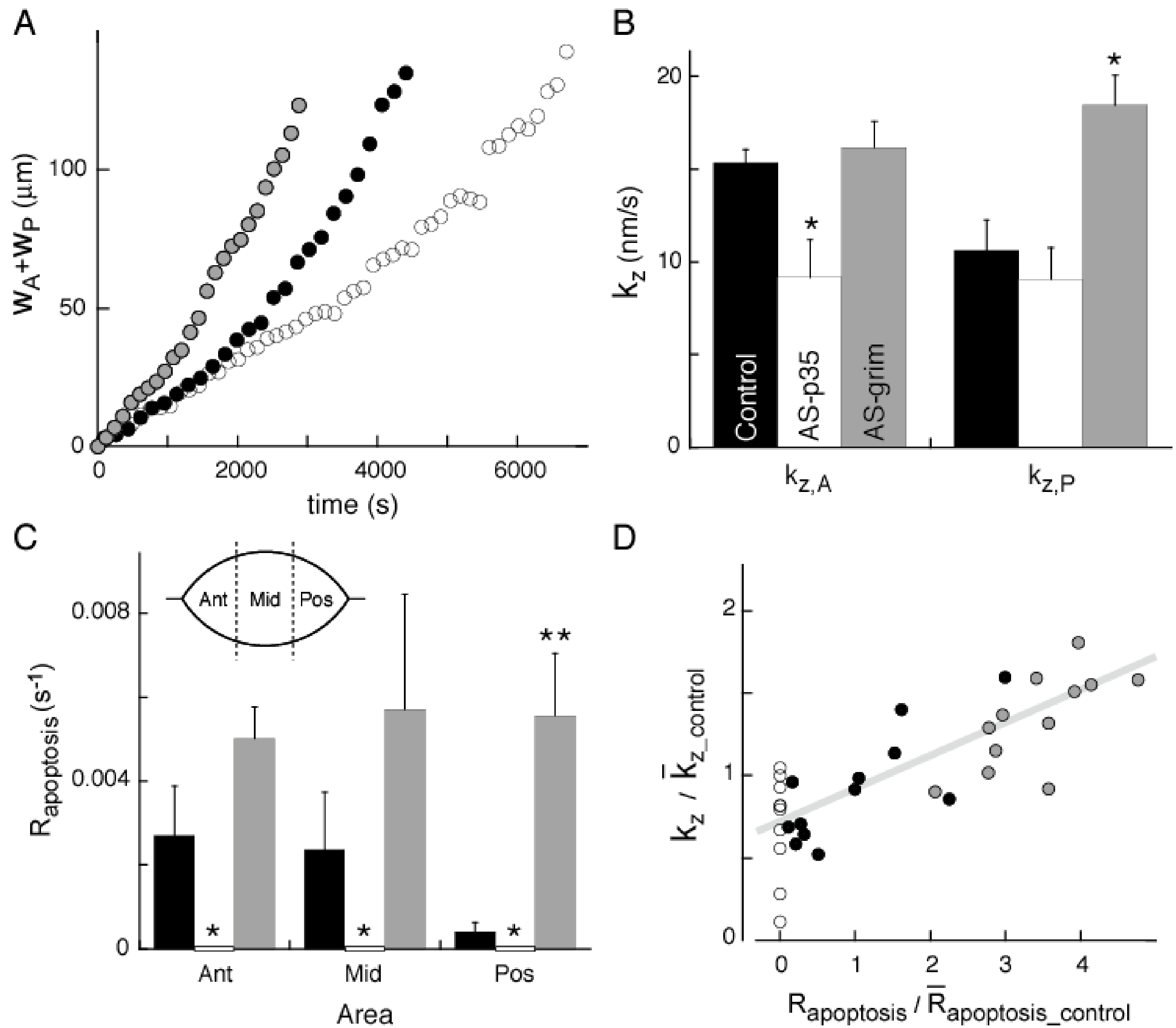


Fig.4



A
w_A+w_P (μm)
time (s)
B
k_z (nm/s)
Control
AS-p35
AS-grim
$k_{z,A}$
$k_{z,P}$
C
Ant
Mid
Pos
$R_{apoptosis}$ (s^{-1})
Area
D
$k_z / \bar{k}_{z\_control}$
$R_{apoptosis} / \bar{R}_{apoptosis\_control}$

We have observed:

1. Ingression is the principle mechanism for persistent loss of apical area in amnioserosa cells.
2. The native A/P asymmetry in the zipping rate constants correlates with the local rate of apoptosis in the amnioserosa.
3. The rate of apoptosis is correlated to the upregulation in amnioserosa force production and the upregulation in the zipping rate constants.

Future research:

1. What is the zipping mechanism?
2. What regulates ingression?
3. What upregulates ingression in response to perturbation?

Collaborators

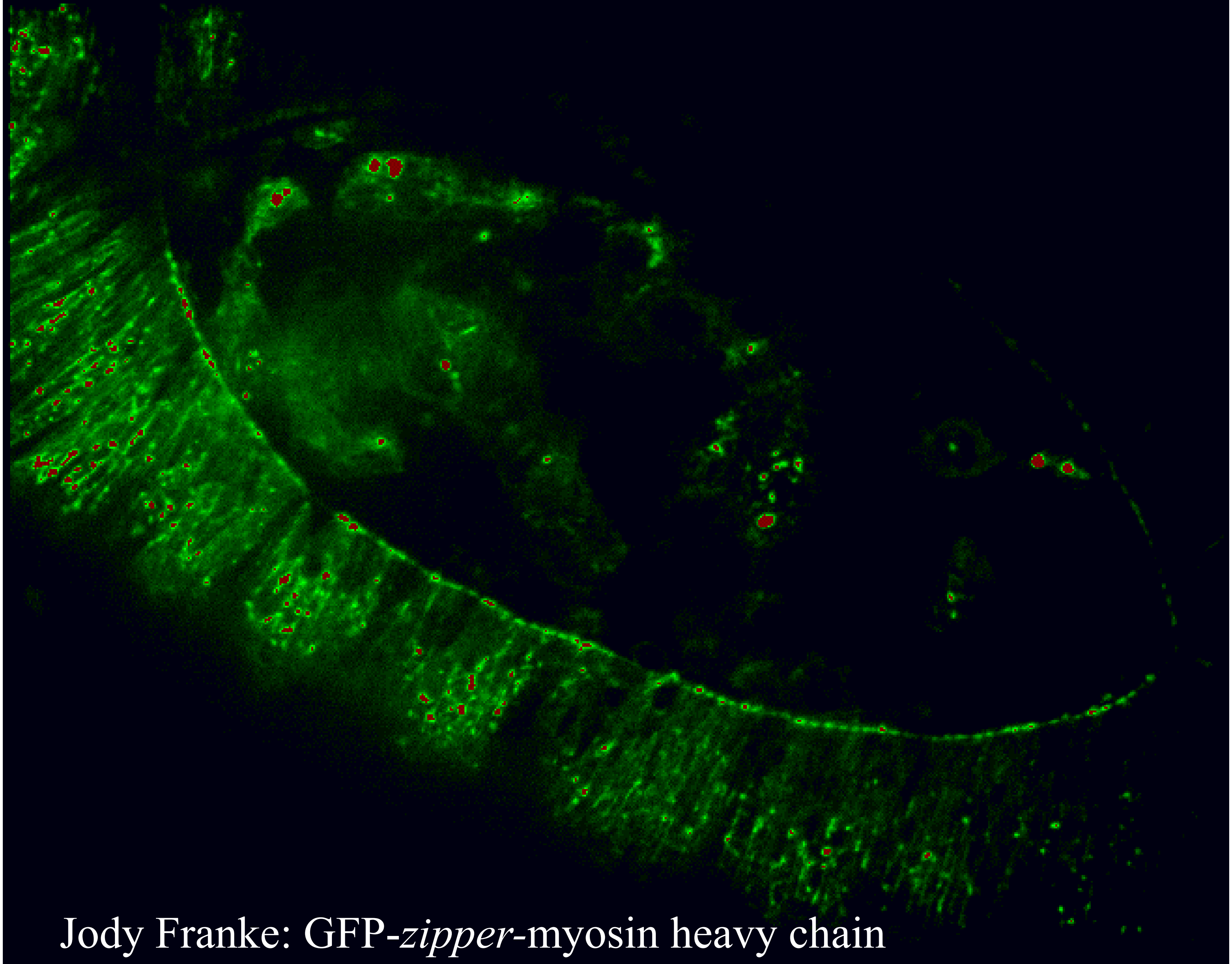
Dan Kiehart and Stephanos Venakides

Shane Hutson, Xomalin Peralta, Alice Rodriquez, Yusuke Toyama, Adam Sokolow, and Heng Lu

Ming-Shien Chen, Yoichiro Tokutake, and Adrienne Wells

Nicole Czakon, Fernando Boschini, David Staub, Brett Rosenthal, and Angela Cai

Haoyang Ren



Jody Franke: GFP-*zipper*-myosin heavy chain