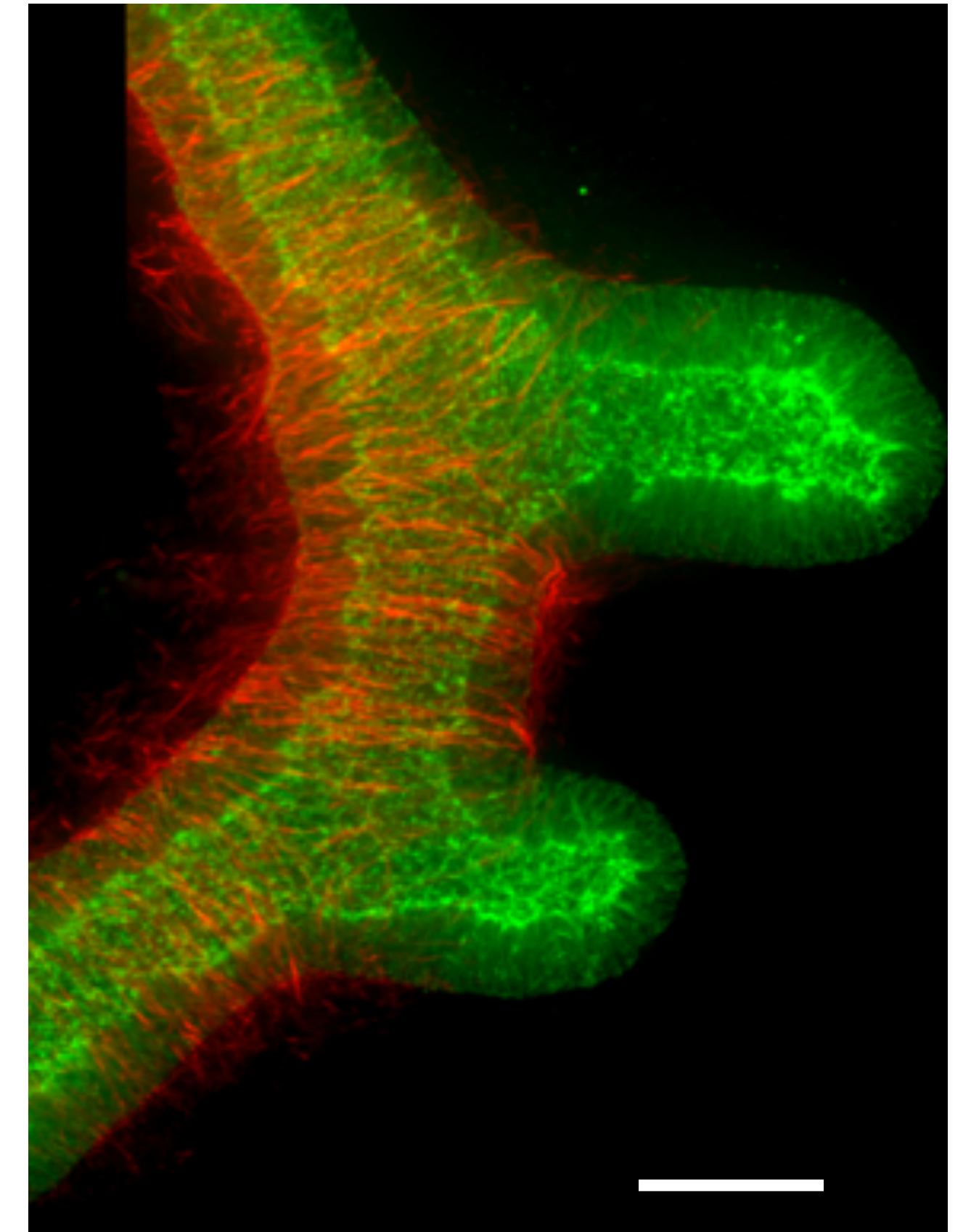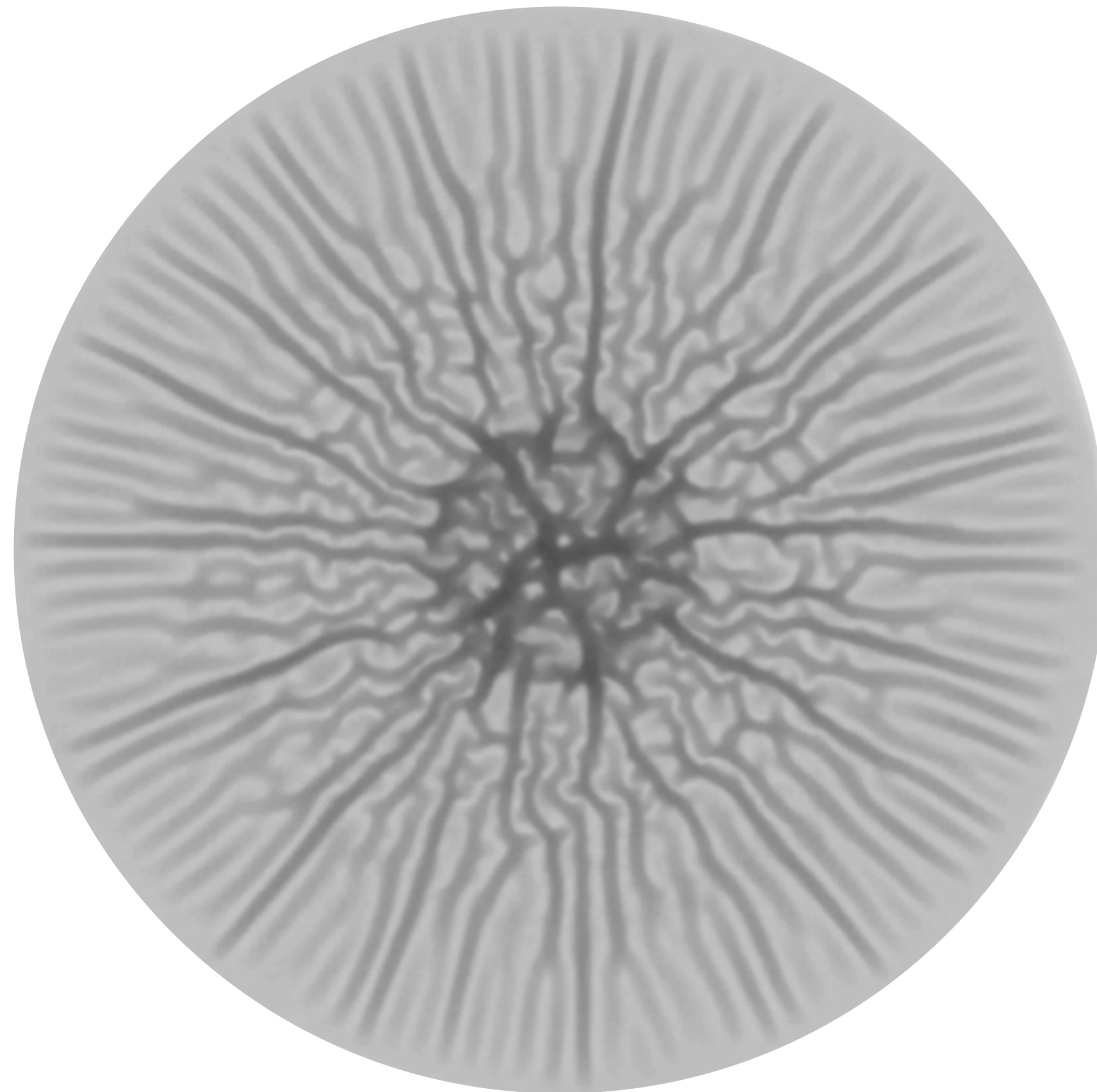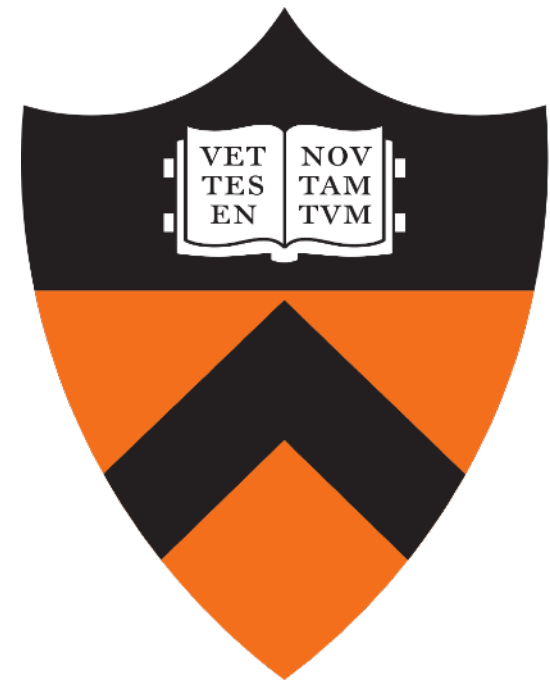# Mechanical instabilities in growing biological systems: wrinkling and branching

## Andrej Košmrlj

PRINCETON

**Department of Mechanical and Aerospace Engineering**
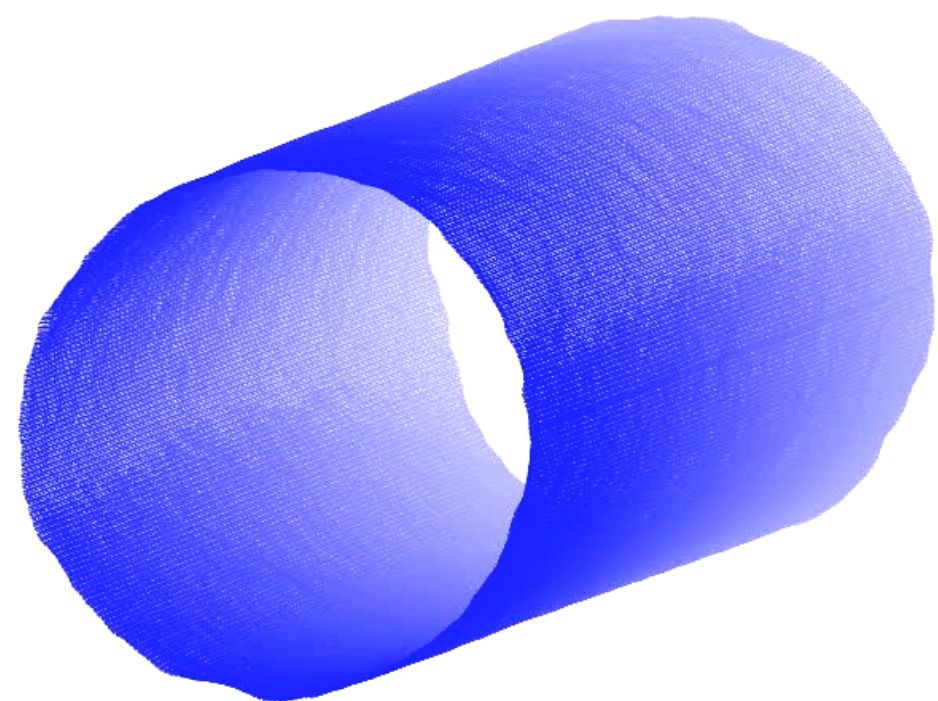


**Workshop "Morphogenesis in Animals and Plants: Search for Principles"**
**KITP, UC Santa Barbara, July 31, 2019**
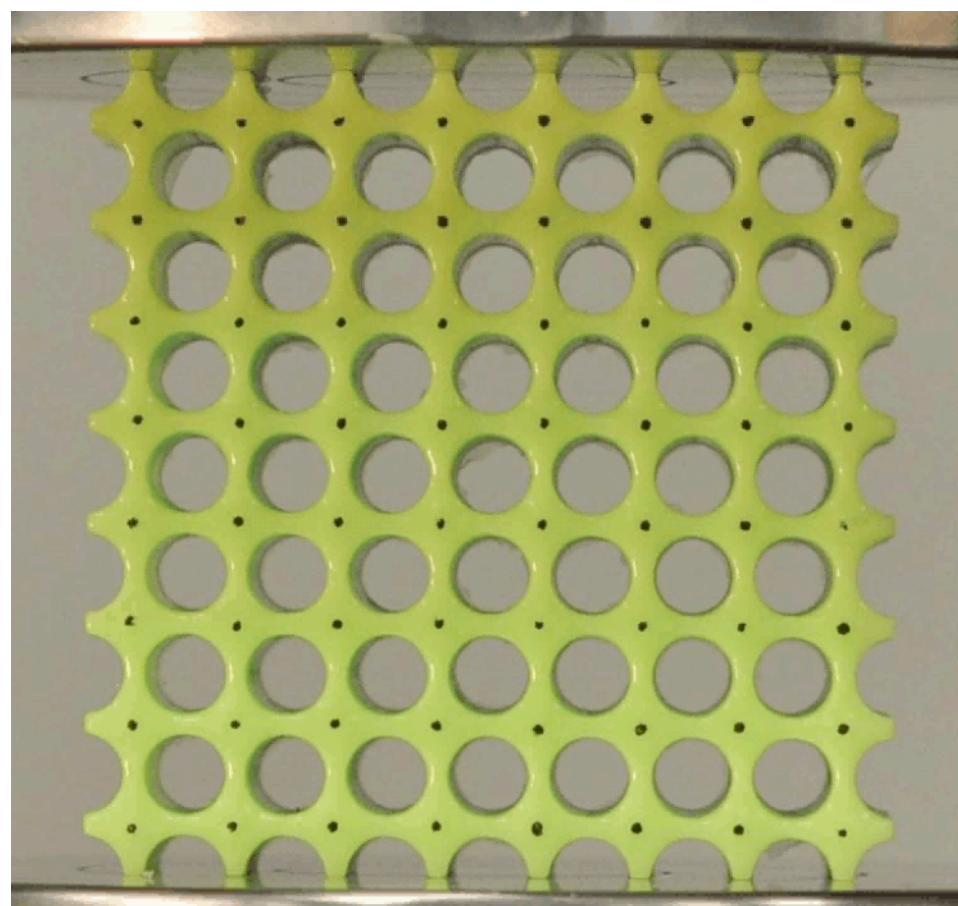
# Research interests

**mechanics of nanostructures**

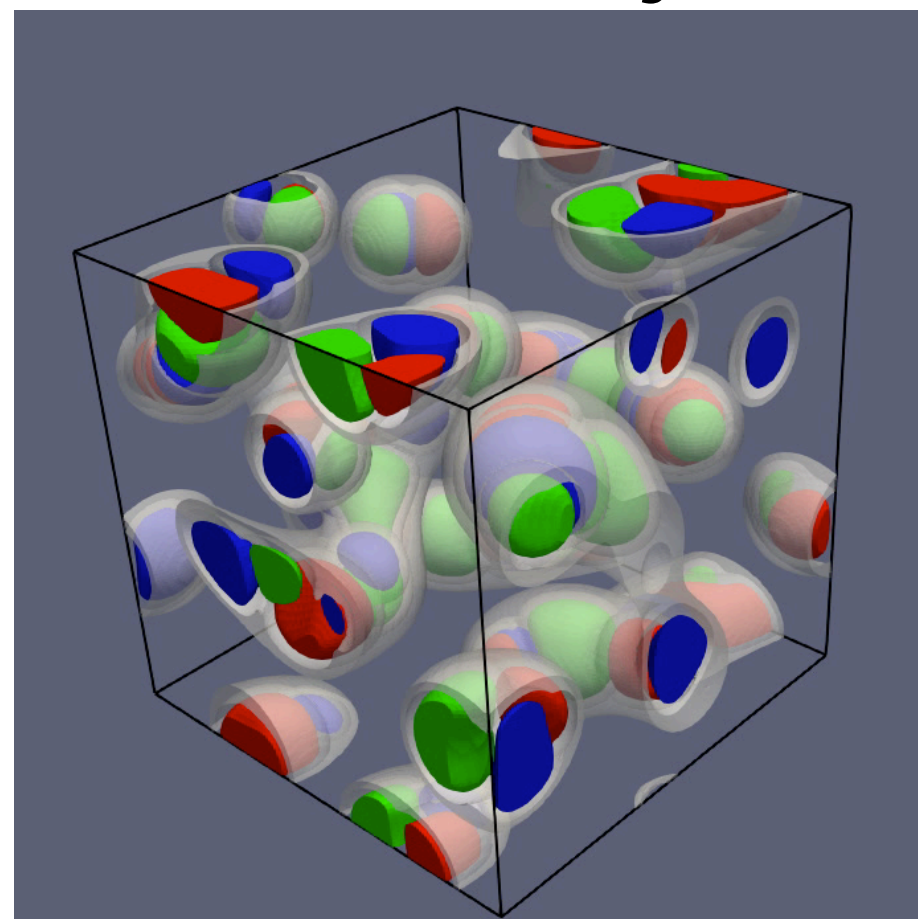D. Nelson, D. Weitz, R. Bruinsma, P. Koumoutsakos
M.J. Abdolhosseini Qomi

**mechanical metamaterials**

K. Bertoldi, M. Brojan

**phase separation**

M. Haataja

**epithelial tissues**

D. Cohen

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S. Taheri-Araghi

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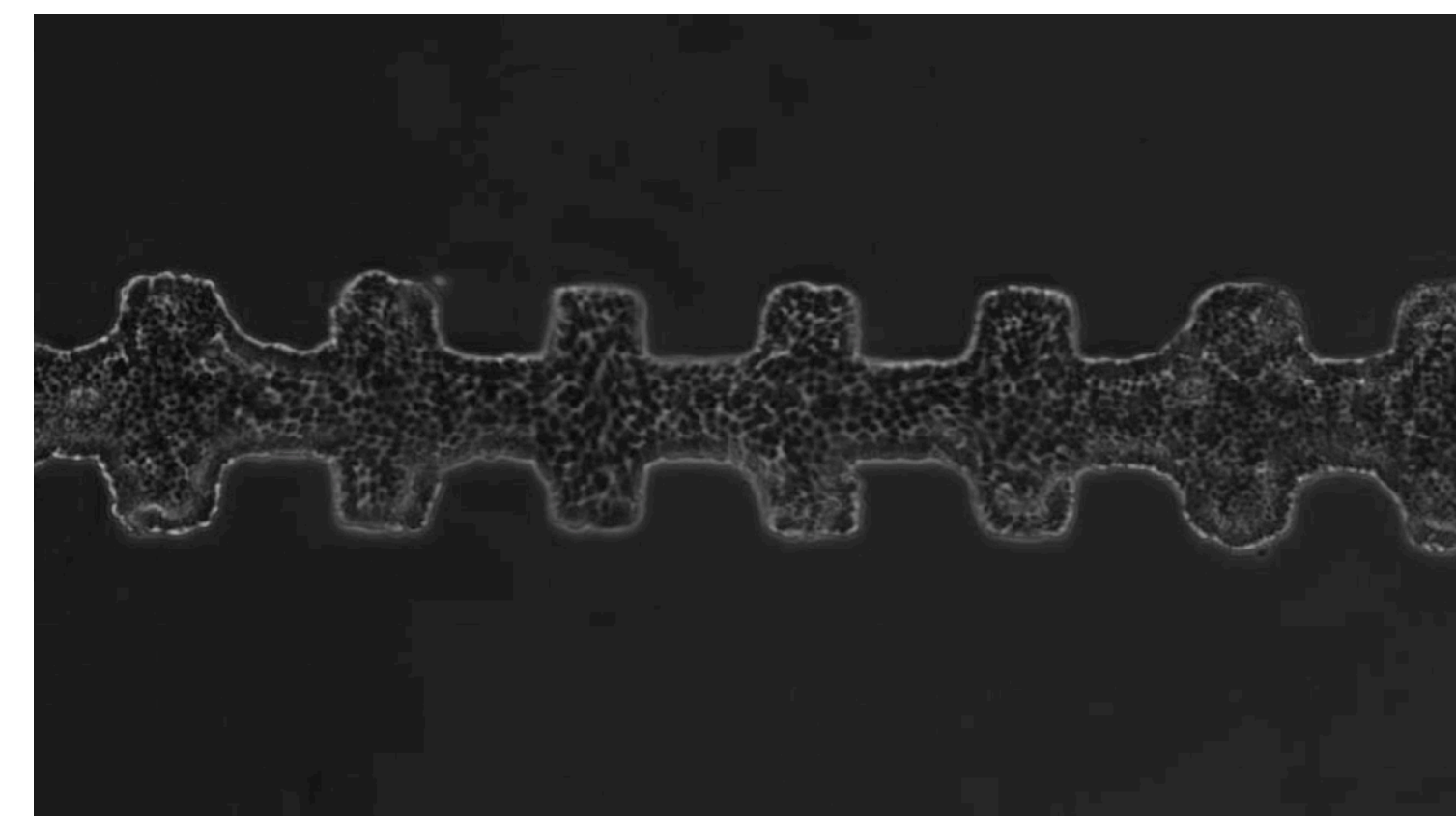**mechanical metamaterials**

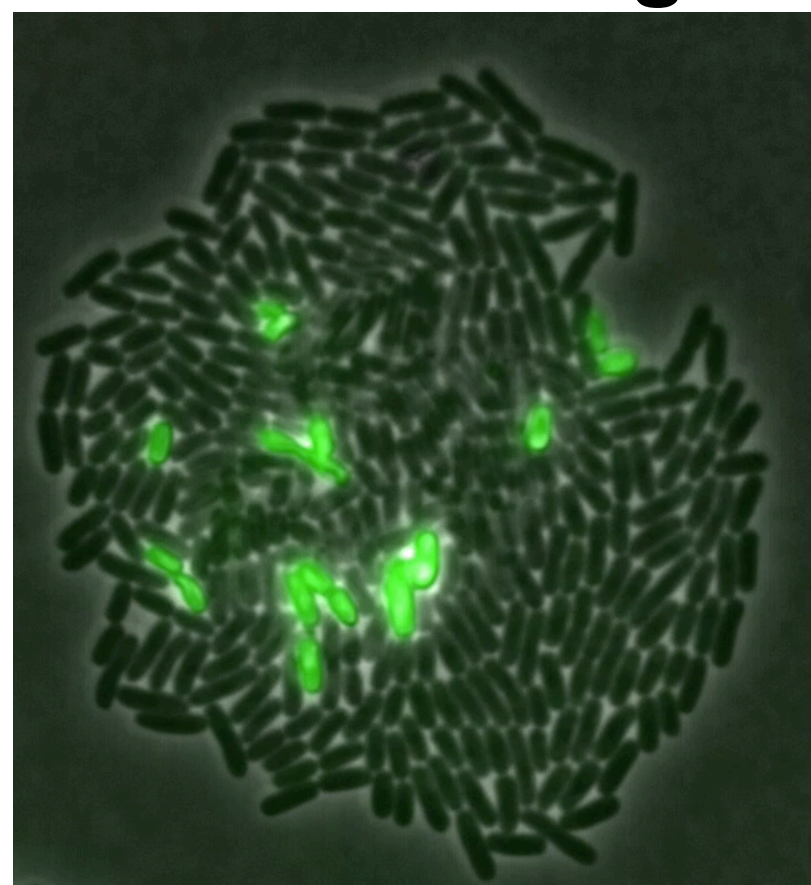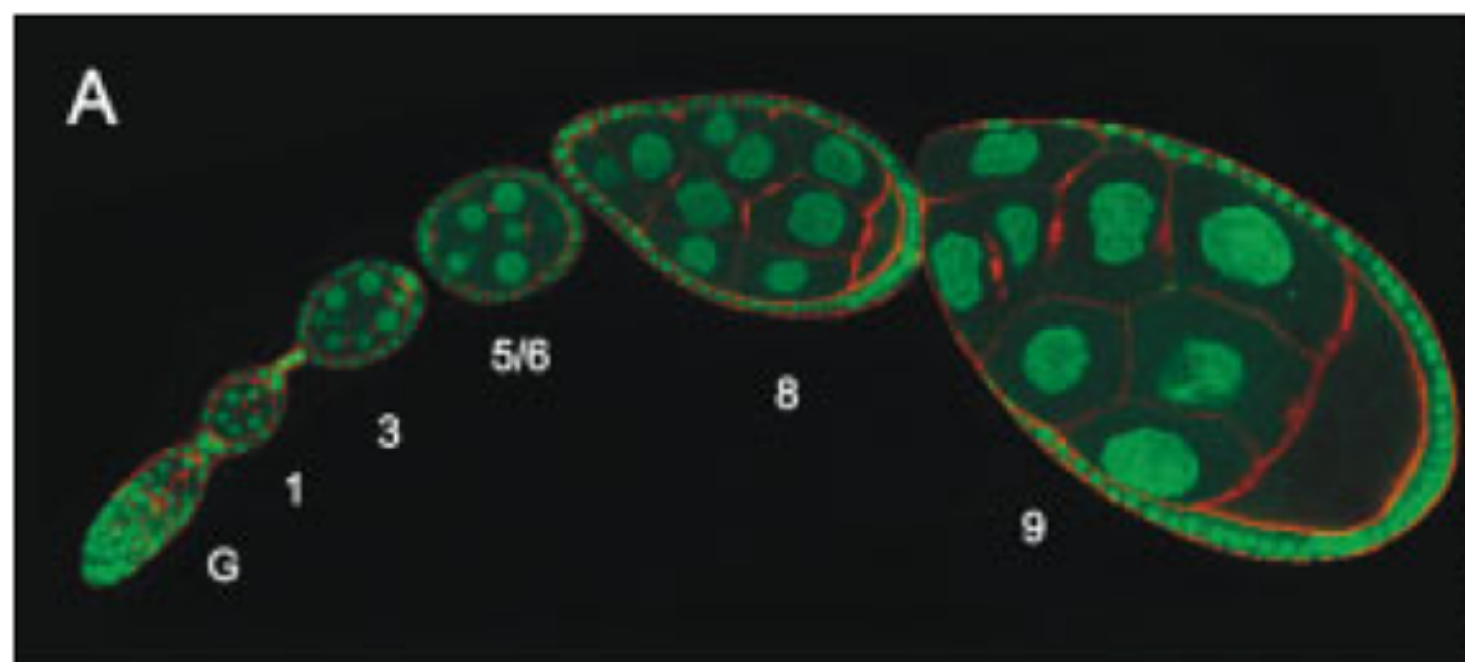K. Bertoldi, M. Brojan

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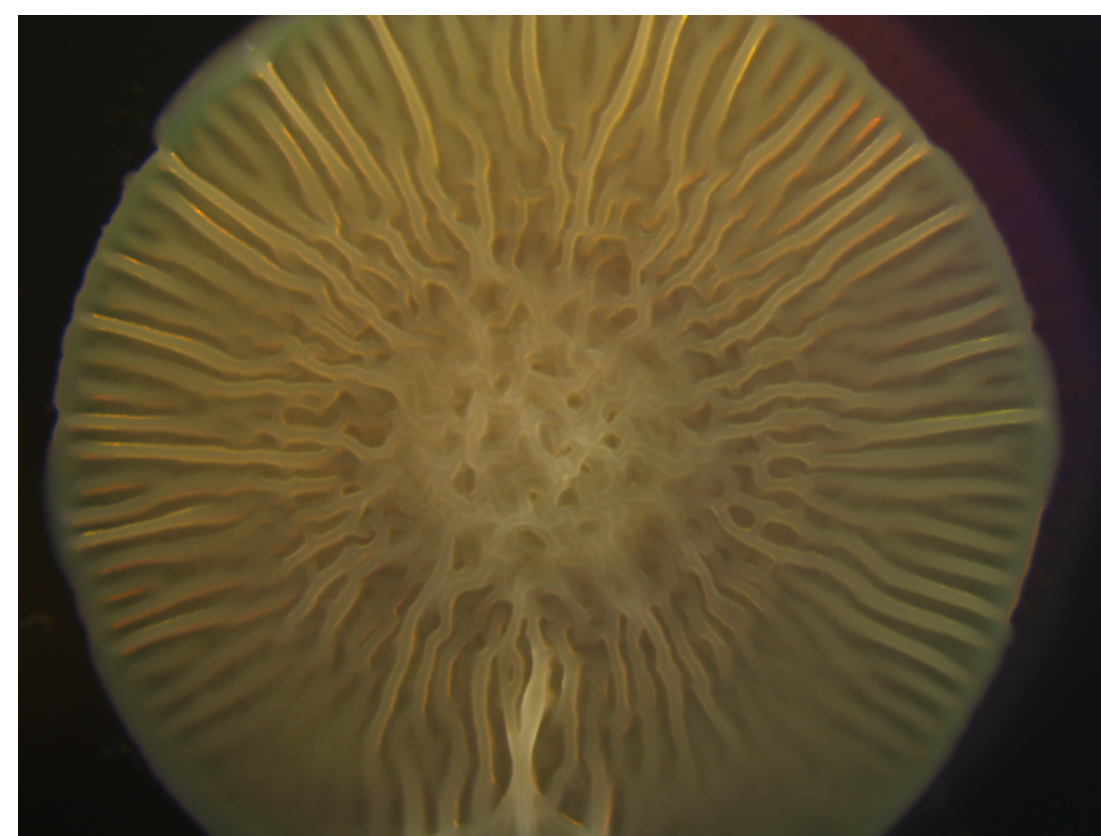**antimicrobial peptides**

S. Taheri-Araghi

**Drosophila egg chamber development**
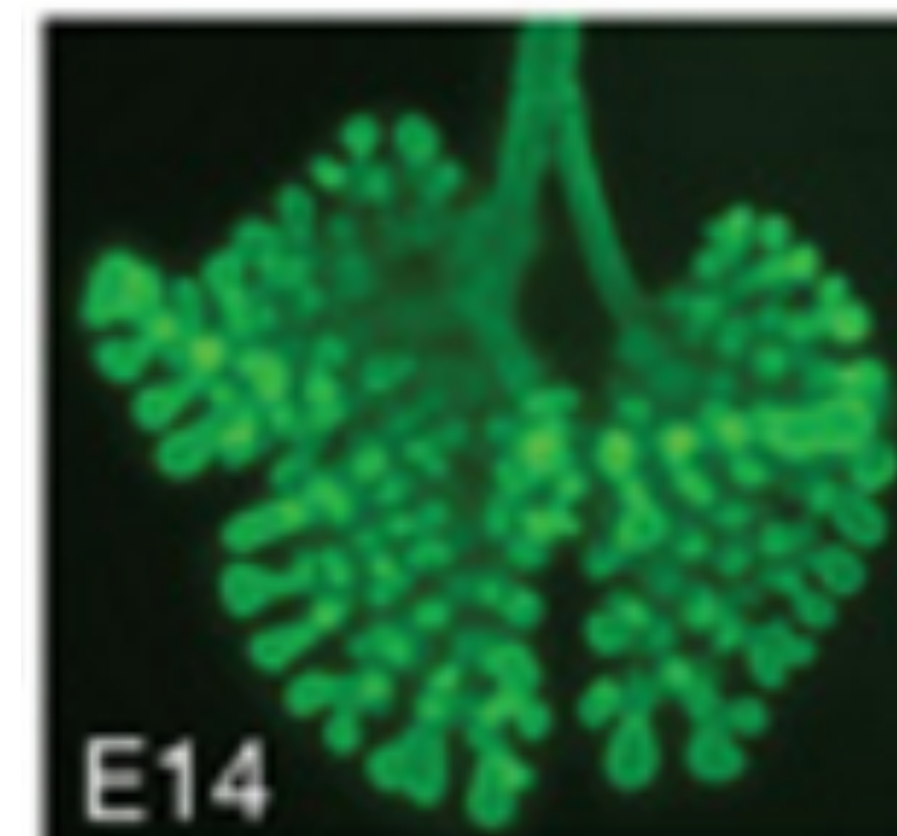
S. Shvartsman

**bacterial biofilms**

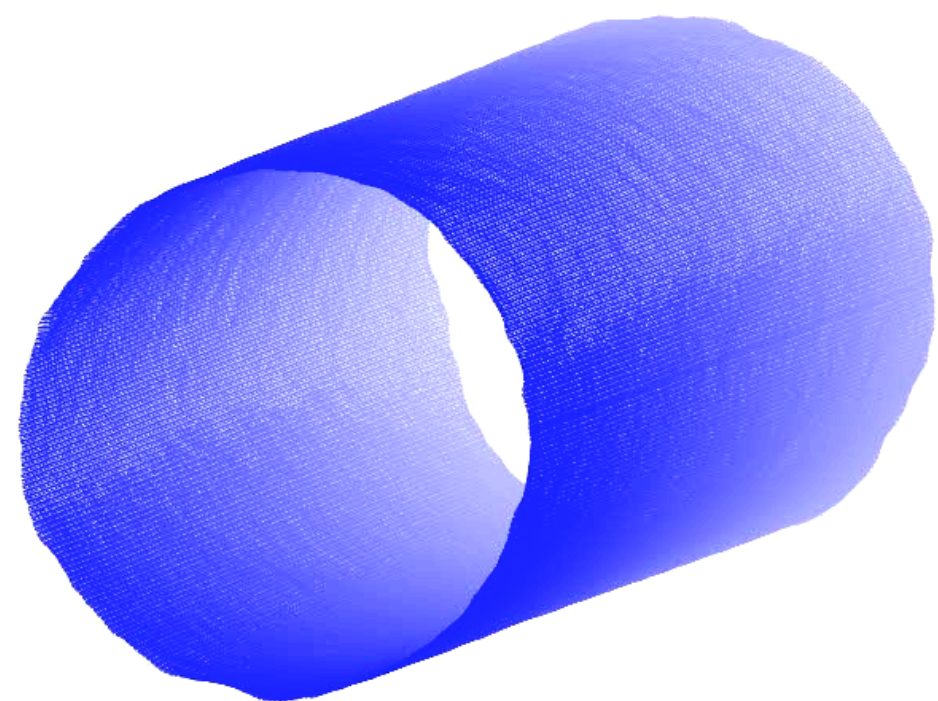B. Bassler, H. Stone, N. Wingreen

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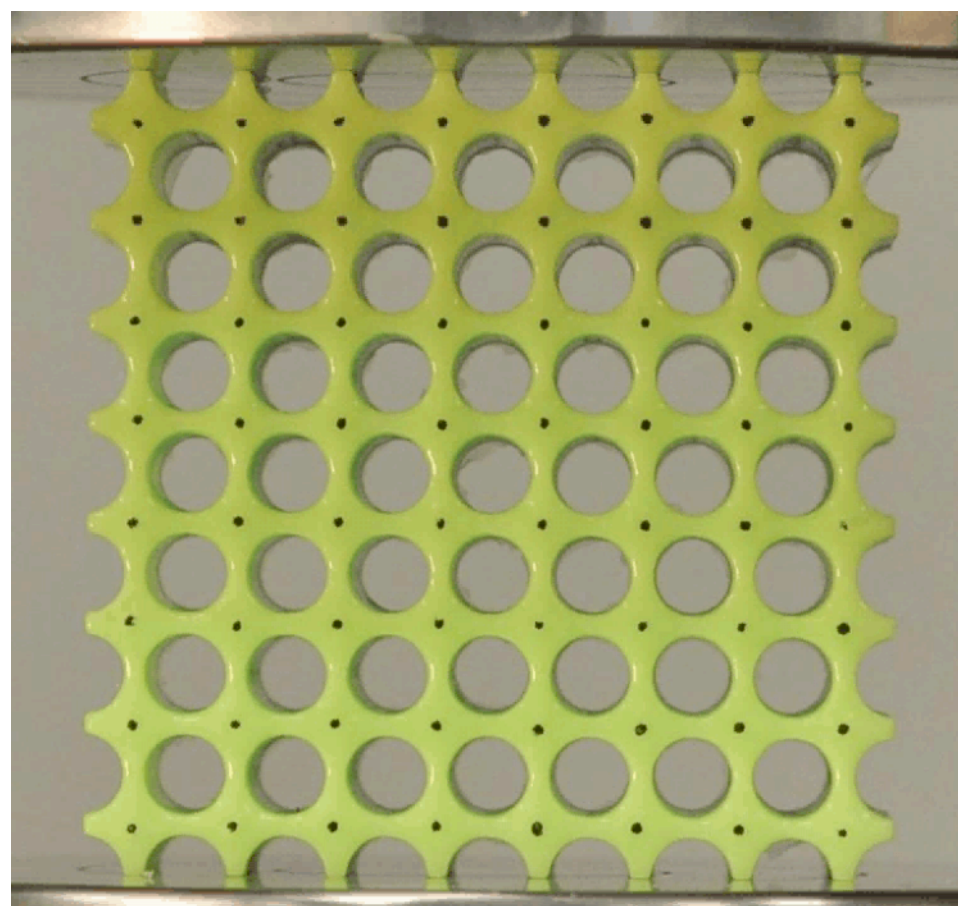
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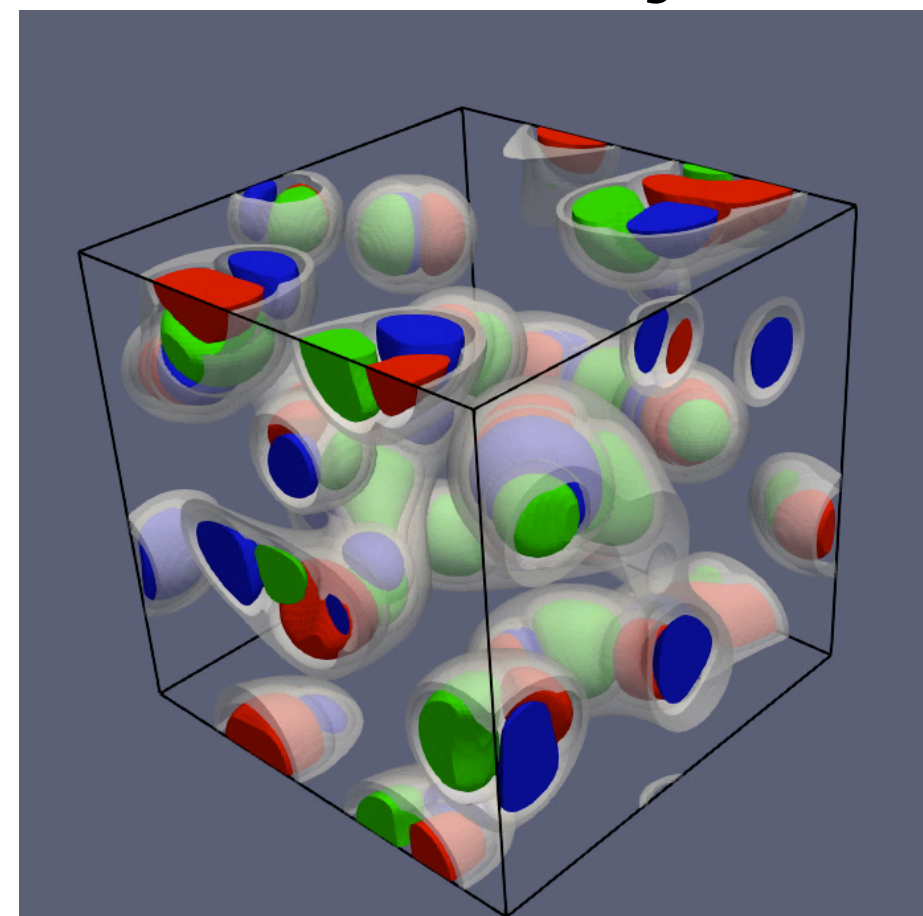D. Nelson, D. Weitz, R. Bruinsma, P. Koumoutsakos M.J. Abdolhosseini Qomi

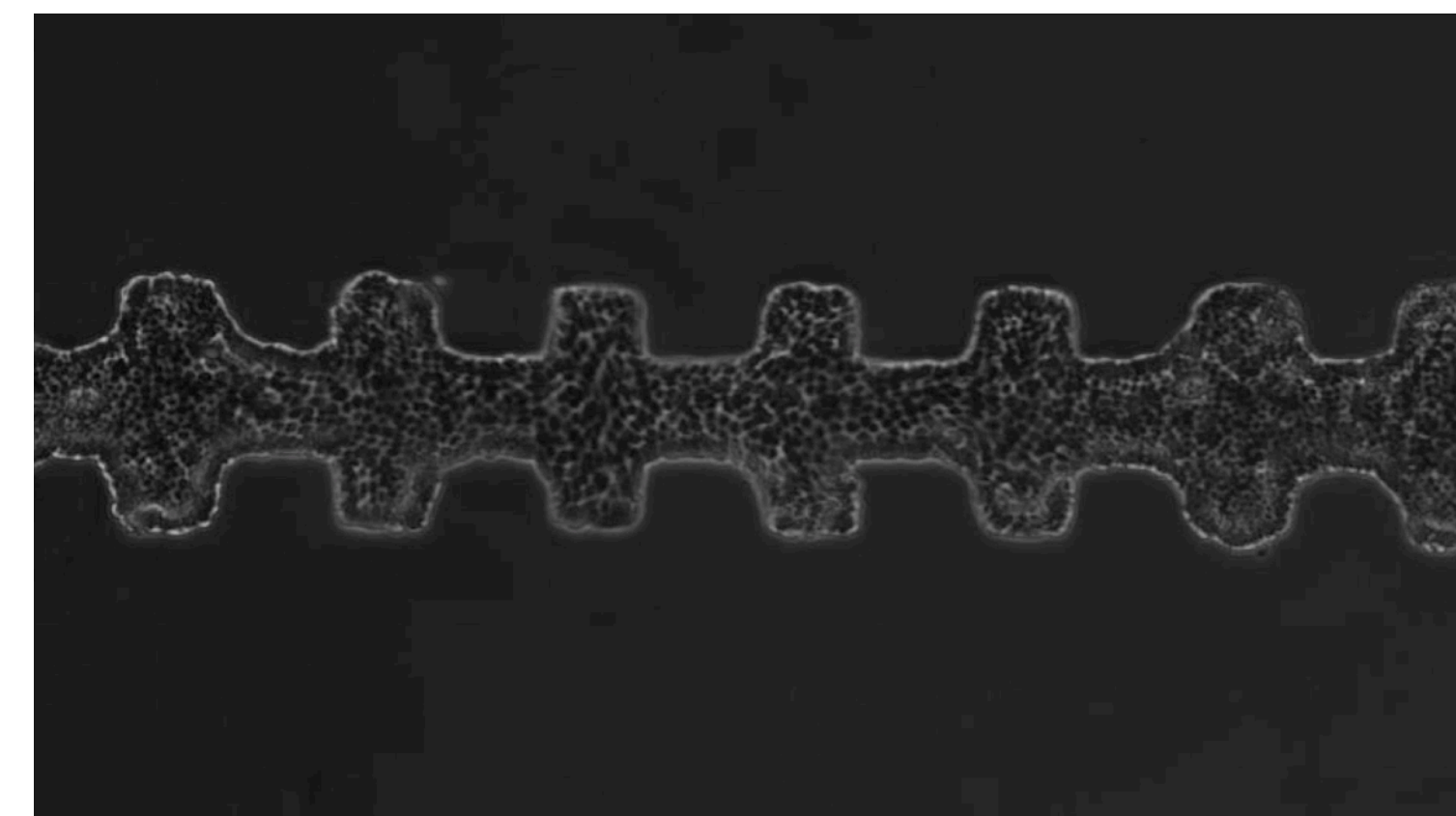**mechanical metamaterials**

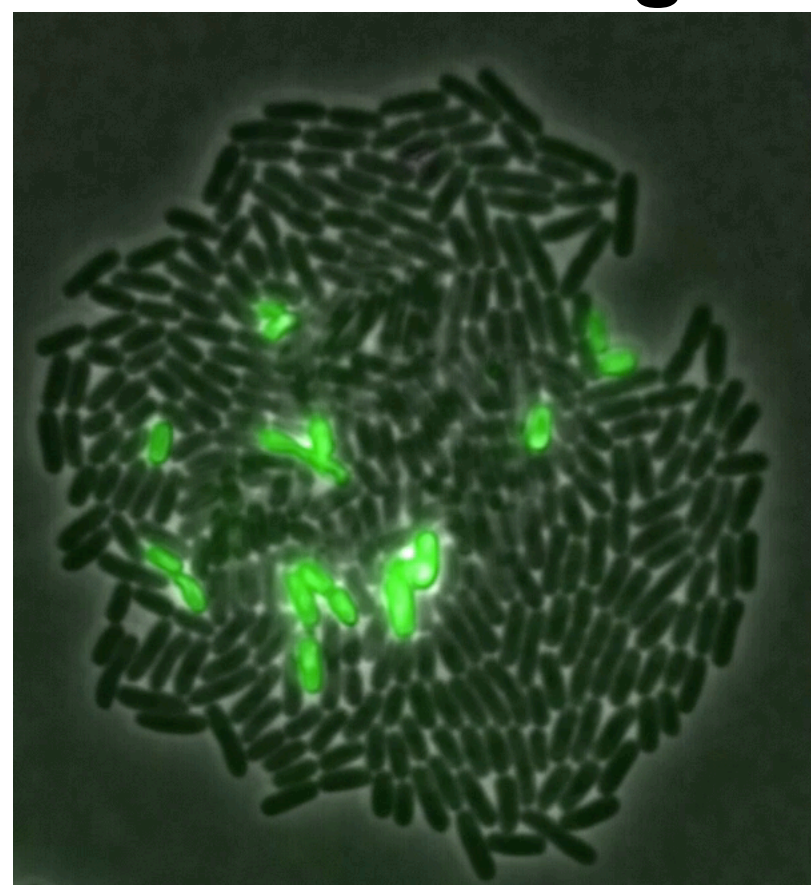K. Bertoldi, M. Brojan

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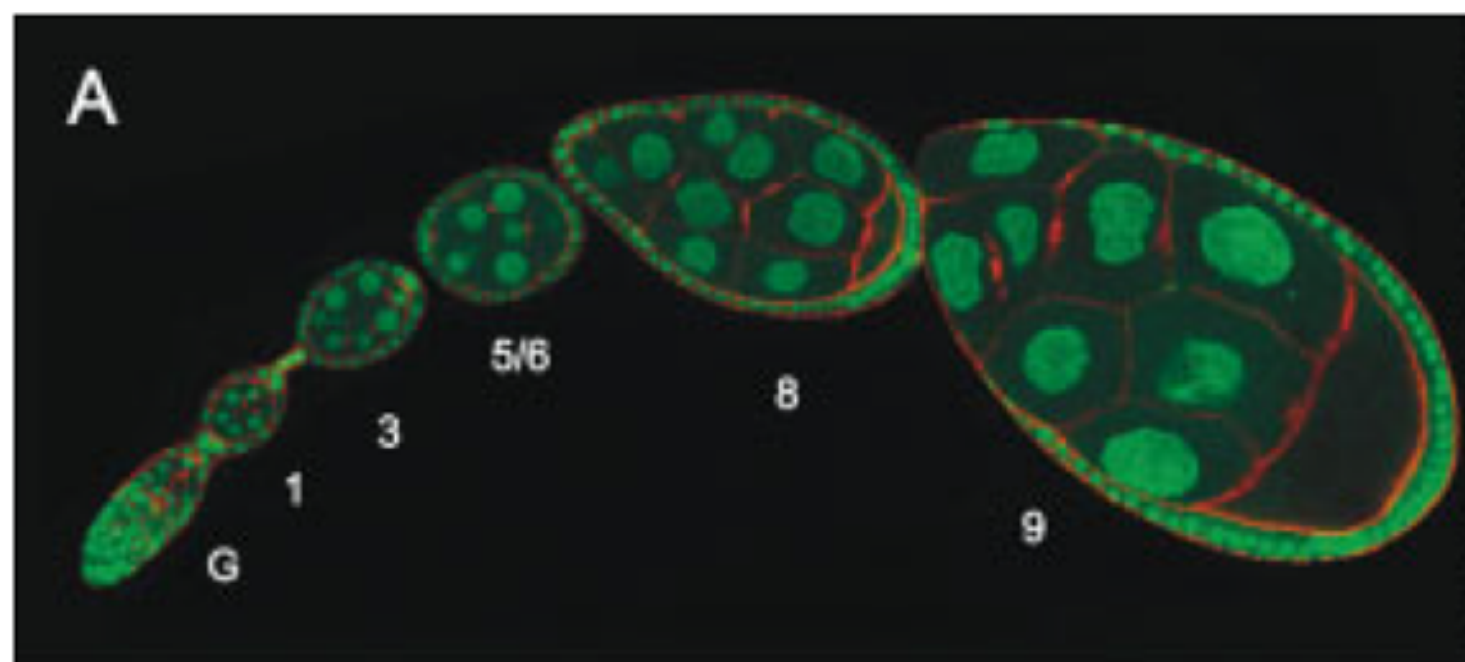
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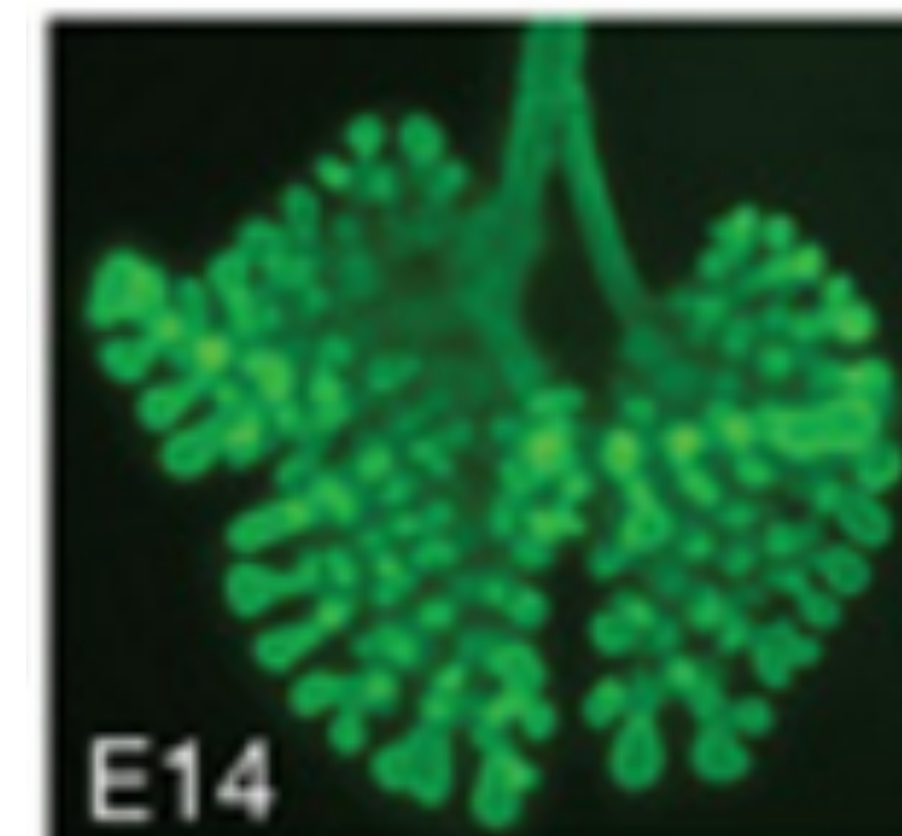
S. Shvartsman

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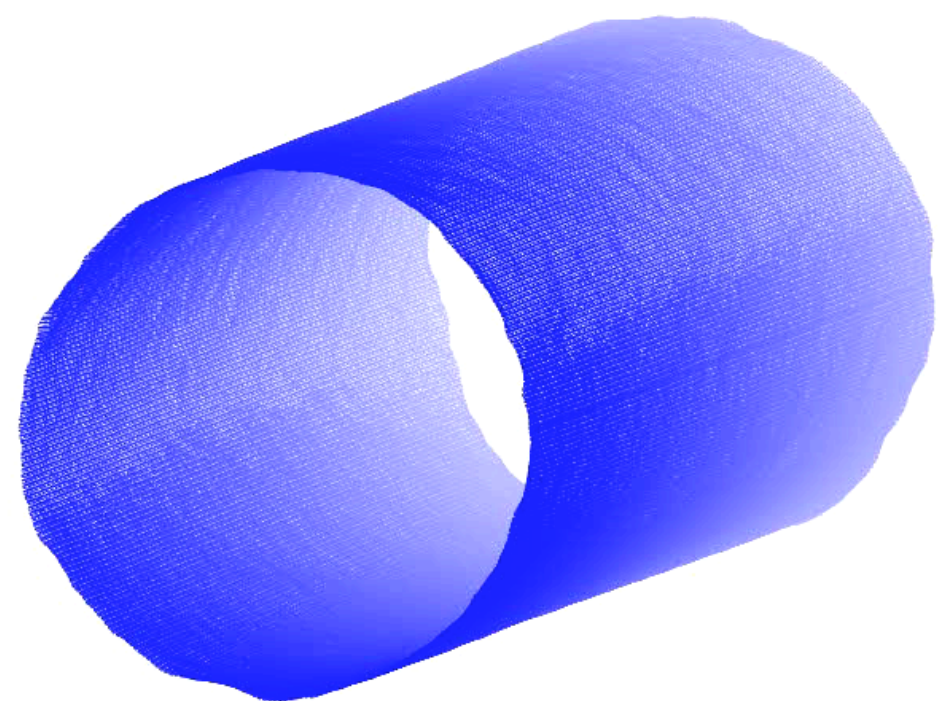B. Bassler, H. Stone, N. Wingreen

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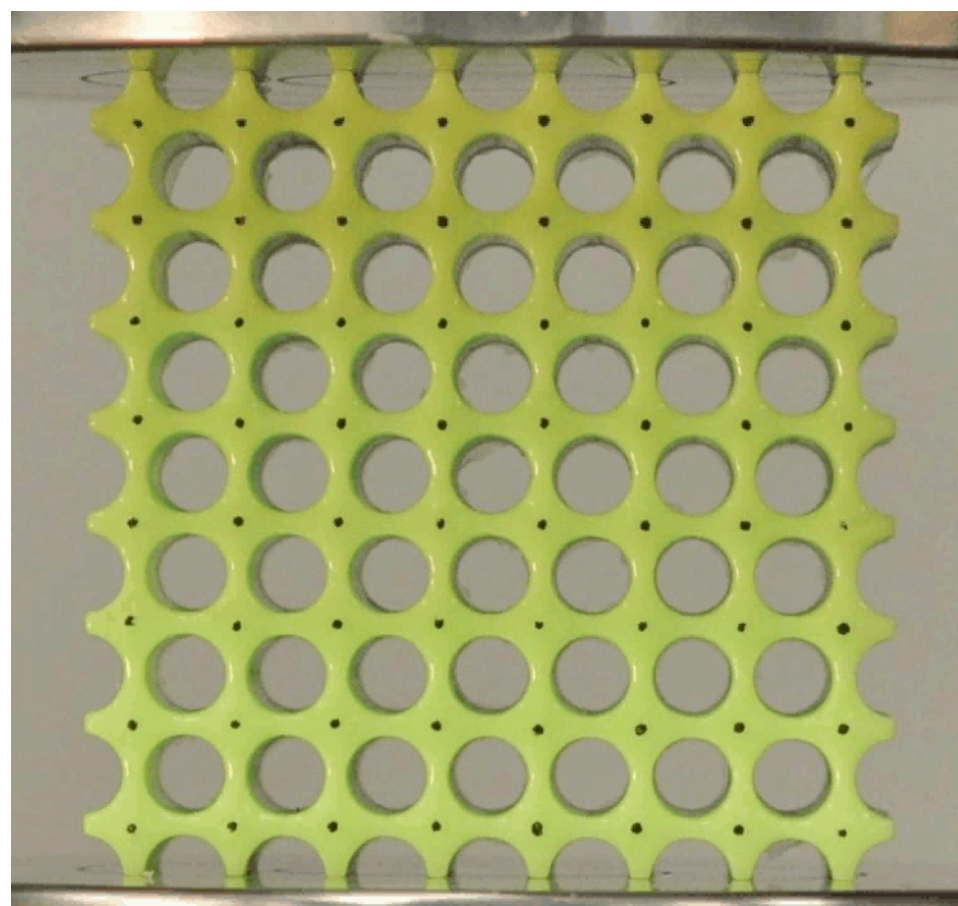C. Nelson

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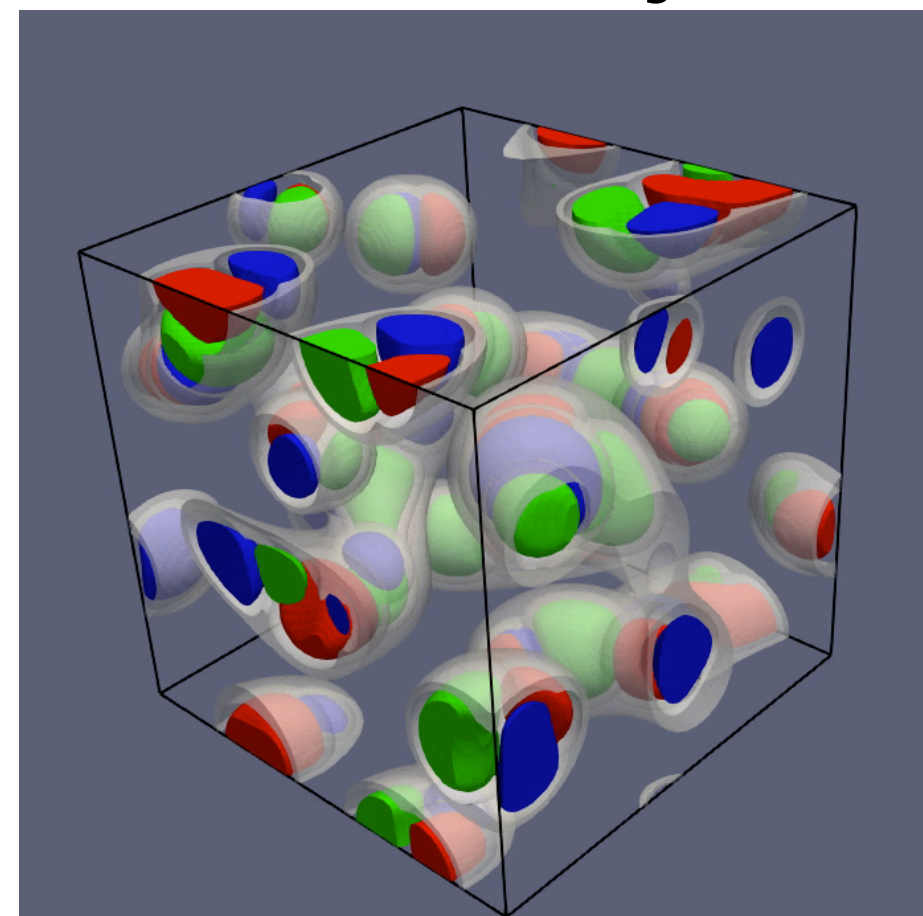D. Nelson, D. Weitz, R. Bruinsma, P. Koumoutsakos M.J. Abdolhosseini Qomi

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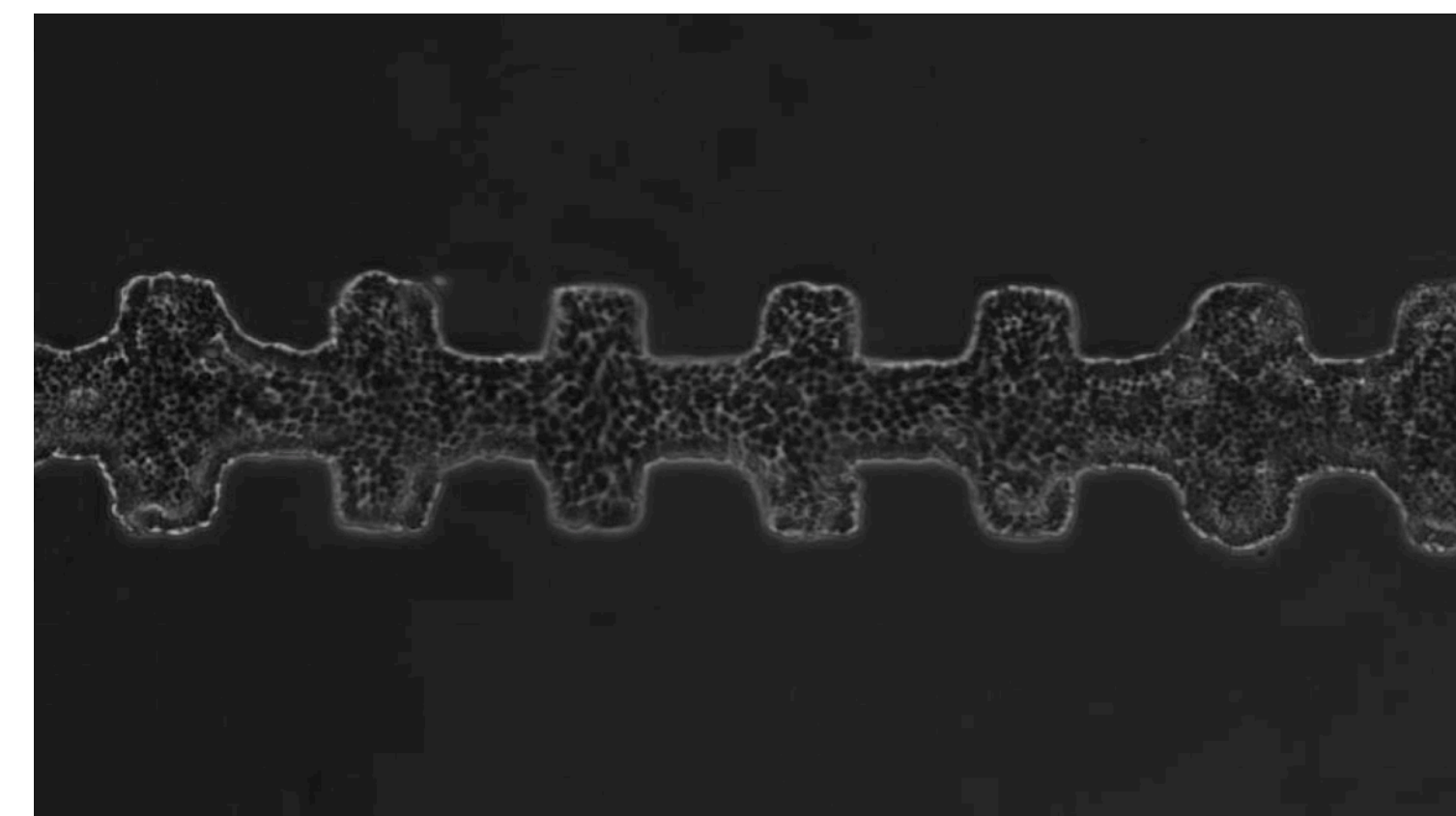
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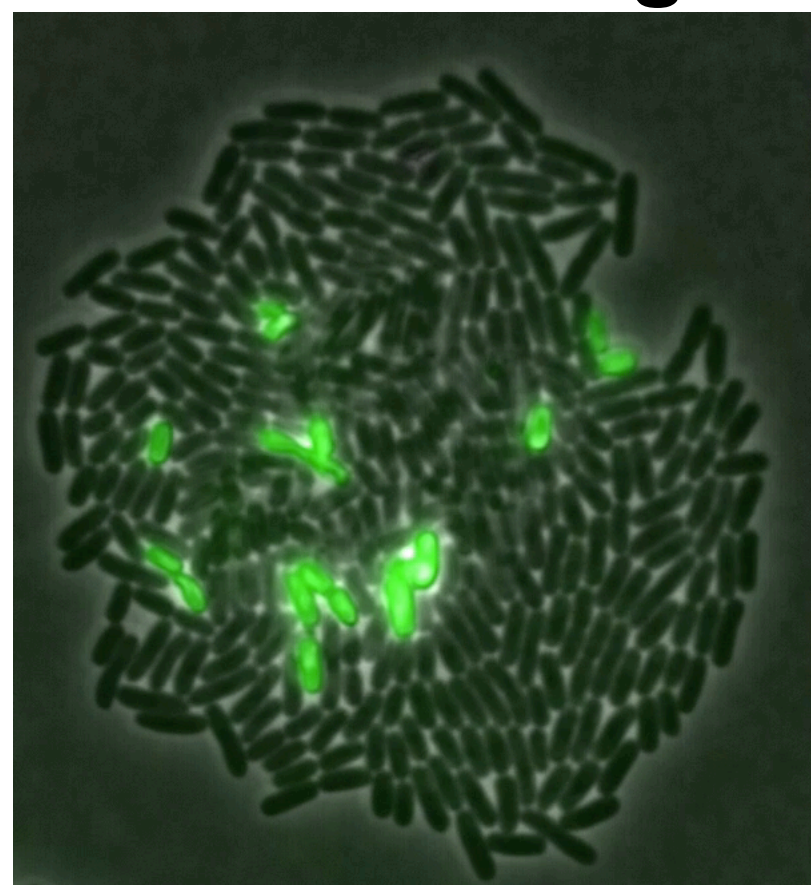
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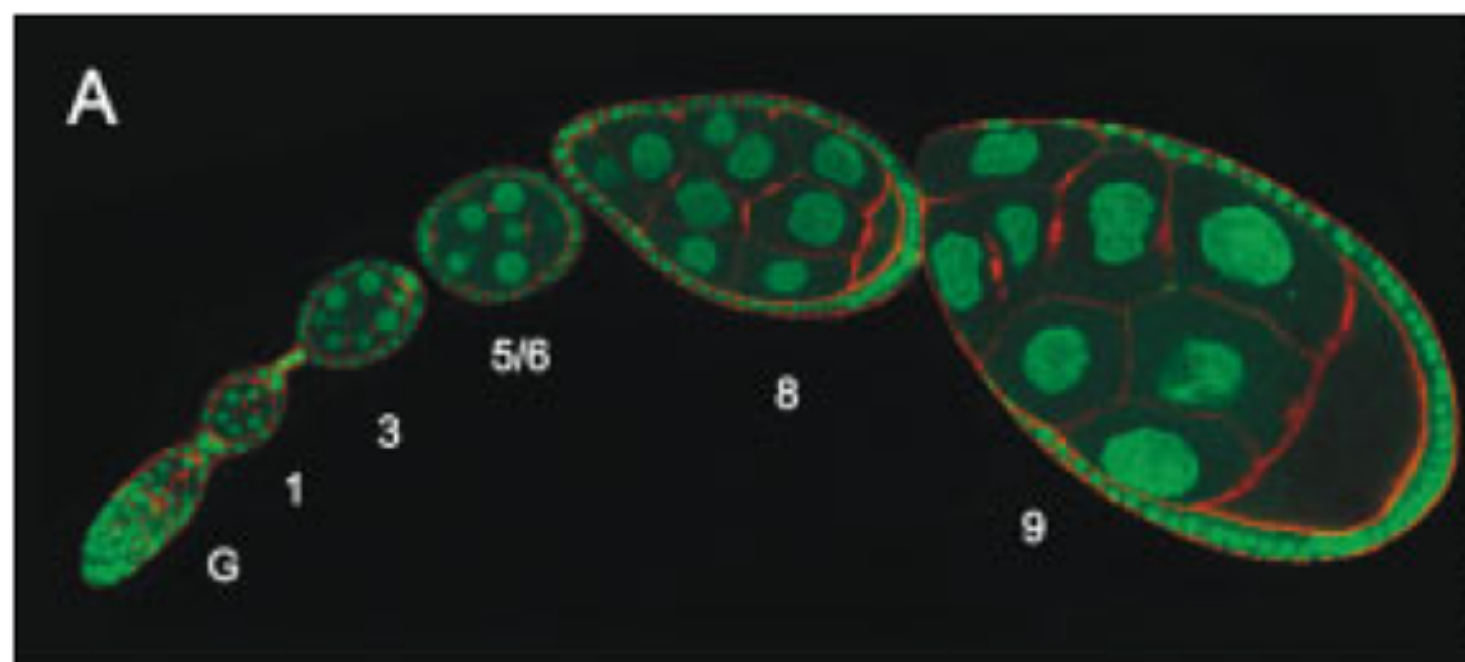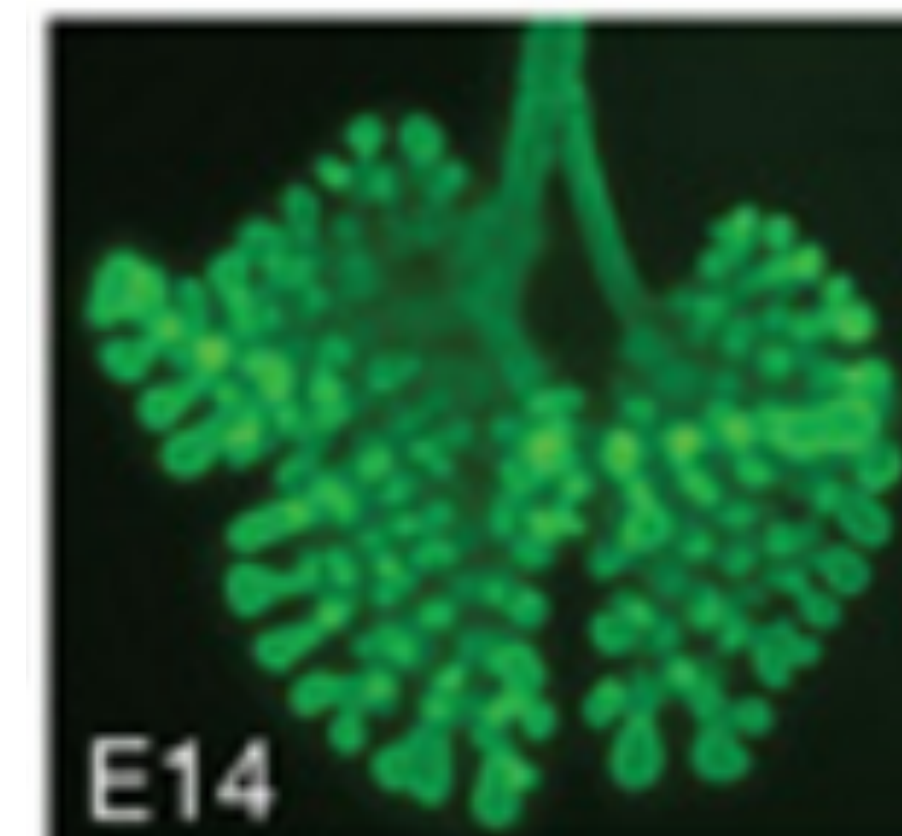M. Haataja

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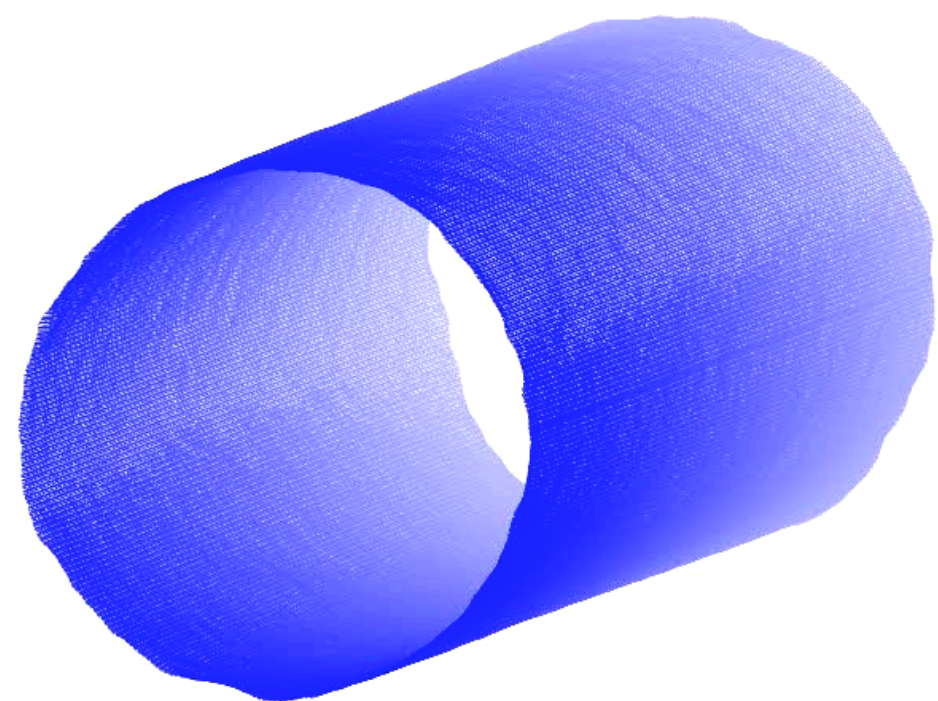B. Bassler, H. Stone, N. Wingreen

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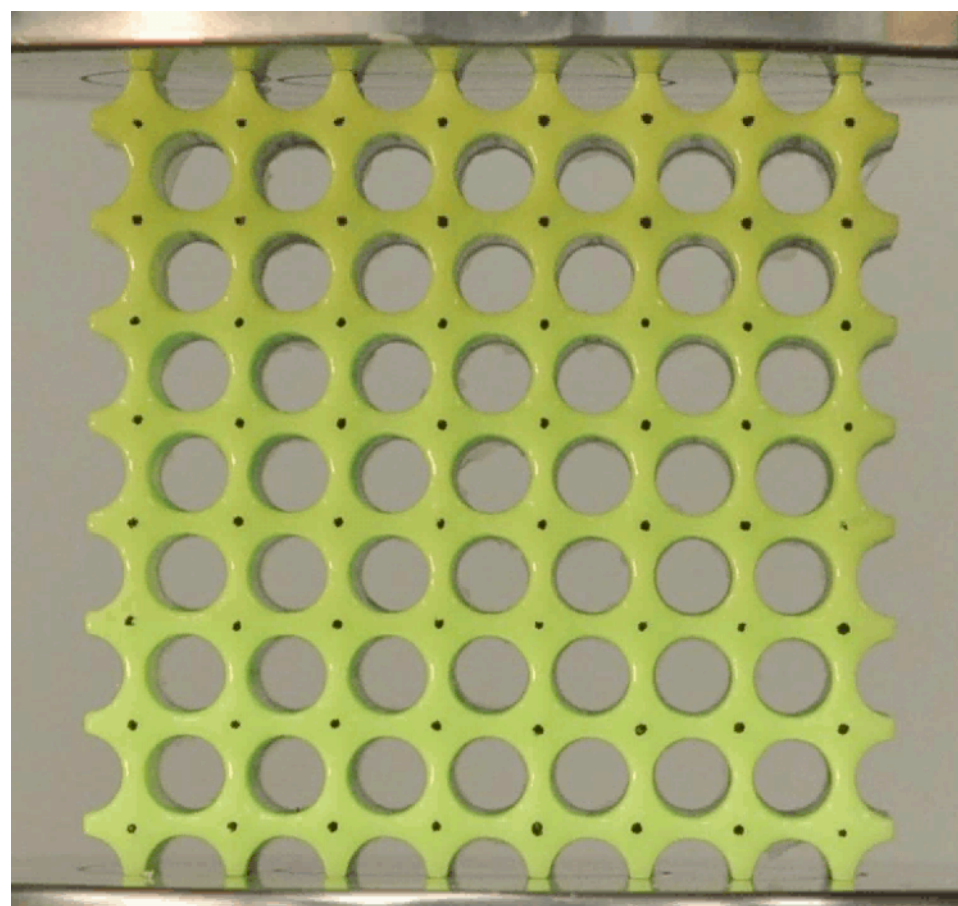
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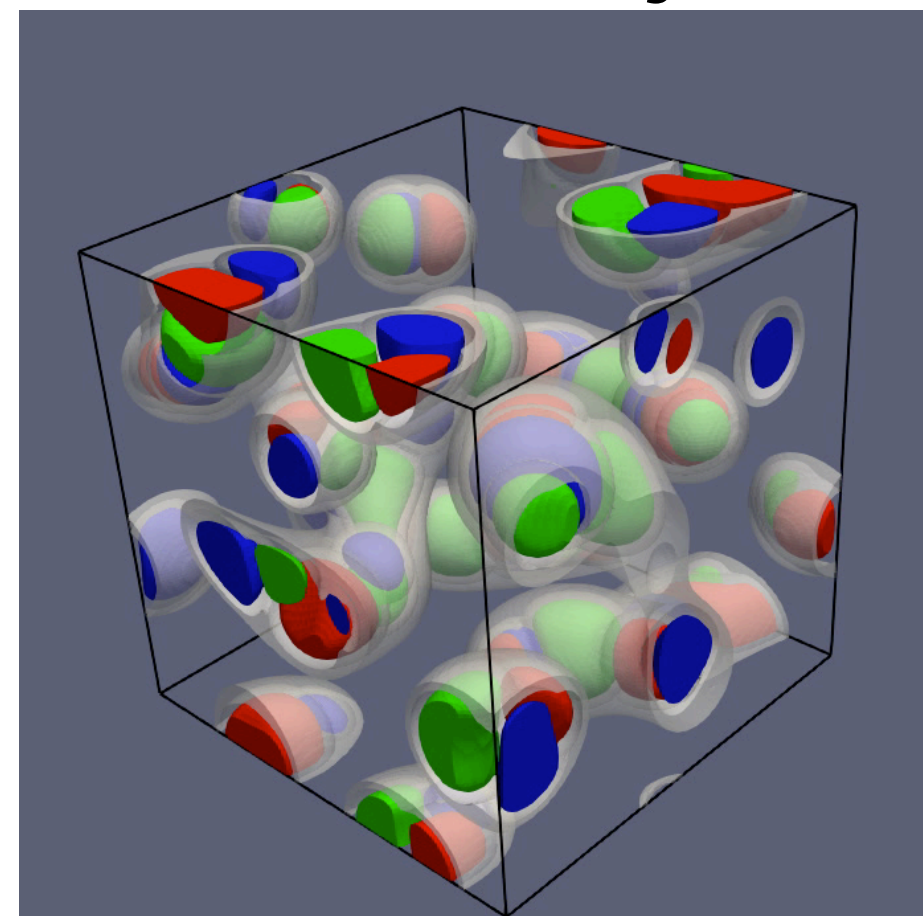D. Nelson, D. Weitz, R. Bruinsma, P. Koumoutsakos M.J. Abdolhosseini Qomi

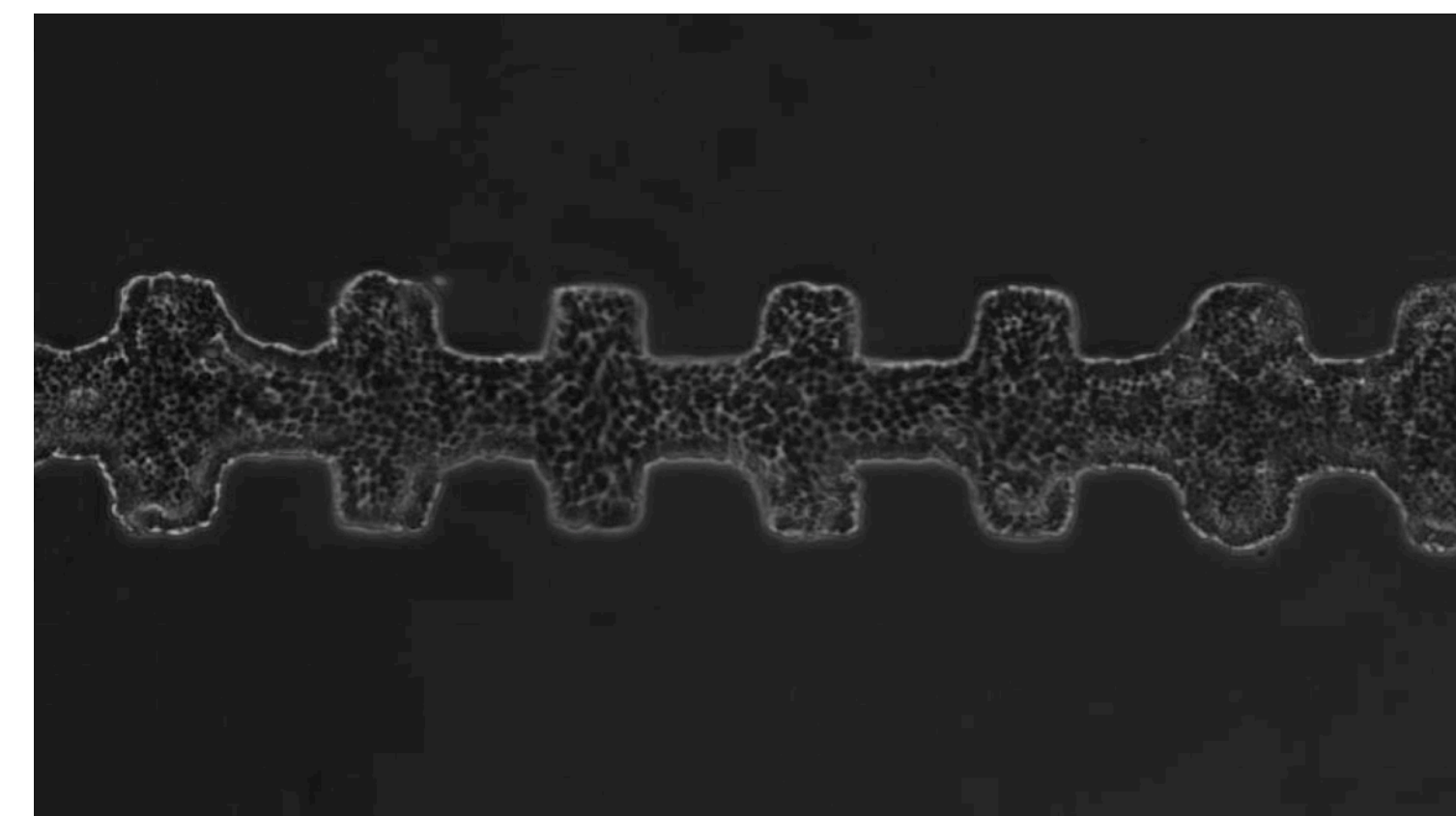**mechanical metamaterials**

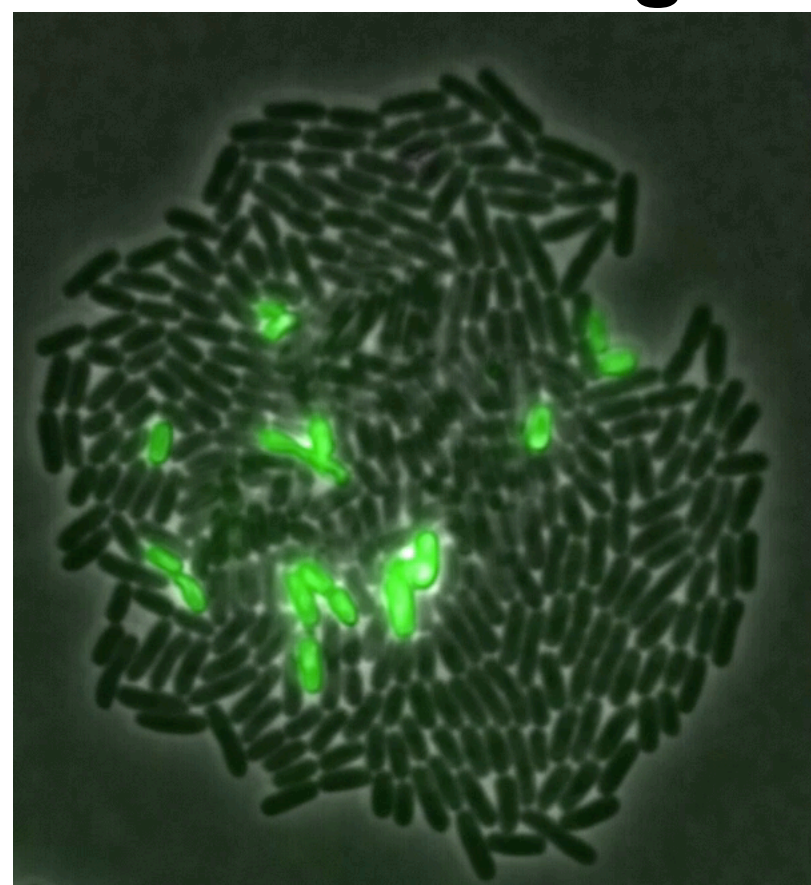K. Bertoldi, M. Brojan

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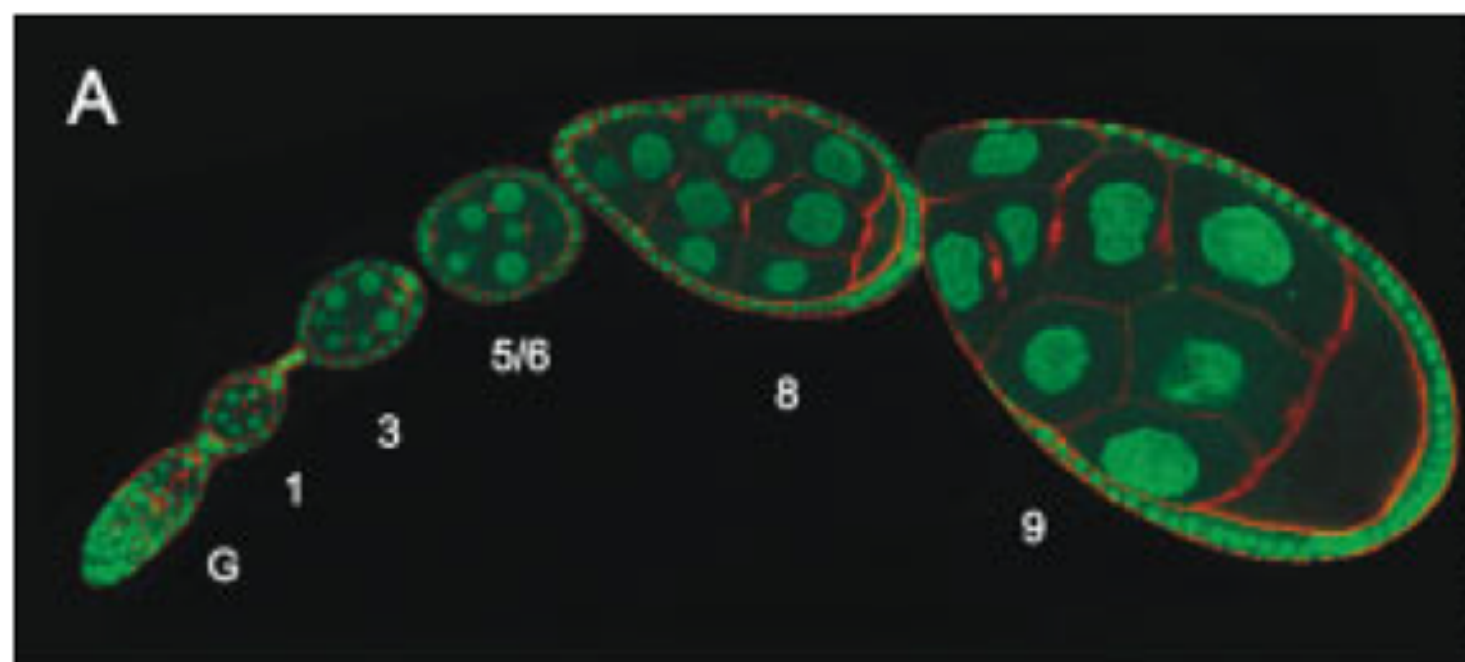
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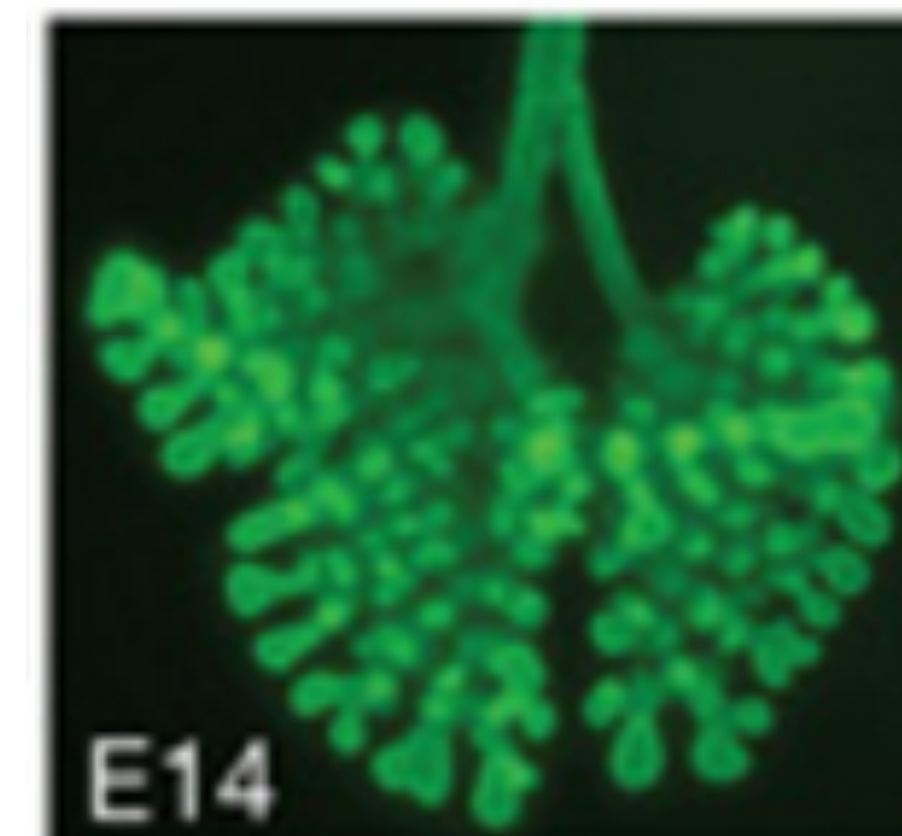
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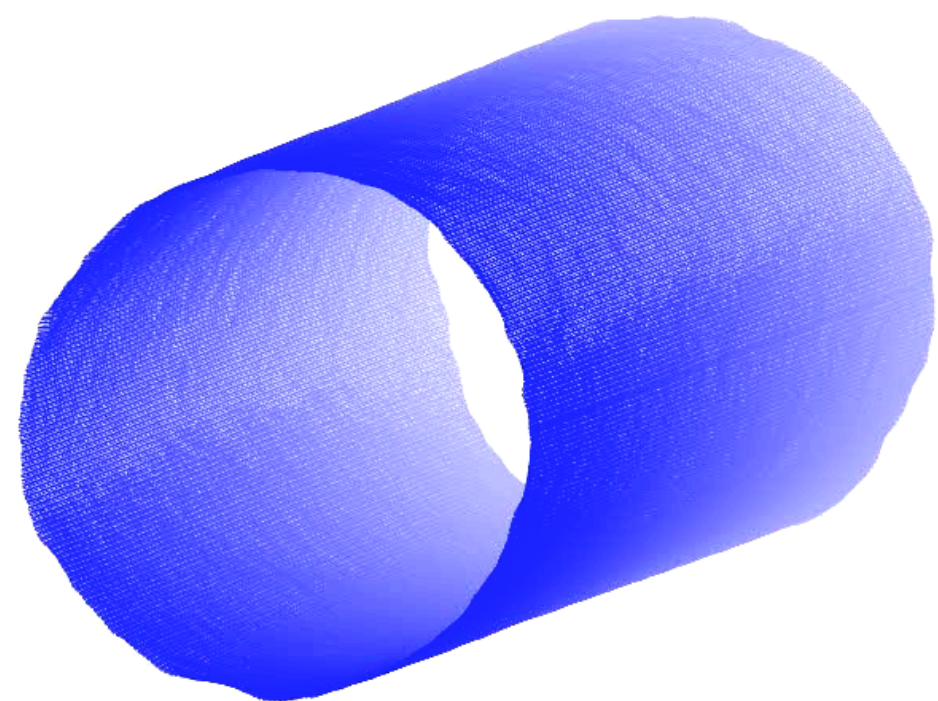B. Bassler, H. Stone, N. Wingreen

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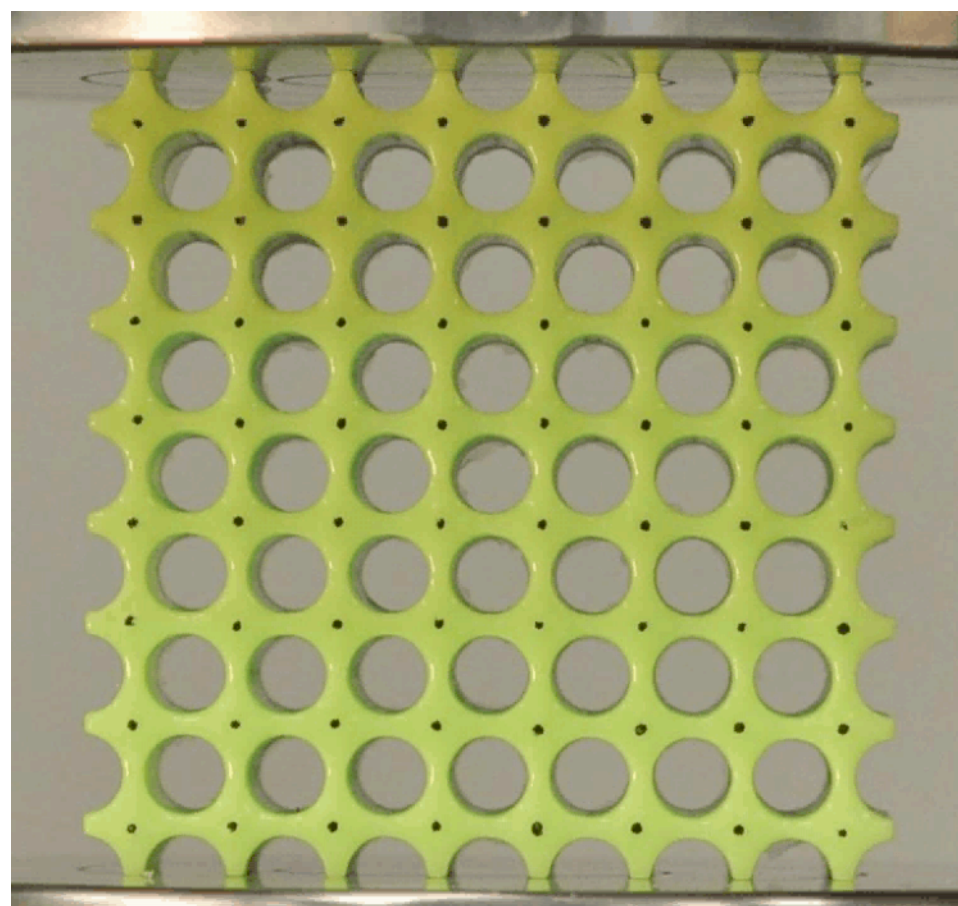C. Nelson

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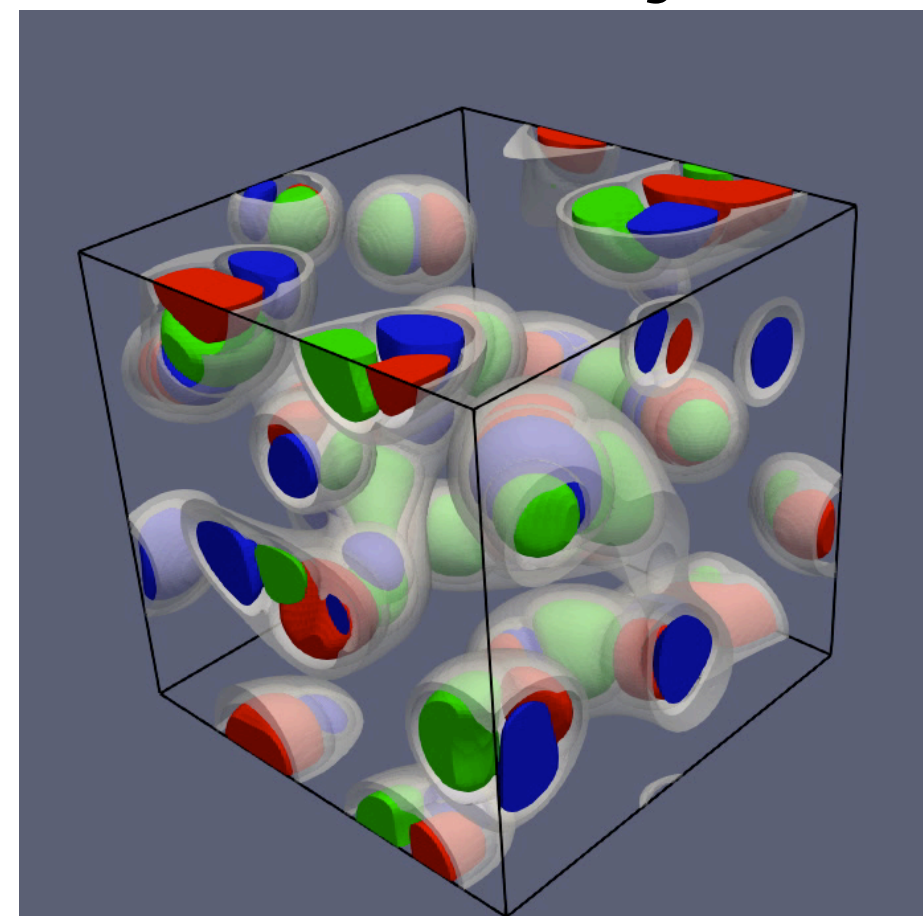D. Nelson, D. Weitz, R. Bruinsma, P. Koumoutsakos M.J. Abdolhosseini Qomi

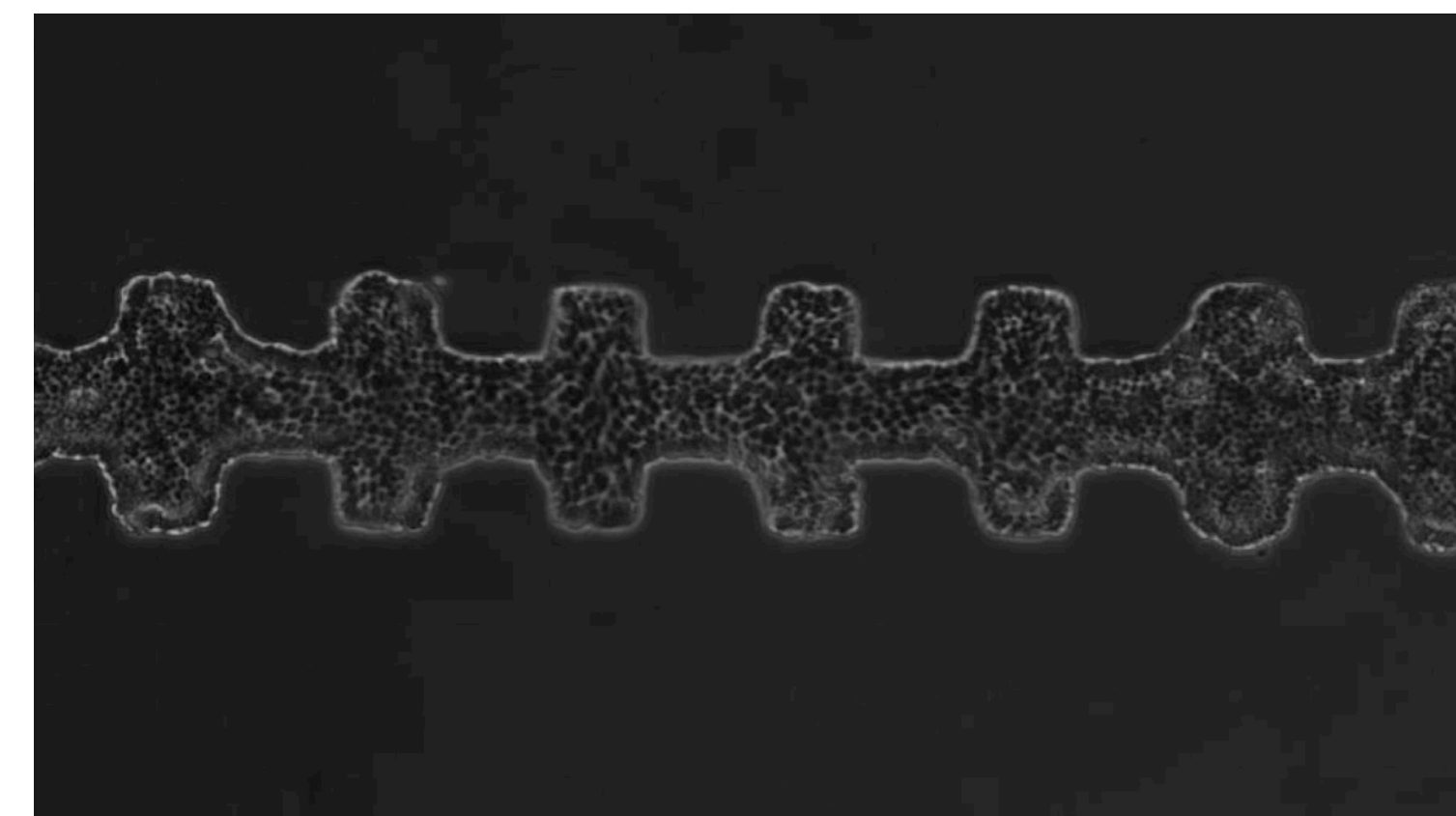**mechanical metamaterials**

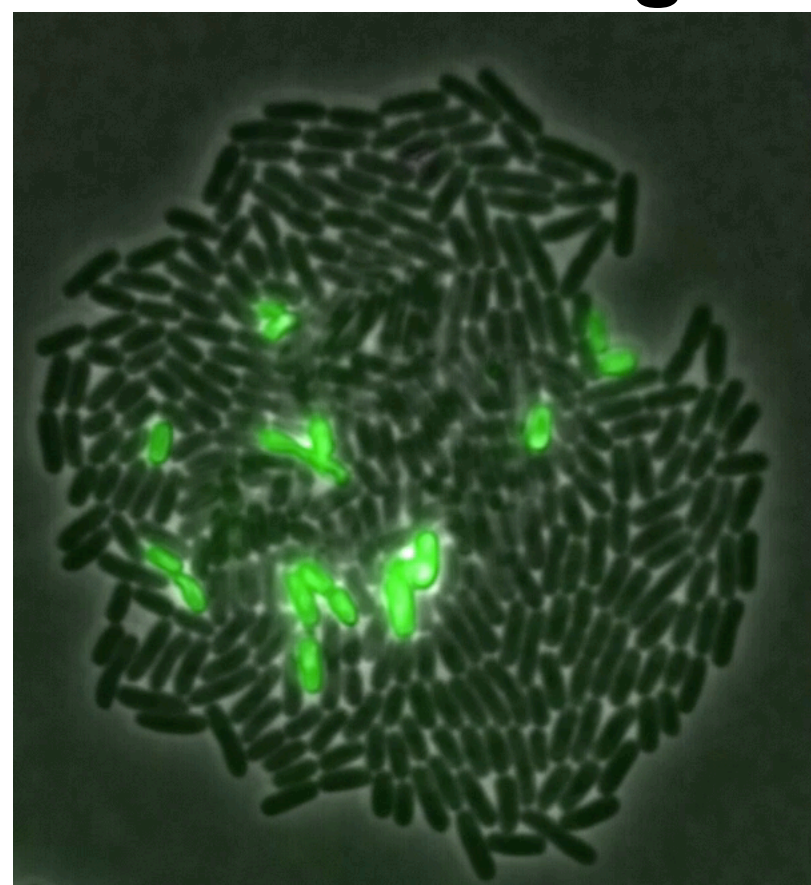K. Bertoldi, M. Brojan

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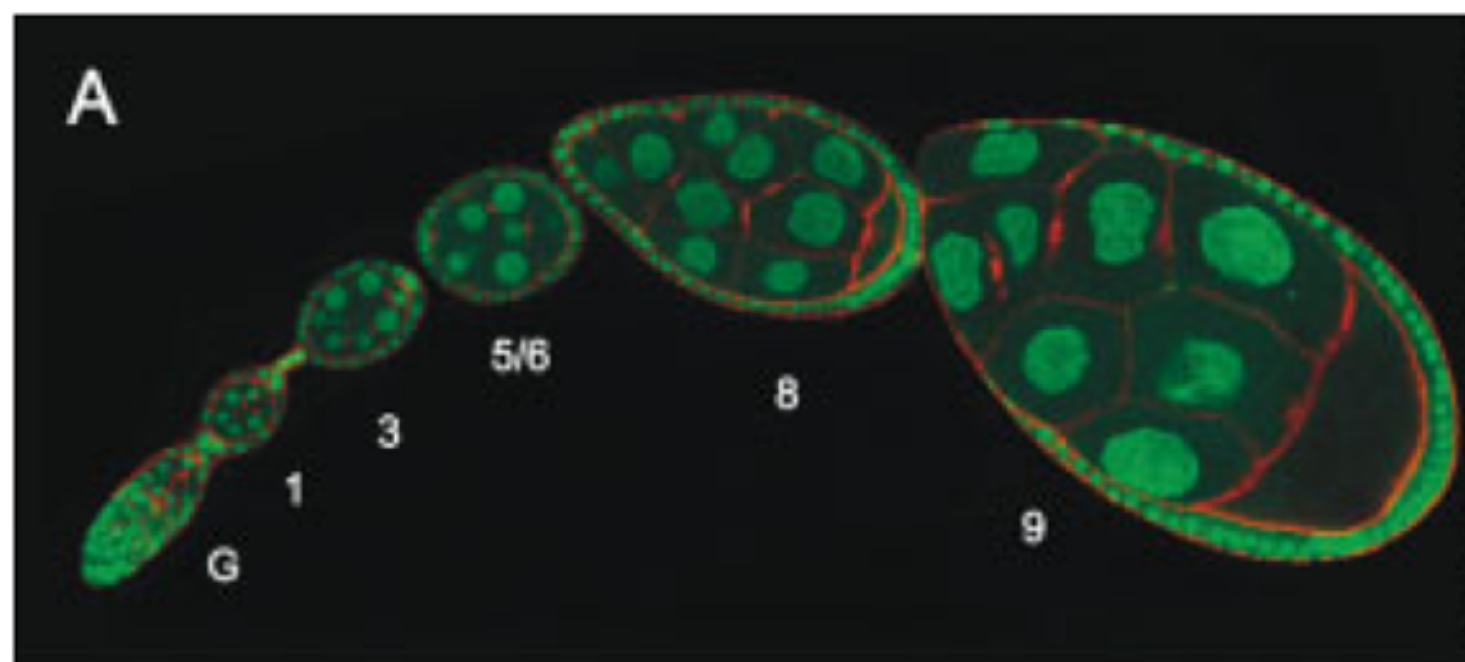
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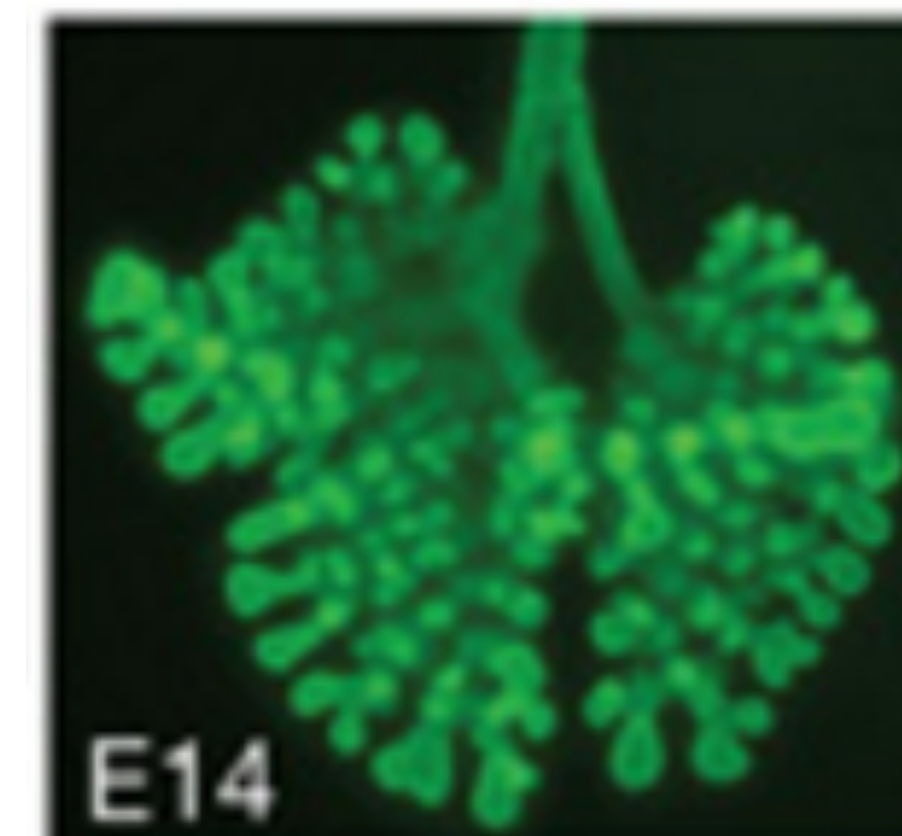S. Taheri-Araghi

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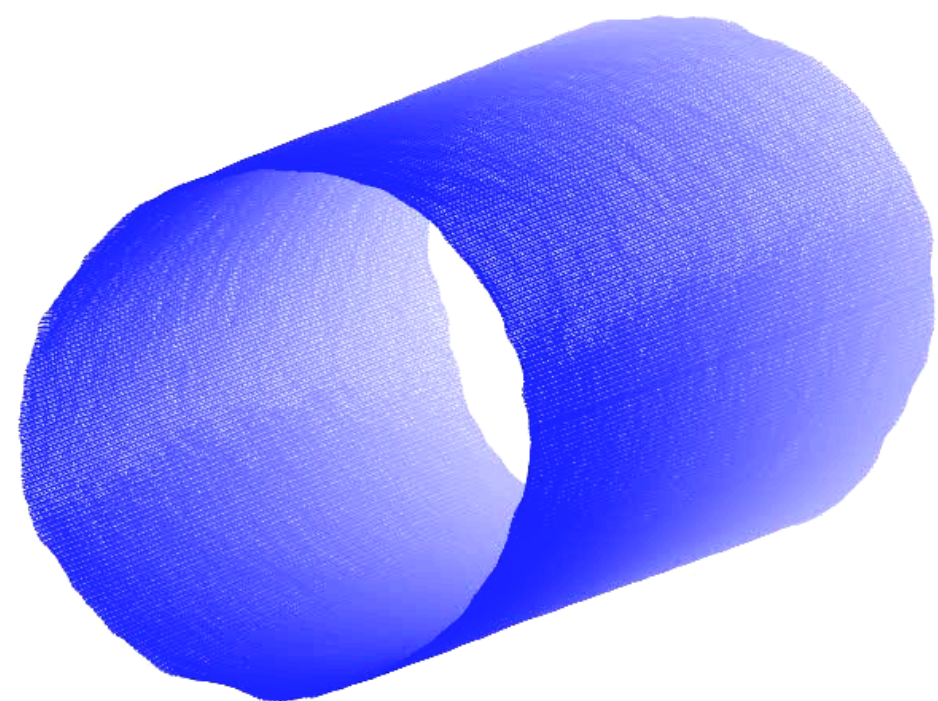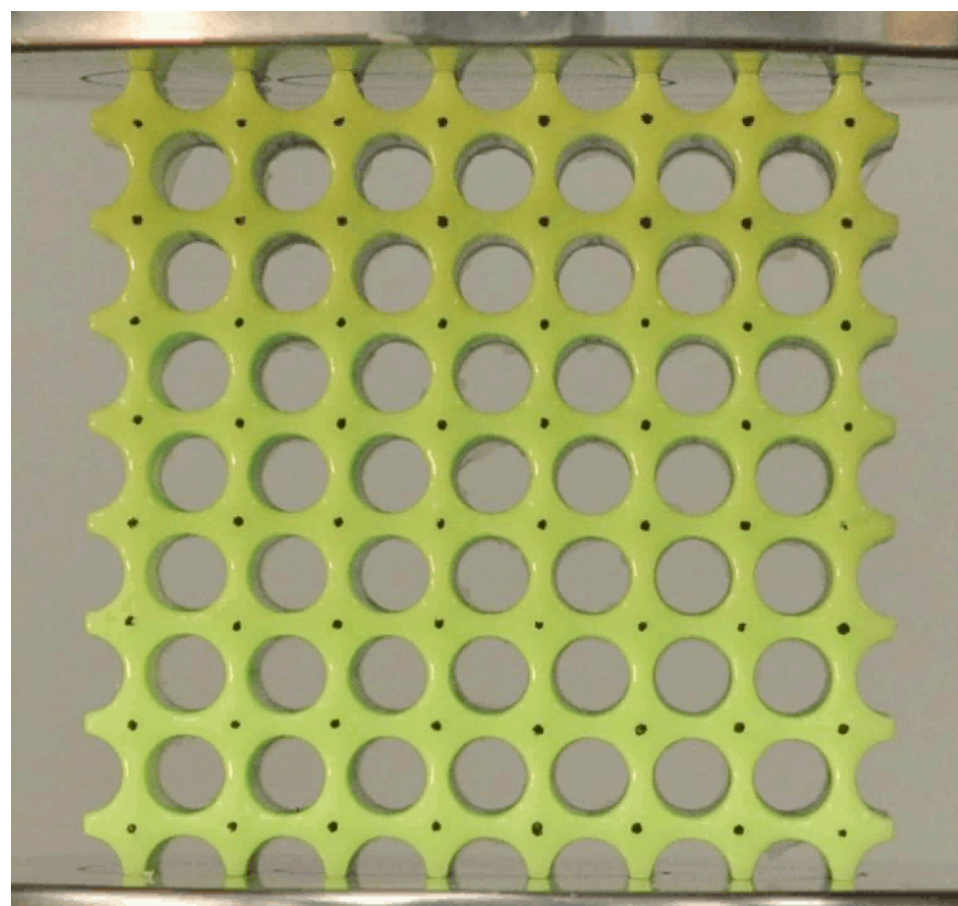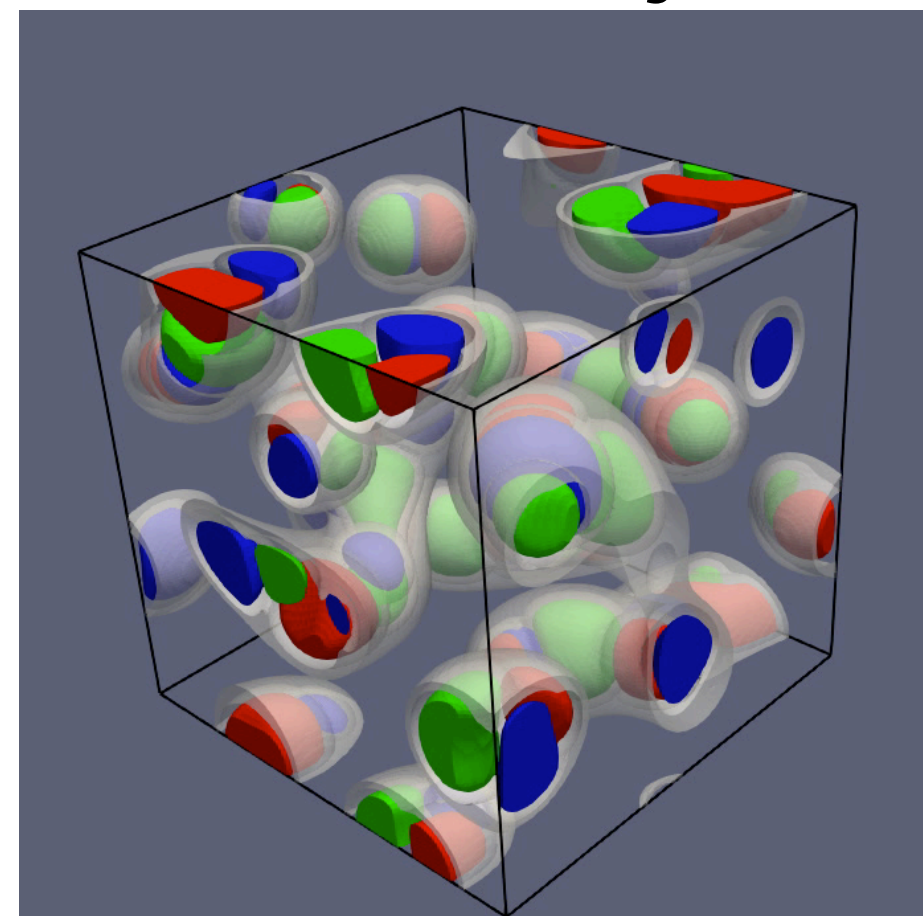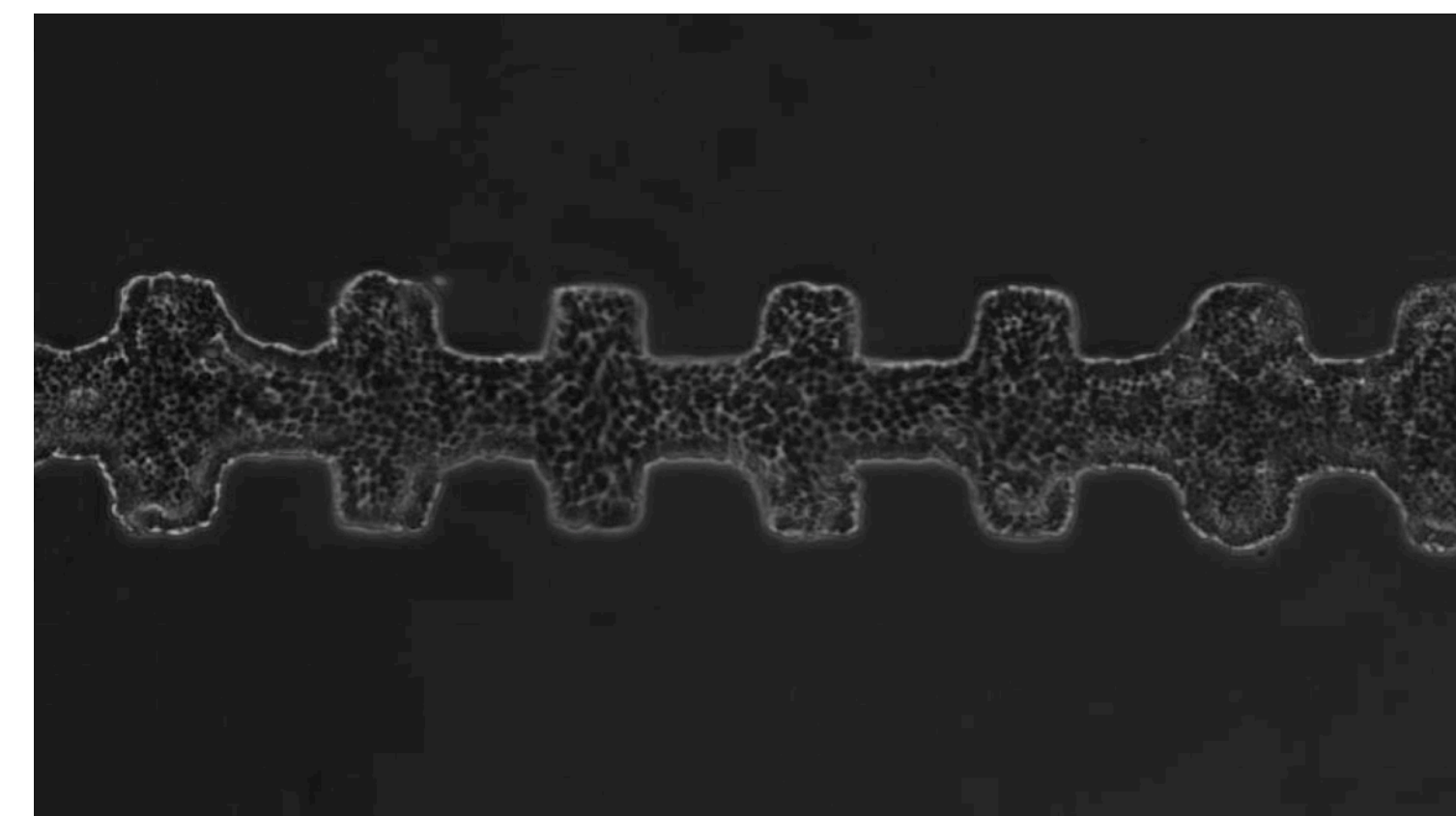
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# Wrinkling and branching in growing biological systems
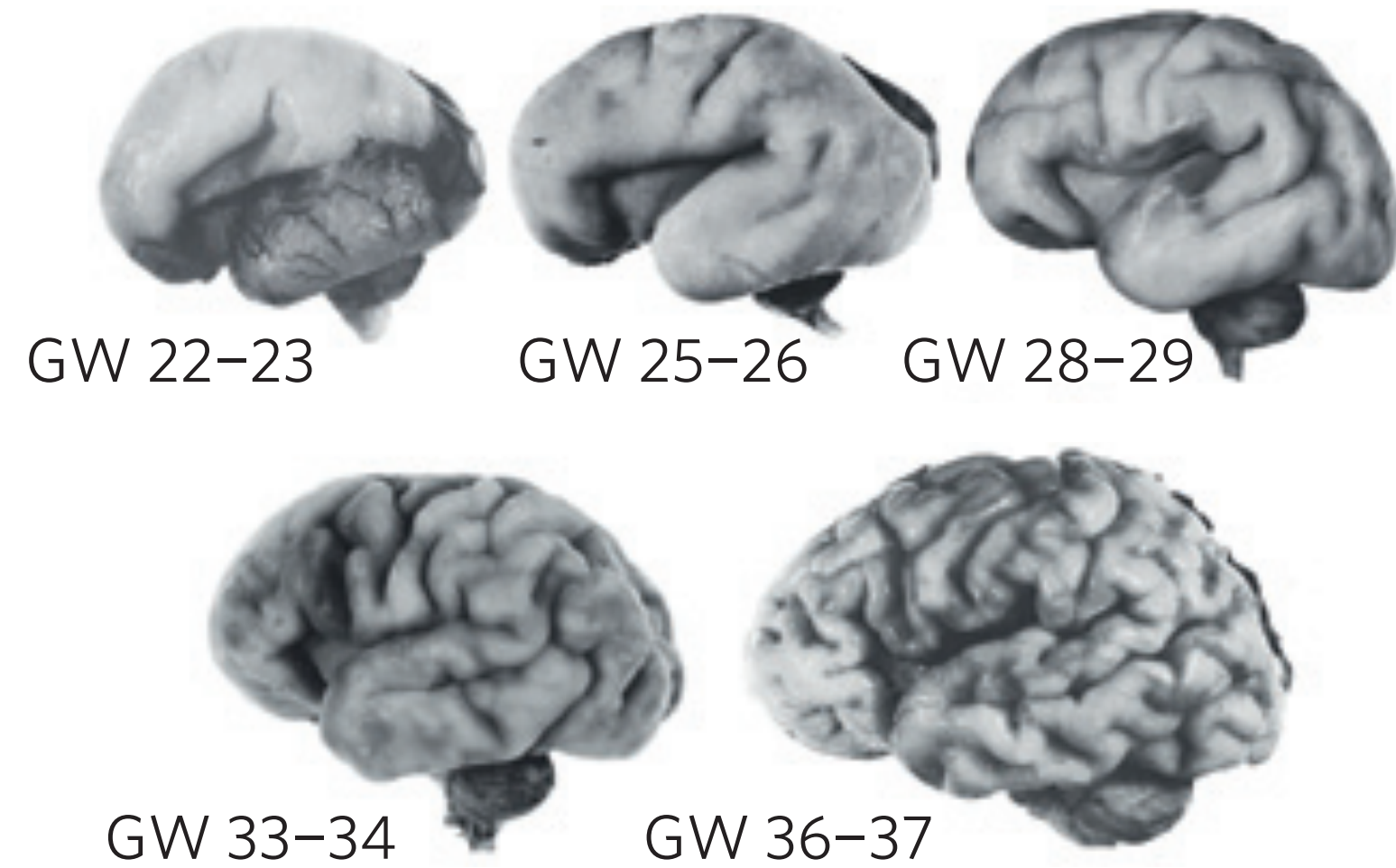
## folding of brain



GW 22–23    GW 25–26    GW 28–29

GW 33–34    GW 36–37

T. Tallinen *et al.*, Nat. Phys. **12**, 588 (2016)

## vili formation in guts



vili

$E6$

$E12$

$E15$

$E17$

$100\,\mu m$
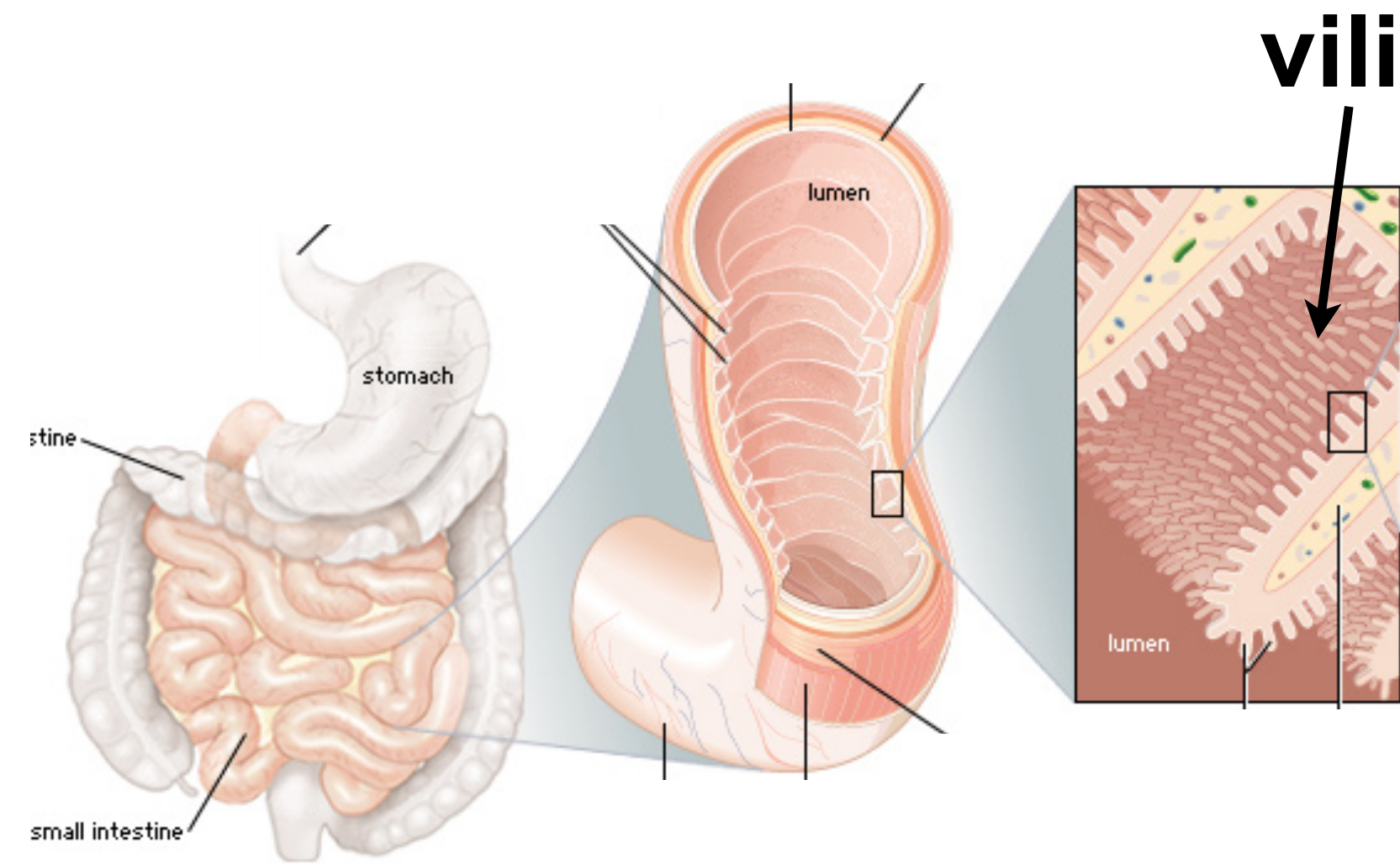
A. Shyer *et al.*, Science **342**, 212 (2013)
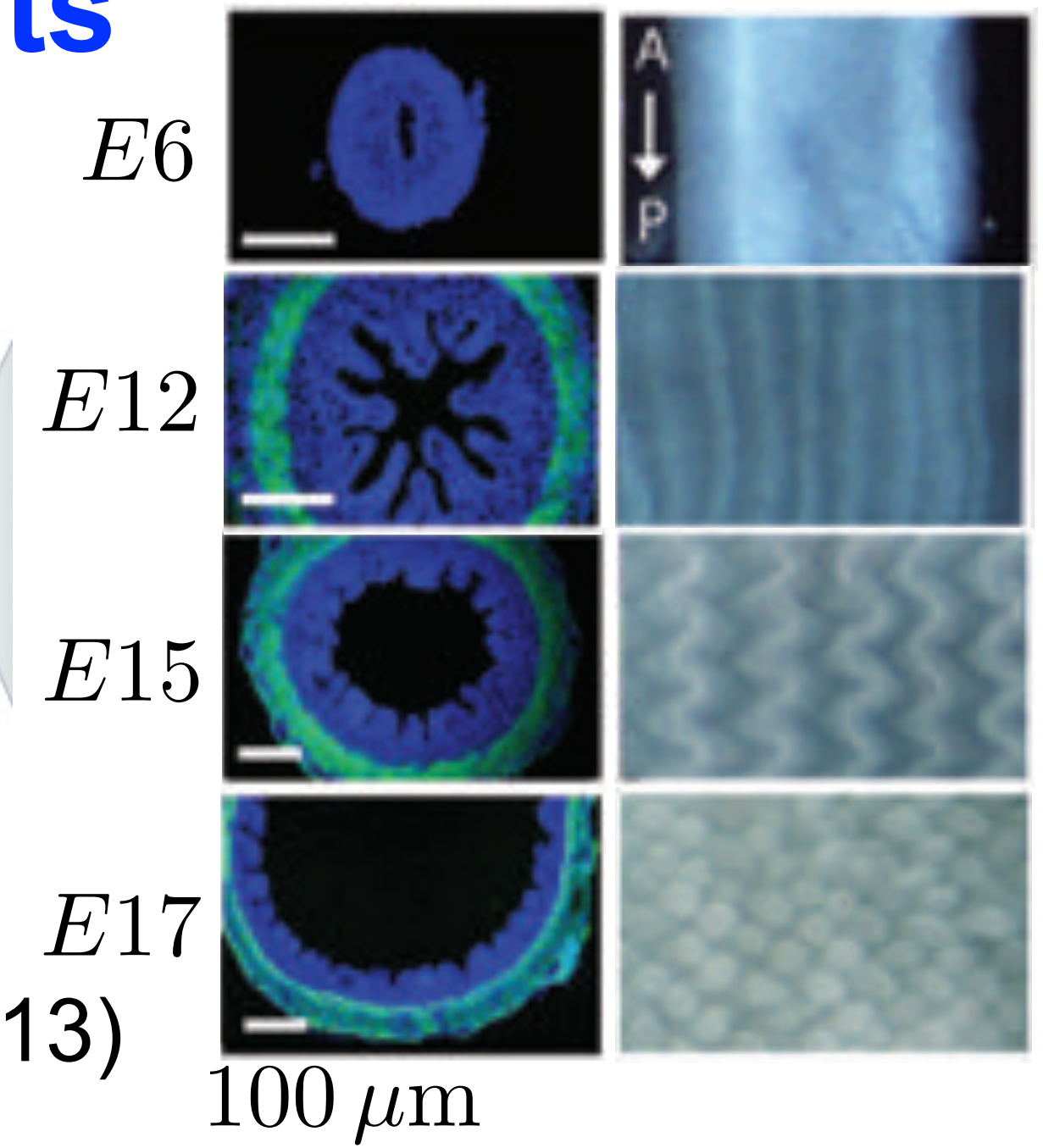
## lungs
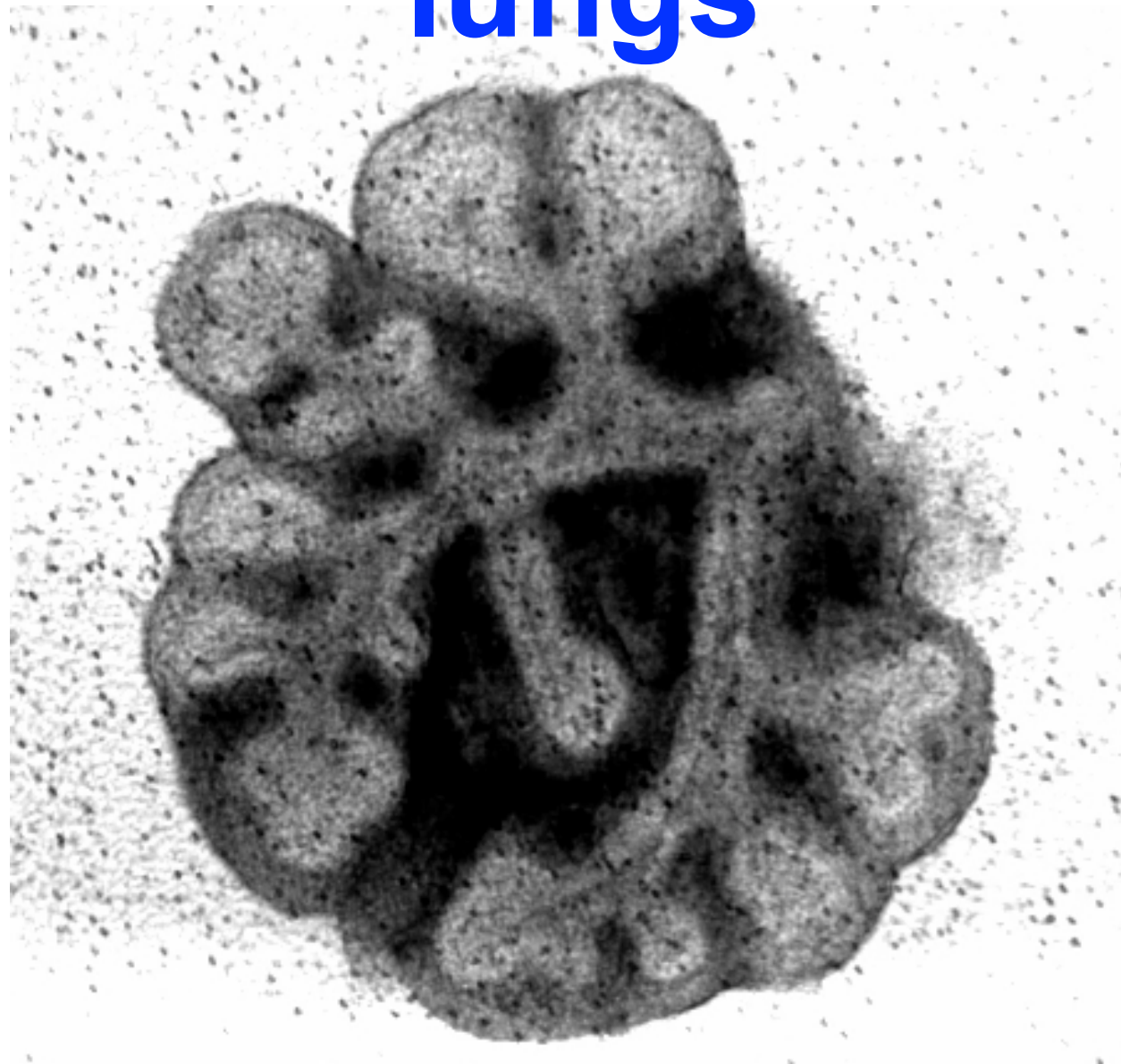


H.Y. Kim et al., Dev. Cell **34**, 719 (2015)

## kidney
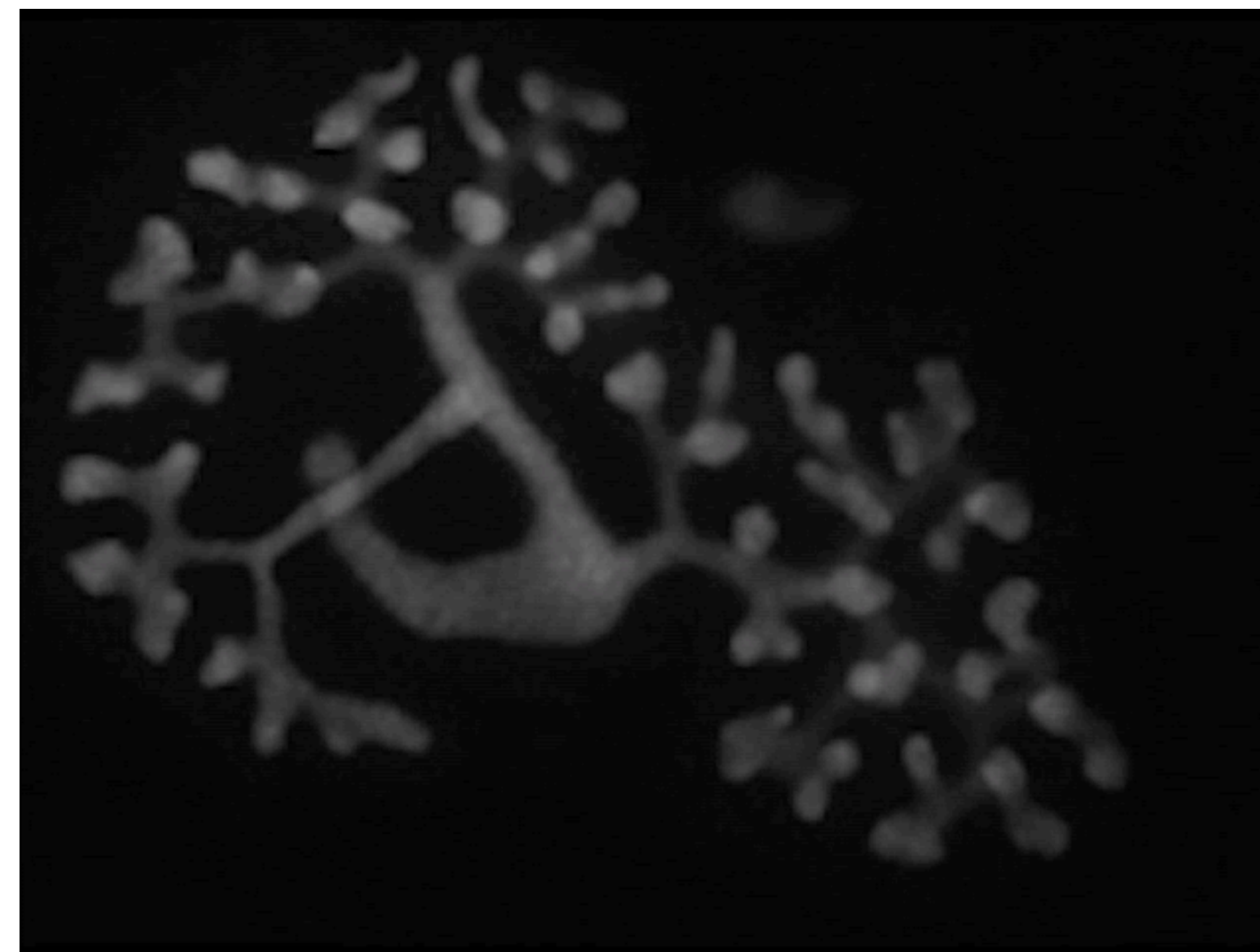


T. Watanabe & F. Constantini, Dev. Biol. **271**, 98 (2004)

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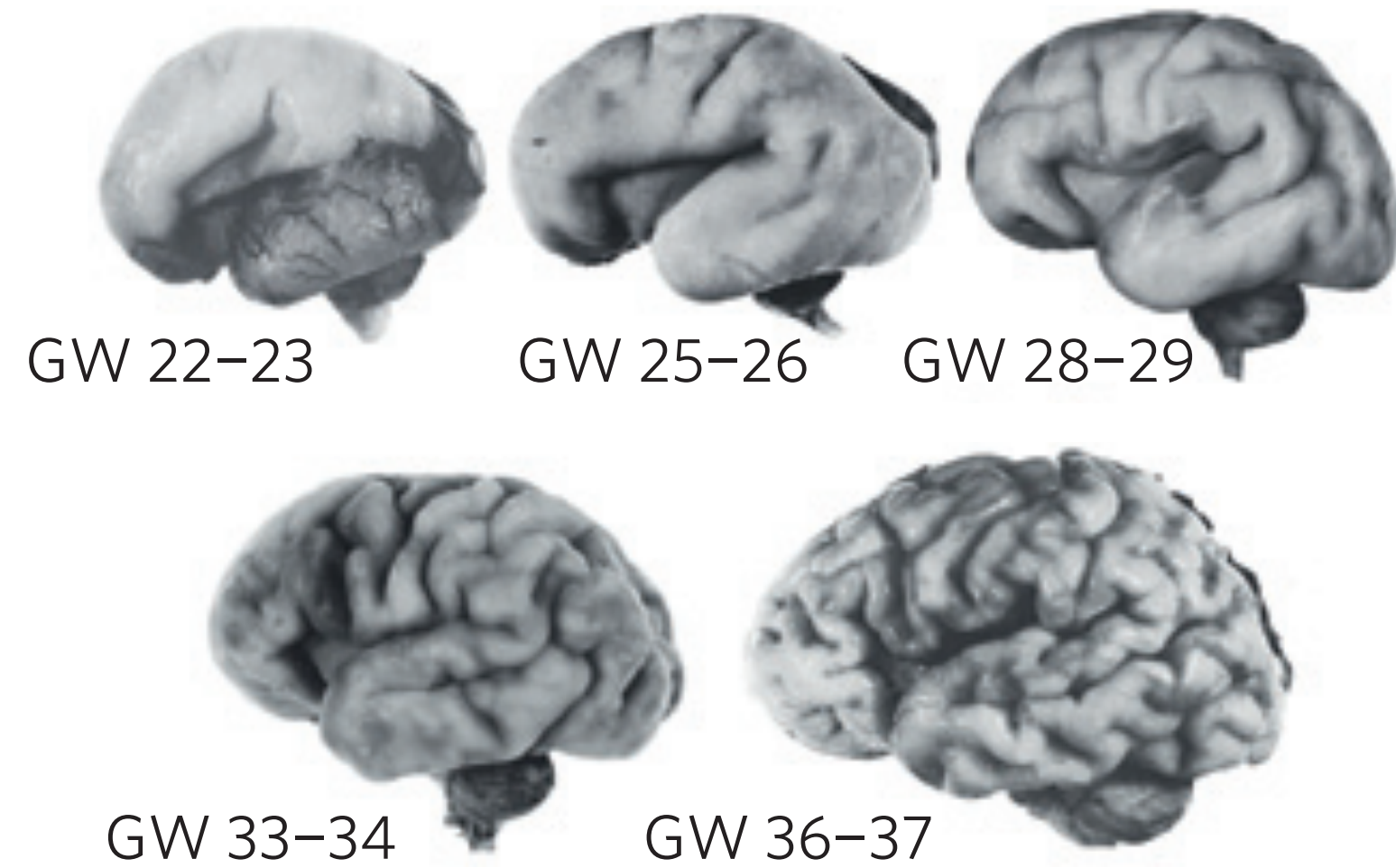
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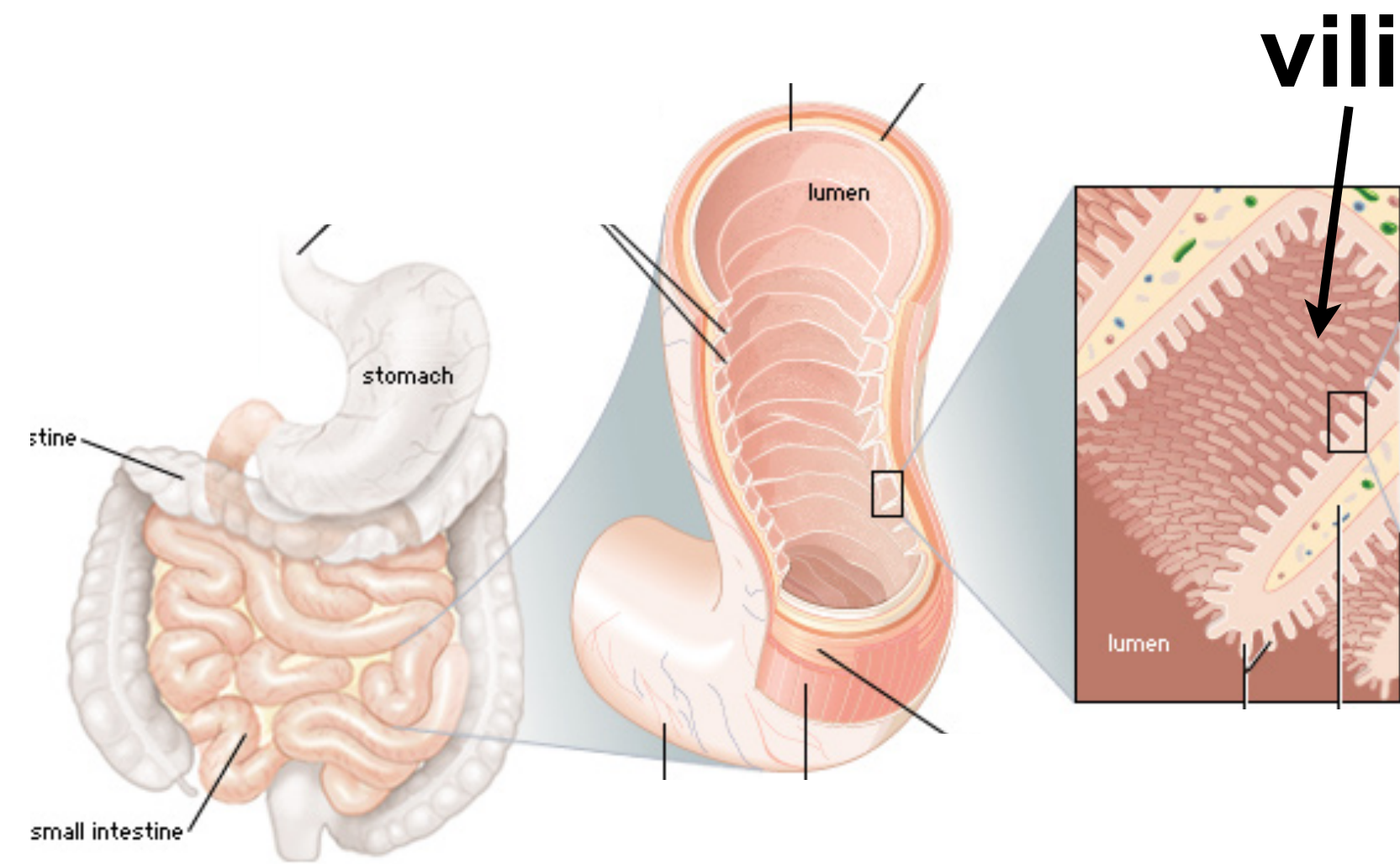
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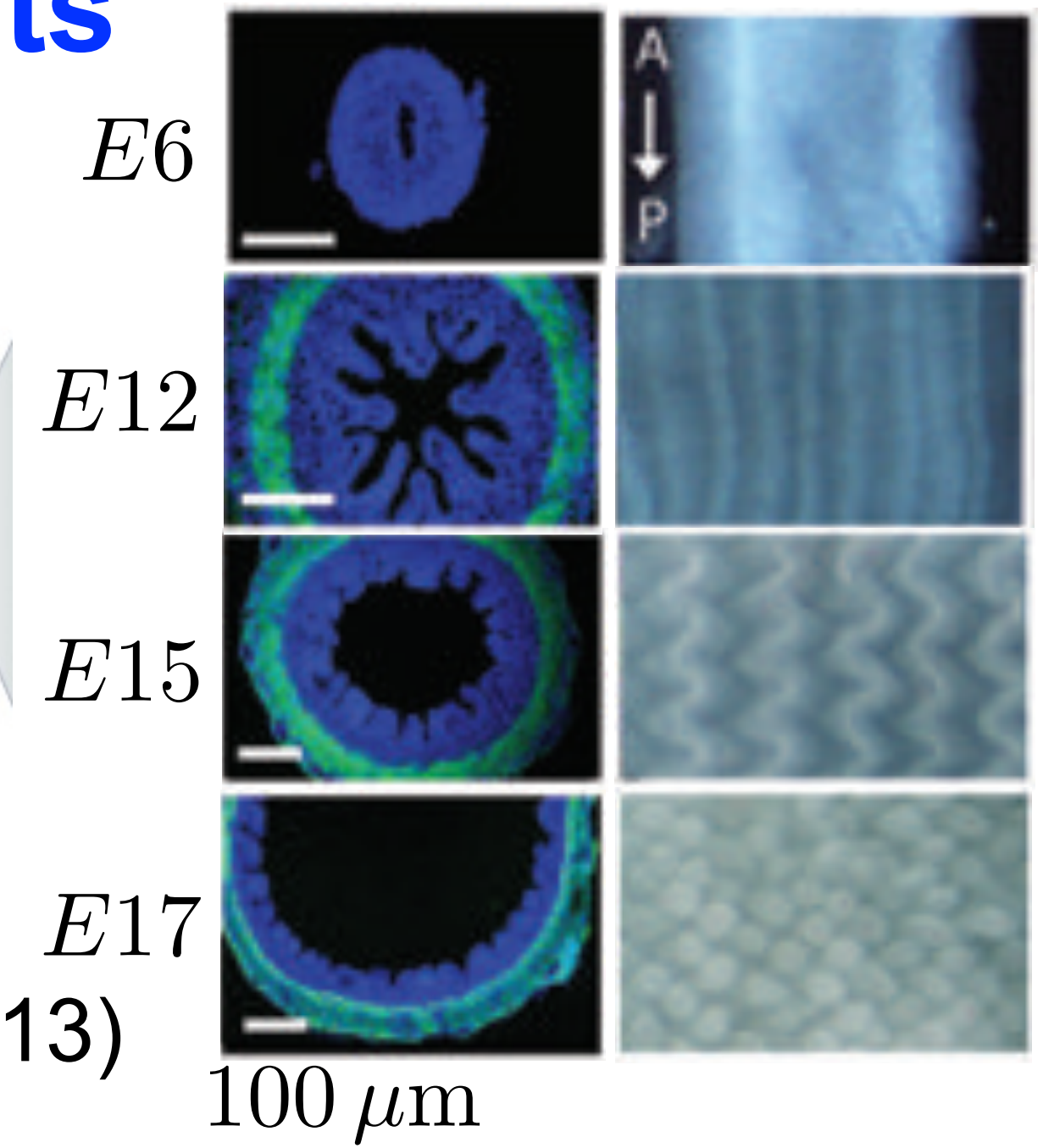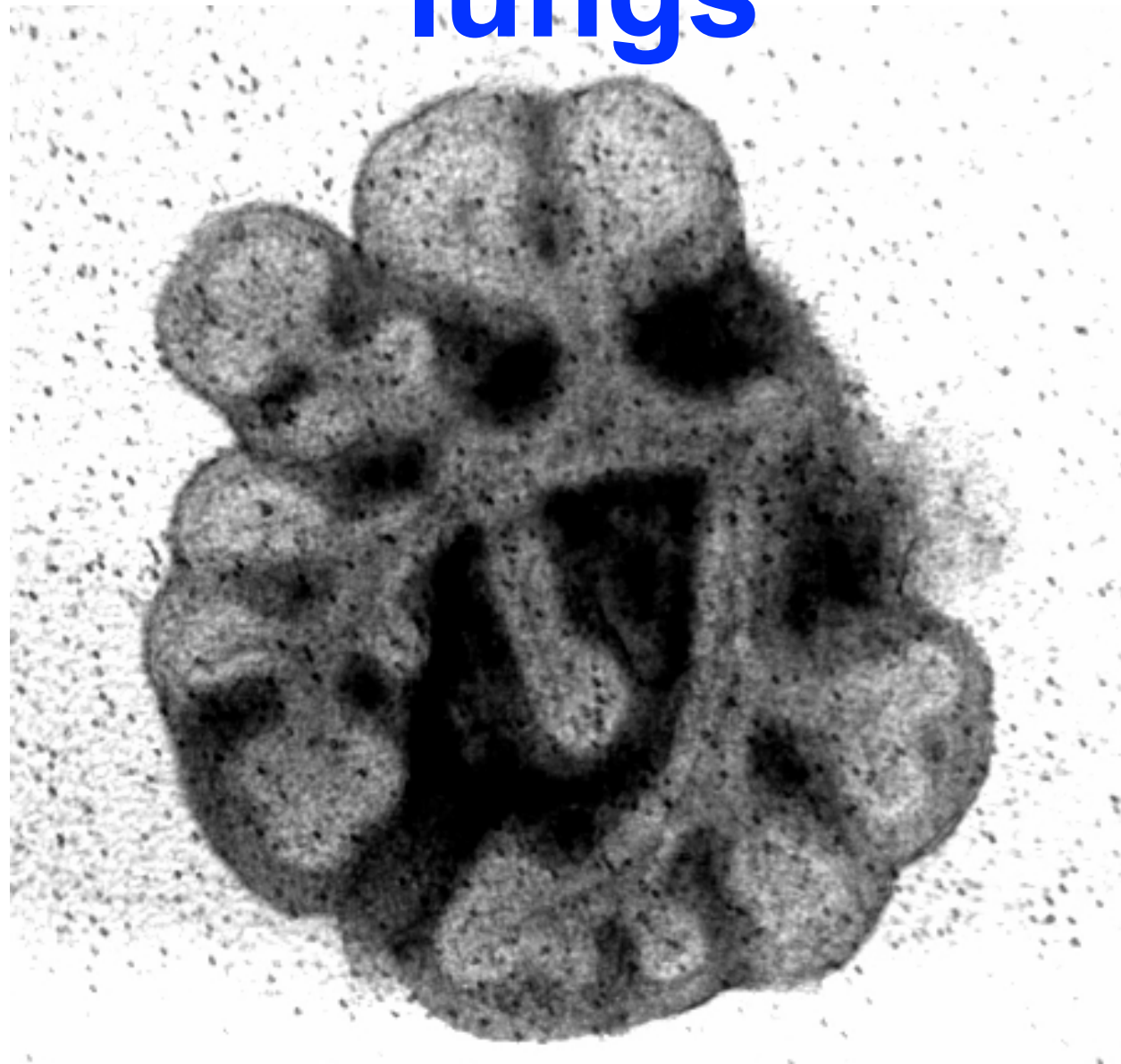
## lungs



H.Y. Kim et al., Dev. Cell **34**, 719 (2015)

## kidney
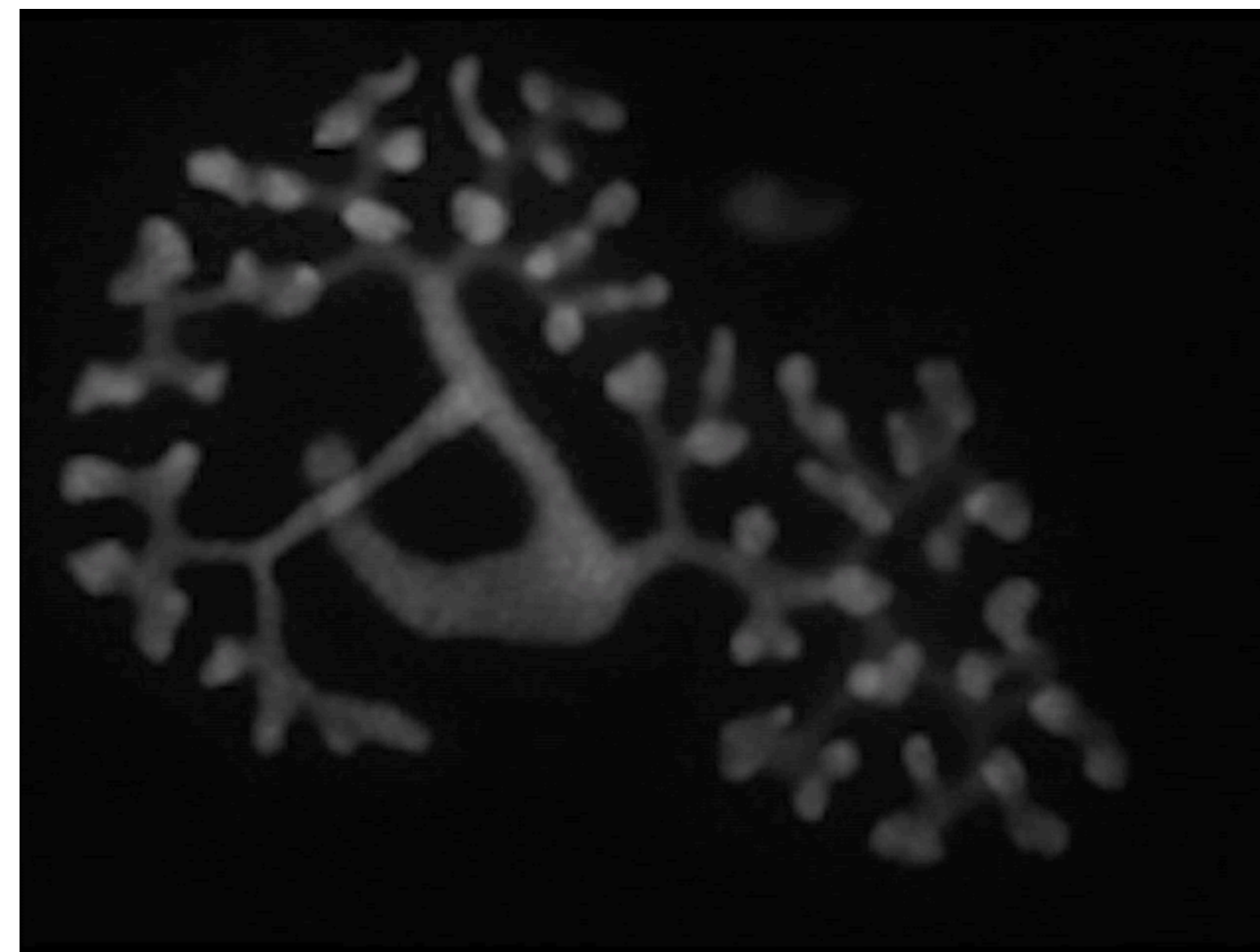


T. Watanabe & F. Constantini, Dev. Biol. **271**, 98 (2004)

# Outline

**wrinkling instability of growing films**
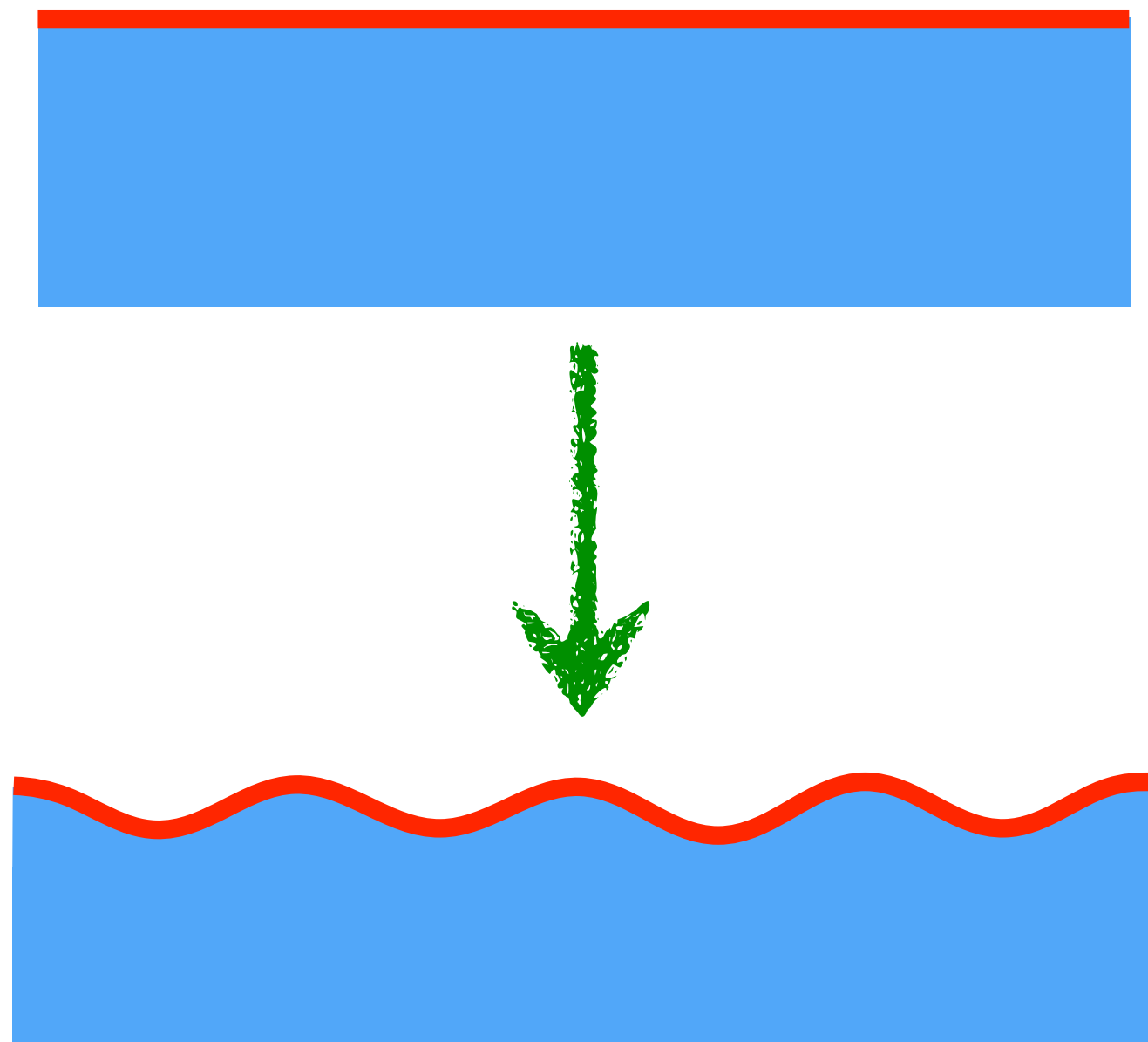
**wrinkling of bacterial biofilm**
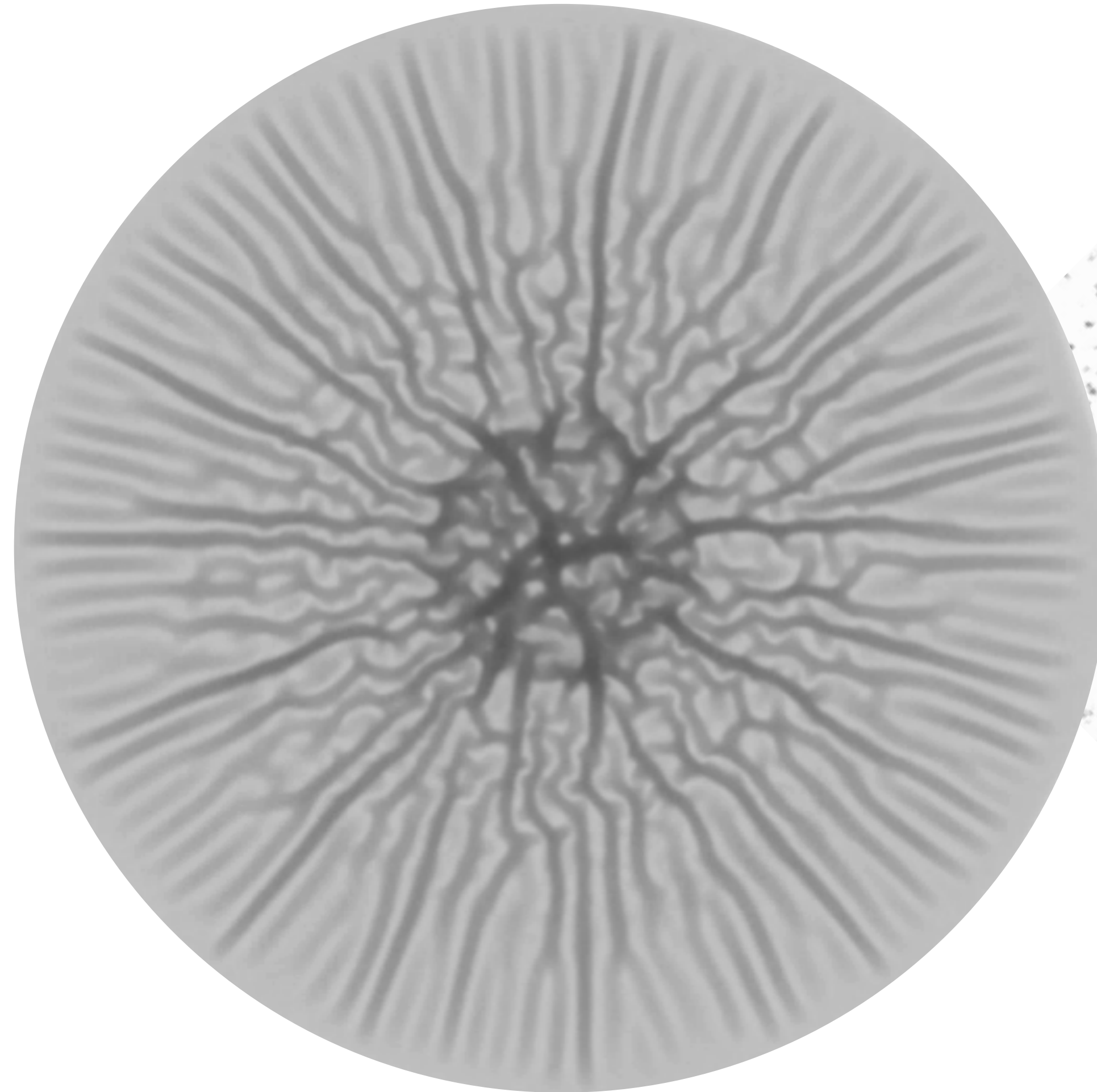
**branching of developing lungs**
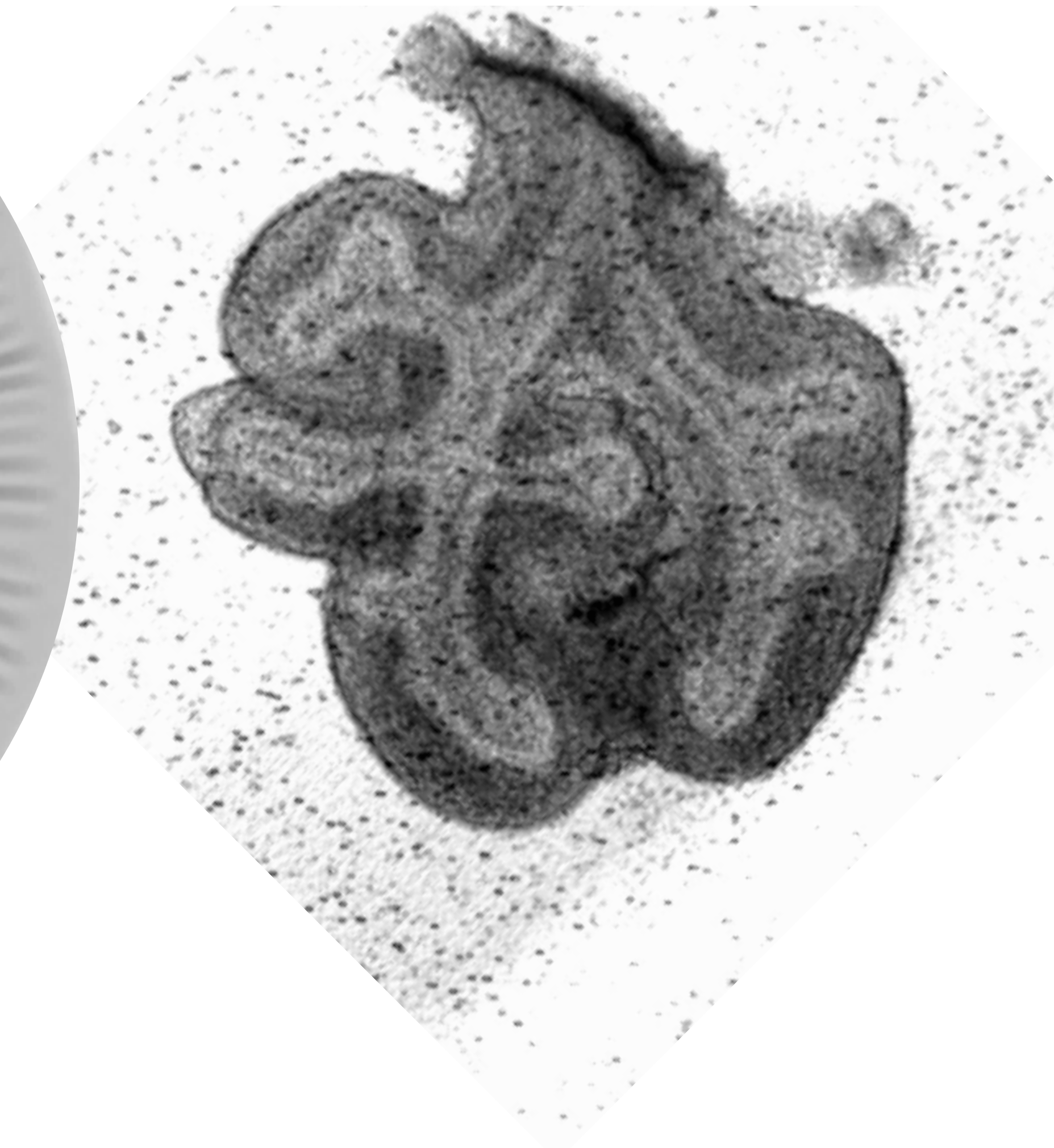
# Compression of stiff thin sheets on liquid and soft elastic substrates



**air**

**stiff sheet**

**liquid or soft substrate**

*Liquid substrate*

*Elastic substrate*

**compression**

$\lambda_0$

$\lambda_0$

**10 $\mu$m thin sheet of polyester on water**

**~10 $\mu$m thin PDMS (stiffer) sheet on PDMS (softer) substrate**

$\lambda_0 = 1.6\,\text{cm}$

$\lambda_0 = 70\,\mu\text{m}$

L. Pocivavsek et al., Science **320**, 912 (2008)

F. Brau et al., Soft Matter **9**, 8177 (2013)
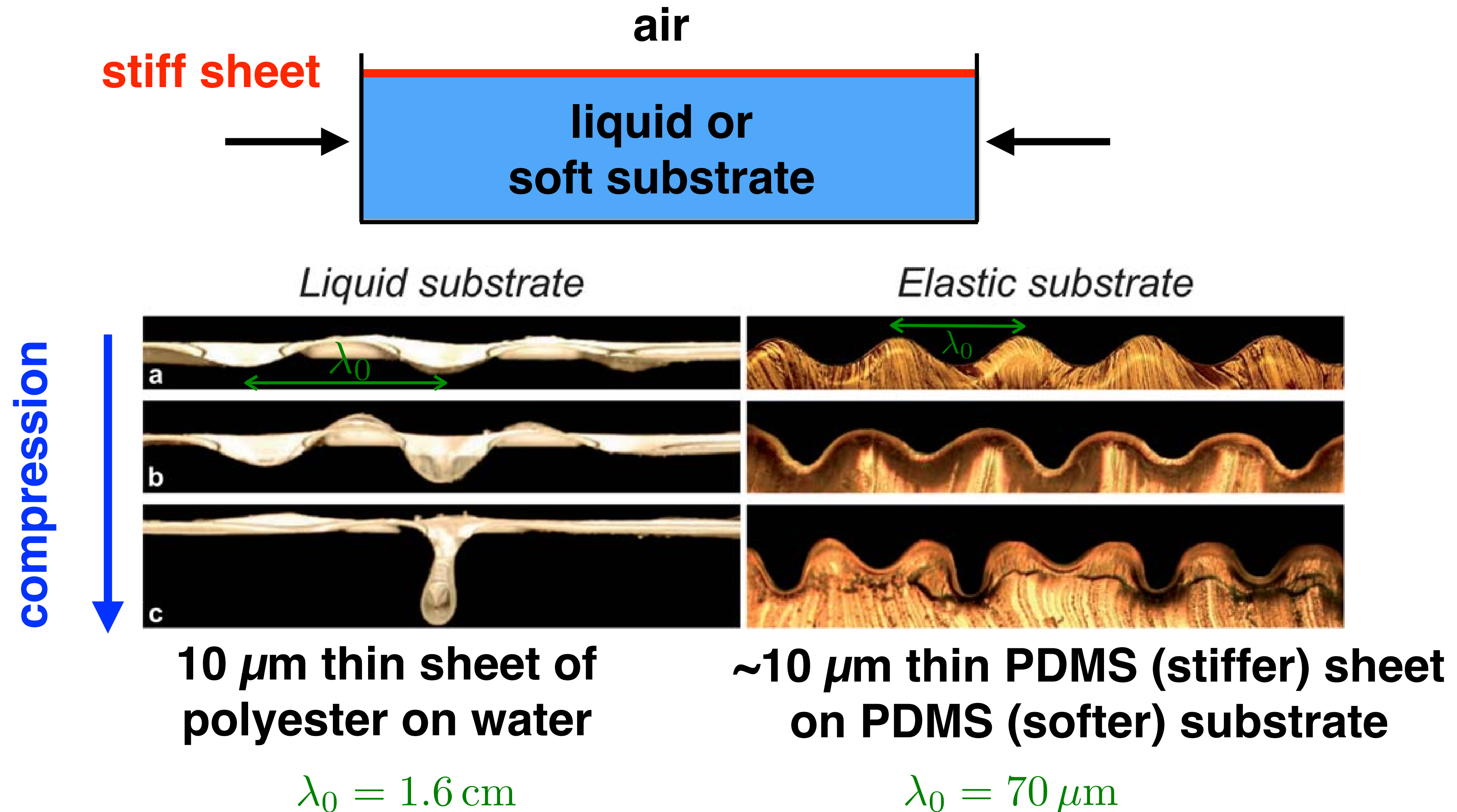
# Buckling vs wrinkling

**Compressed thin sheets buckle**
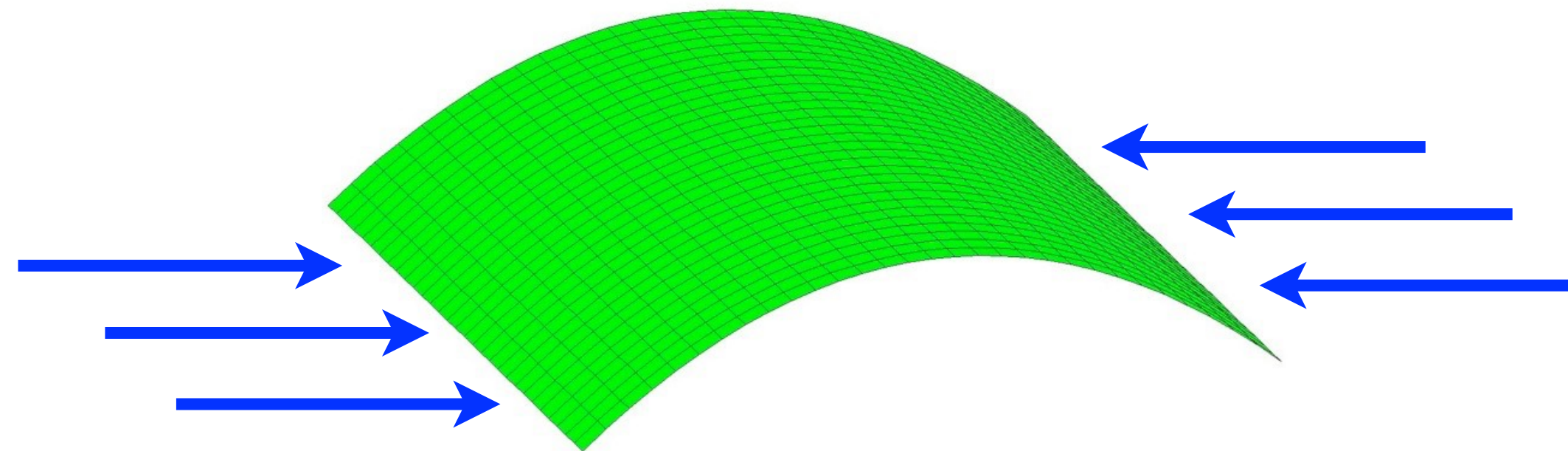


**Compressed thin sheets on liquid
and soft elastic substrates wrinkle**

*Liquid substrate*      *Elastic substrate*
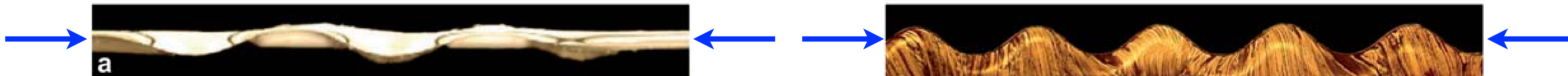


**In compressed thin sheets on liquid and soft elastic substrates global buckling is suppressed, because it would result in very large energy cost associated with deformation of the liquid or soft elastic substrate!**

# Wrinkling of growing thin films on soft substrates
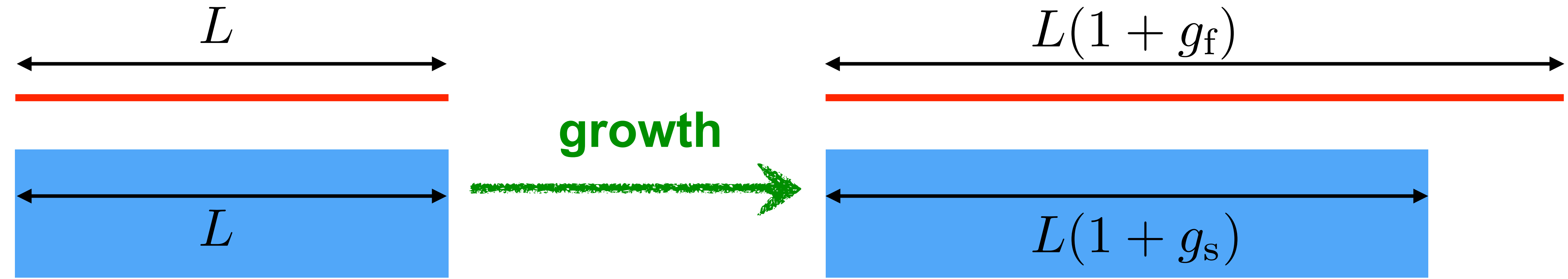
# Wrinkling of growing thin films on soft substrates
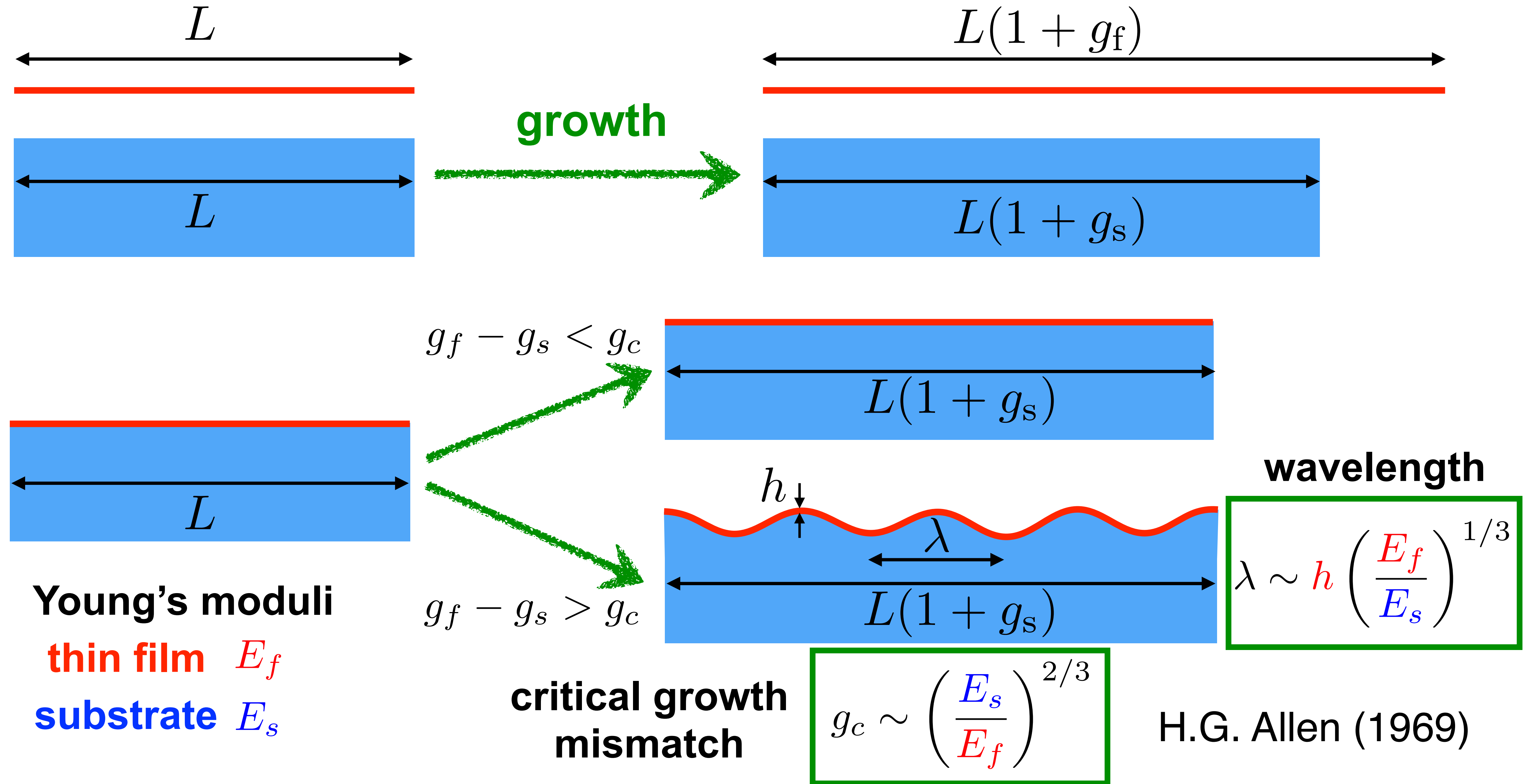
# Isotropic swelling of stiff thin sheets on soft elastic substrates

**critical swelling strain**

$$\epsilon_c \sim \left( \frac{E_s}{E_f} \right)^{2/3}$$
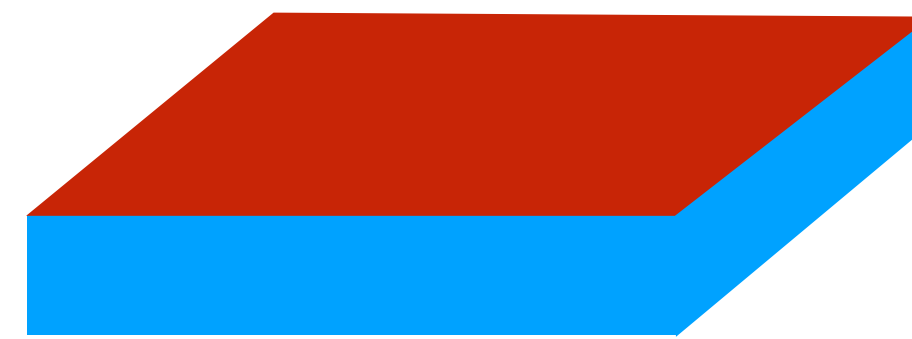


**hexagonal crystal of bumps**

| $\epsilon/\epsilon_c$ | $\lesssim 1$ | $\gtrsim 1$ | $1.3$ |
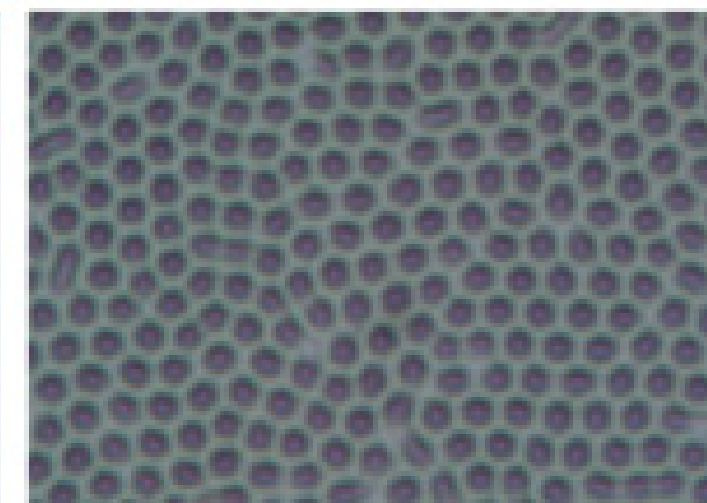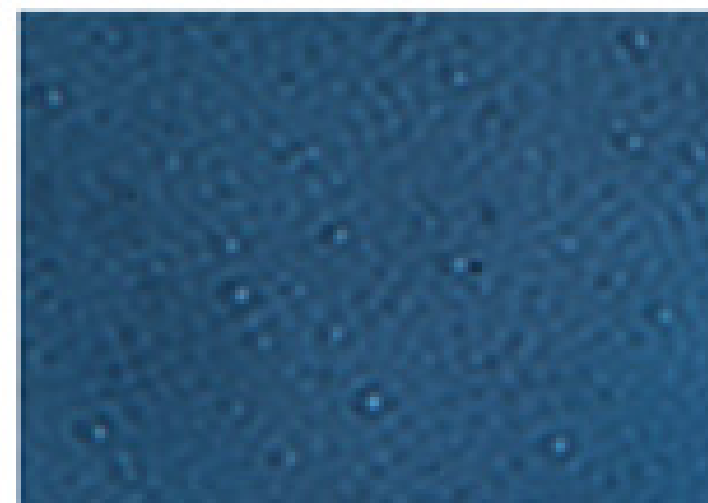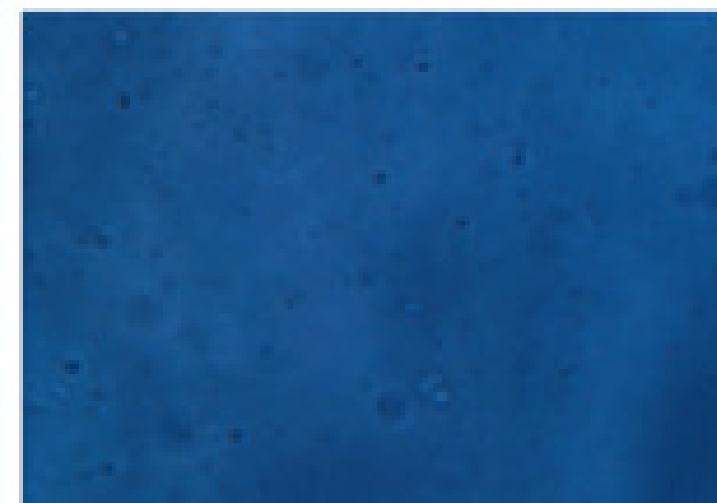
**characteristic wavelength**

$$\lambda \sim h \left( \frac{E_f}{E_s} \right)^{1/3}$$

| $\epsilon/\epsilon_c$ | $1.7$ | $3.0$ | $4.1$ |
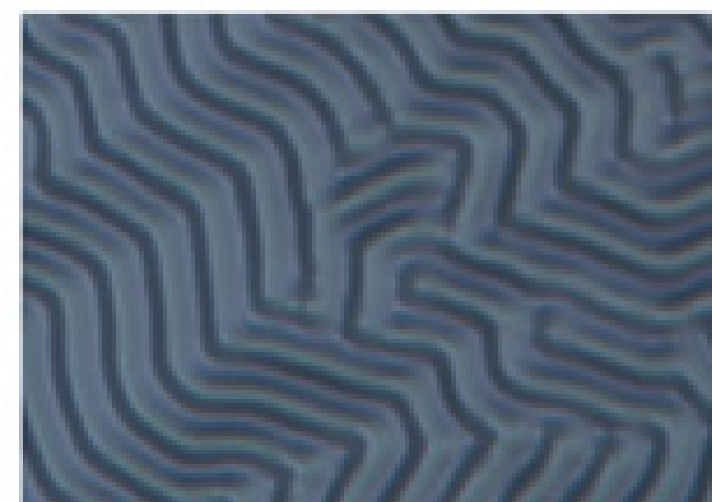
250 um

**herringbone pattern**

S. Cai et al., J. Mech. Phys. Solids **59**, 1094 (2011)

# Compression of stiff thin film on spherical soft substrates



$p_e$

$p_i$

$R$

$h$

**Spherical shells are compressed by reducing internal pressure**

$R = 20\text{mm}$

**hexagonal phase**

**bistable phase**

**wrinkled phase**

$R/h$

**characteristic wavelength is almost independent of radius R**

$$\lambda \sim h \left( \frac{E_f}{E_s} \right)^{1/3}$$

...o et al ...terials **14**, 337 (2015)

# Compression of stiff thin film on spherical soft substrates



Film

Substrate

$h$

$p_i$

$R$

$p_i$

$\sigma$

$\lambda$

$u$

$R$

$\sigma$

$p_e$

**hexagonal phase**

**bistable phase**

**wrinkled phase**

$R/h$

**Spherical shells are compressed by reducing internal pressure**

**characteristic wavelength is almost independent of radius R**

$$\lambda \sim h \left( \frac{E_f}{E_s} \right)^{1/3}$$

$R = 20\mathrm{mm}$

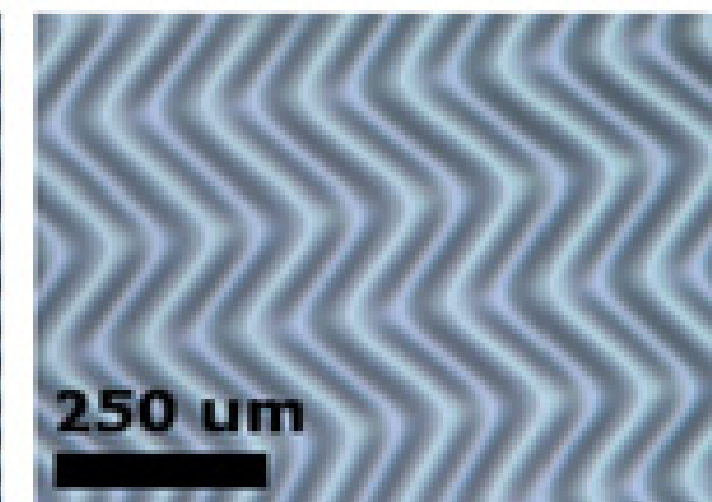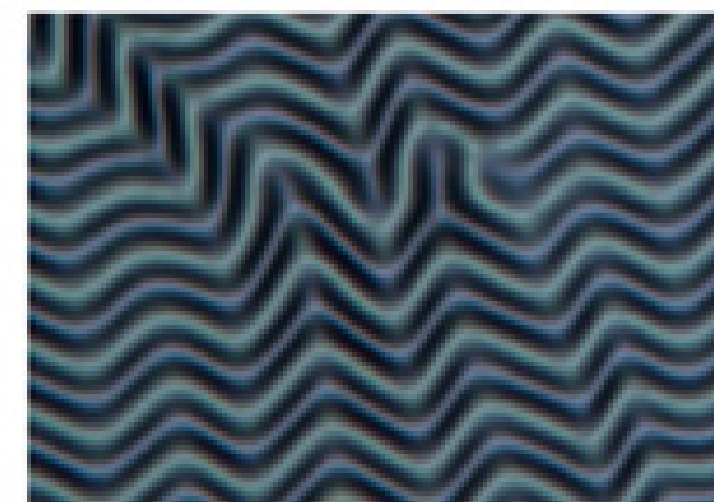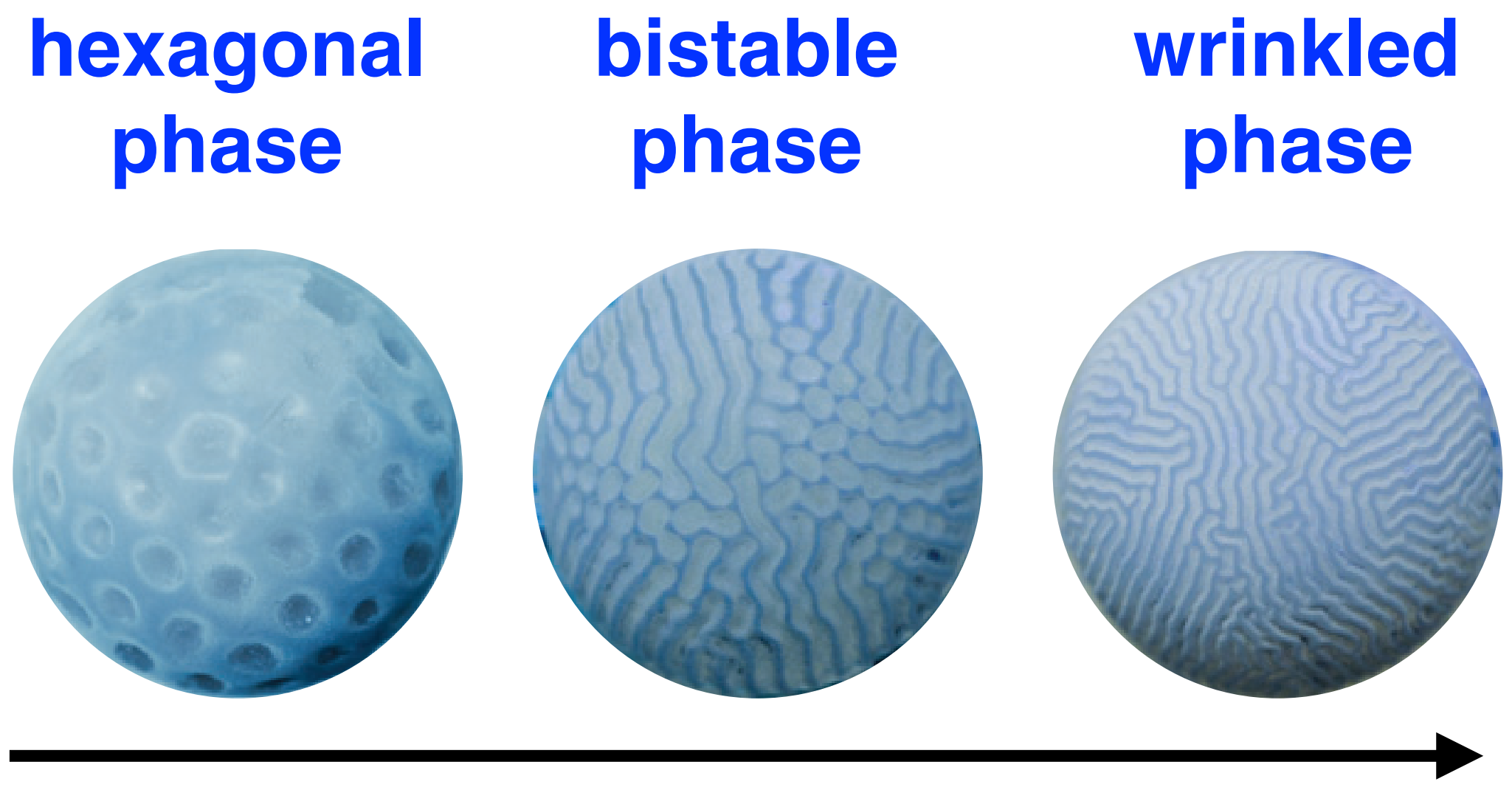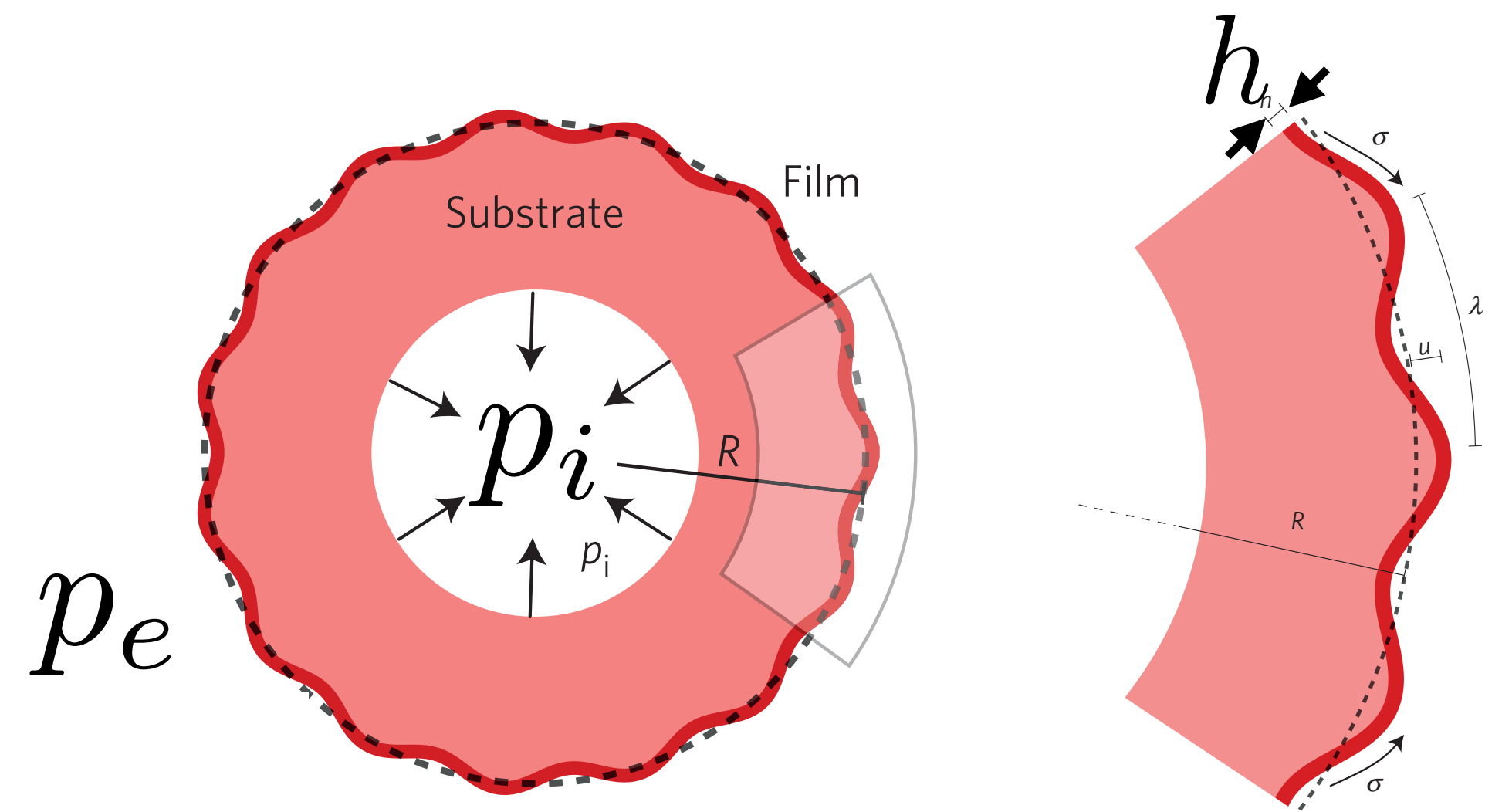Substrate

Film

$R$

a

b

$h$

$\sigma$

...o et al ...terials **14**, 337 (2015)
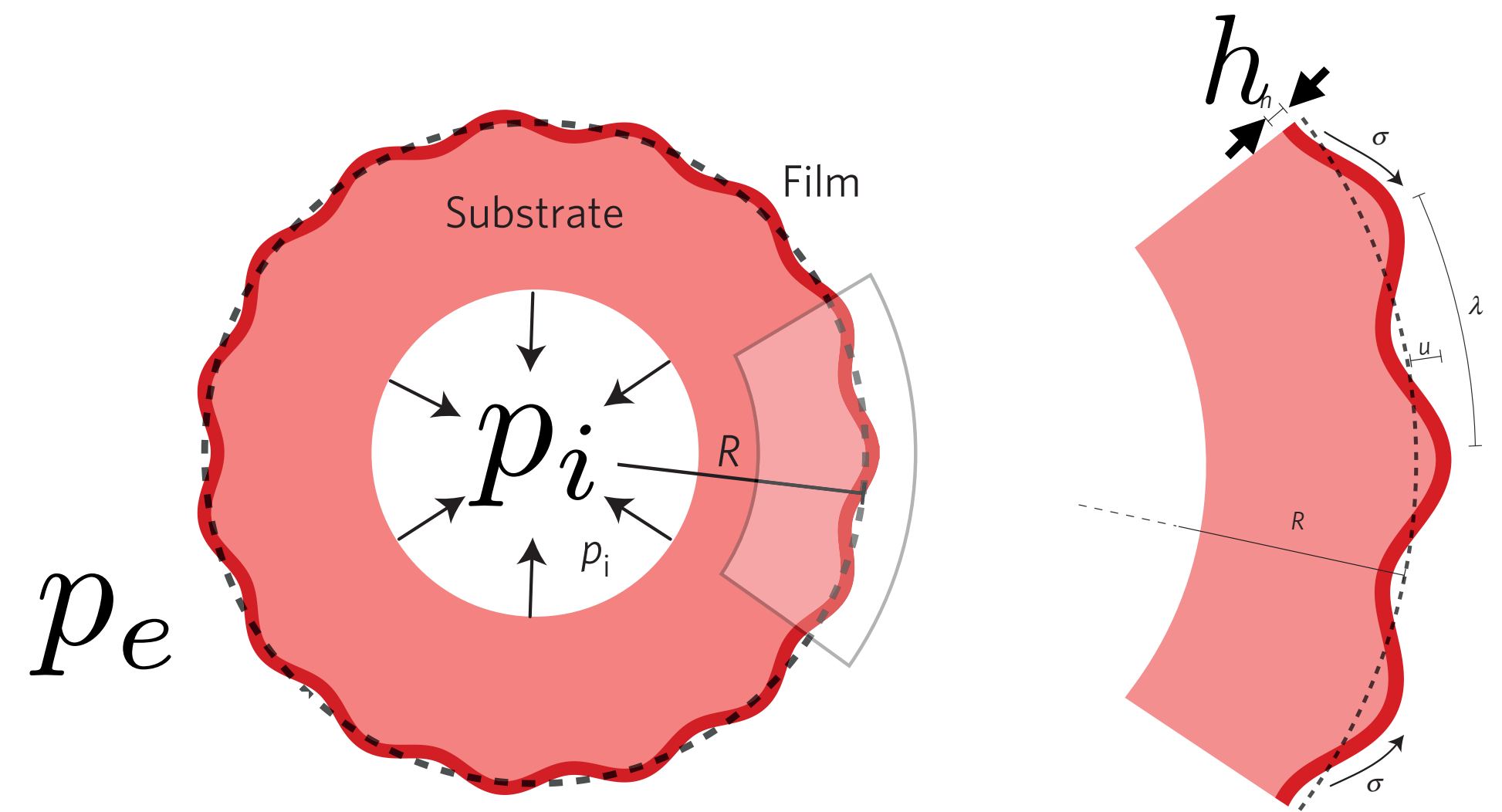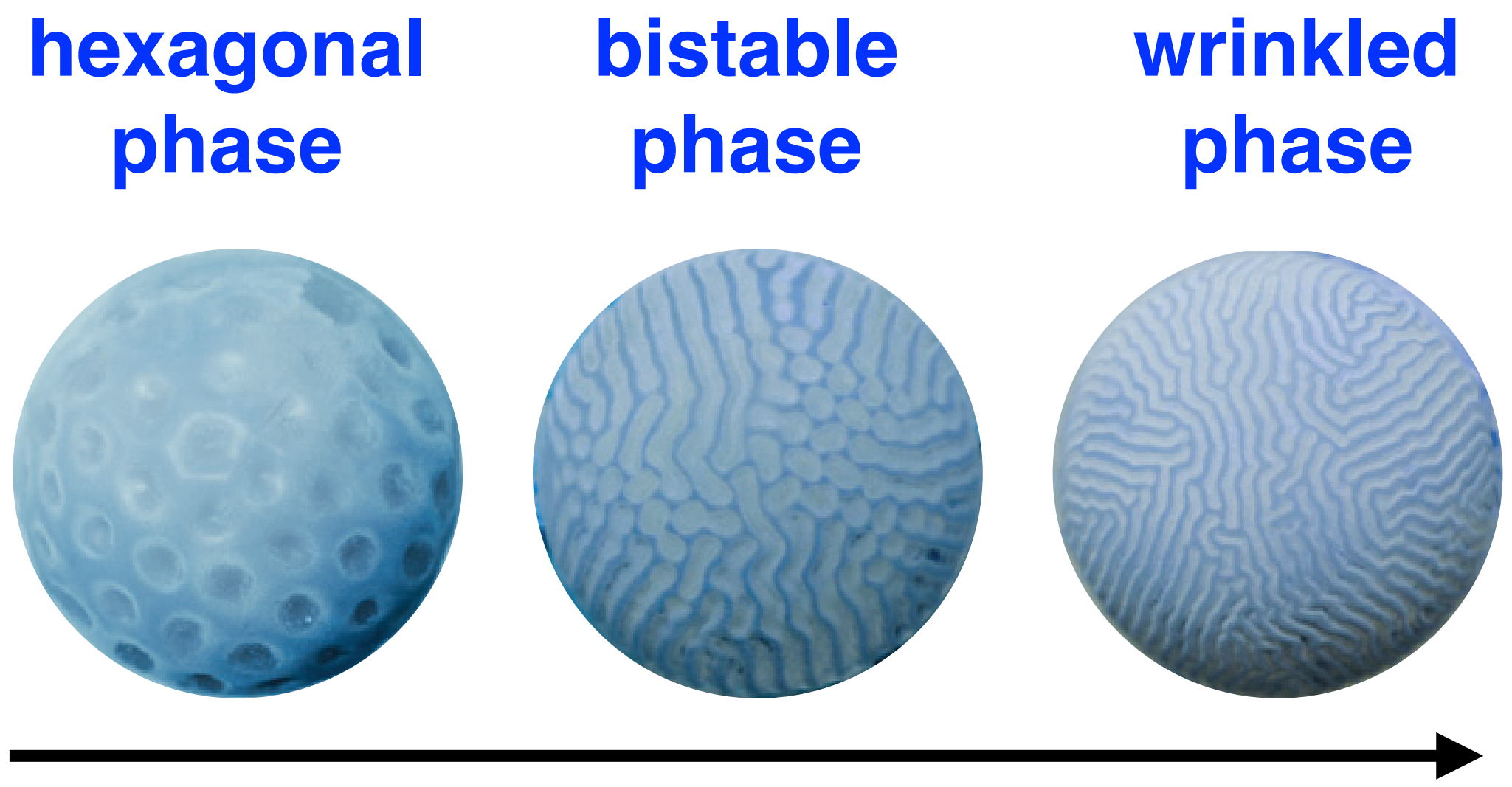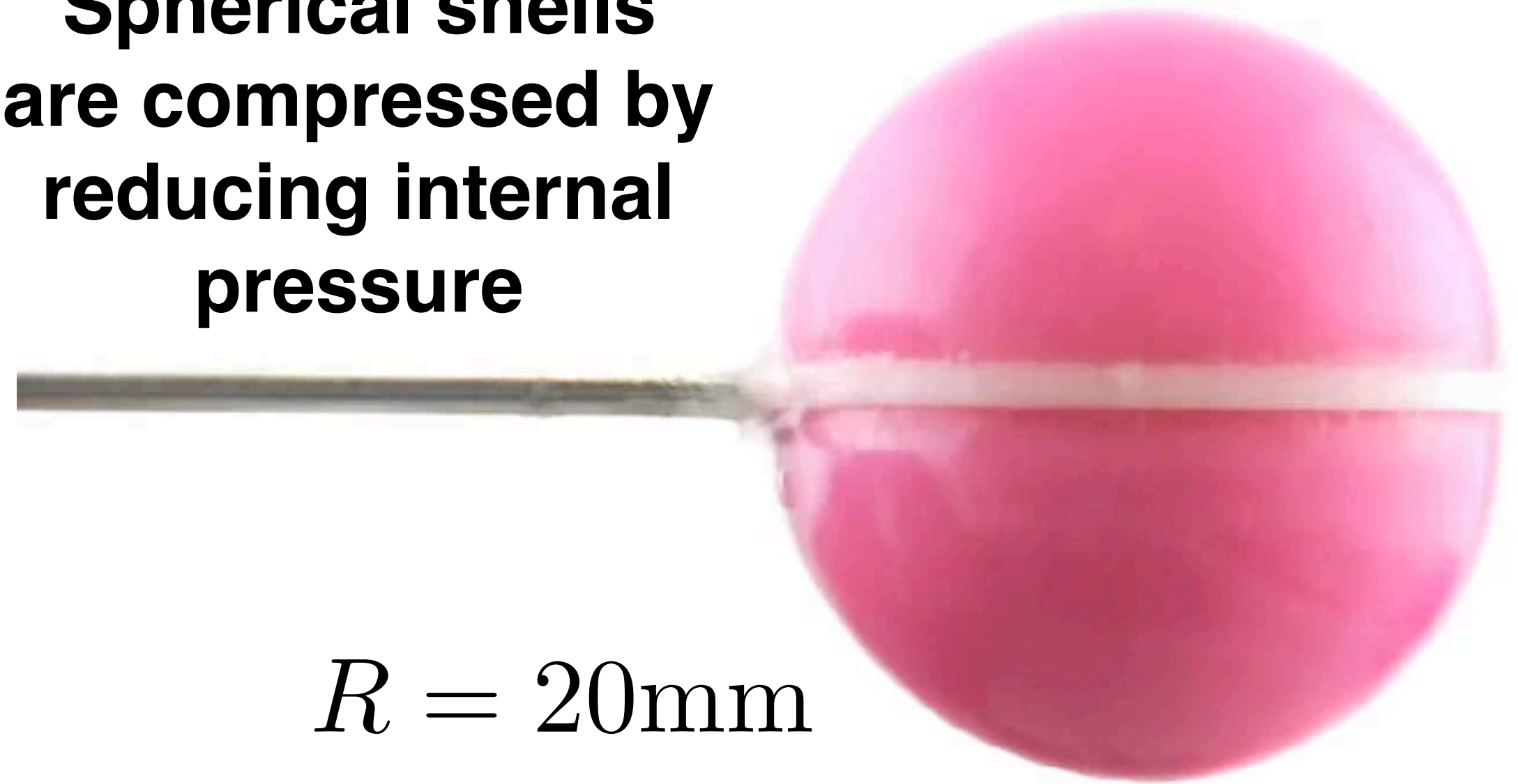
# Outline

**wrinkling instability of growing films**
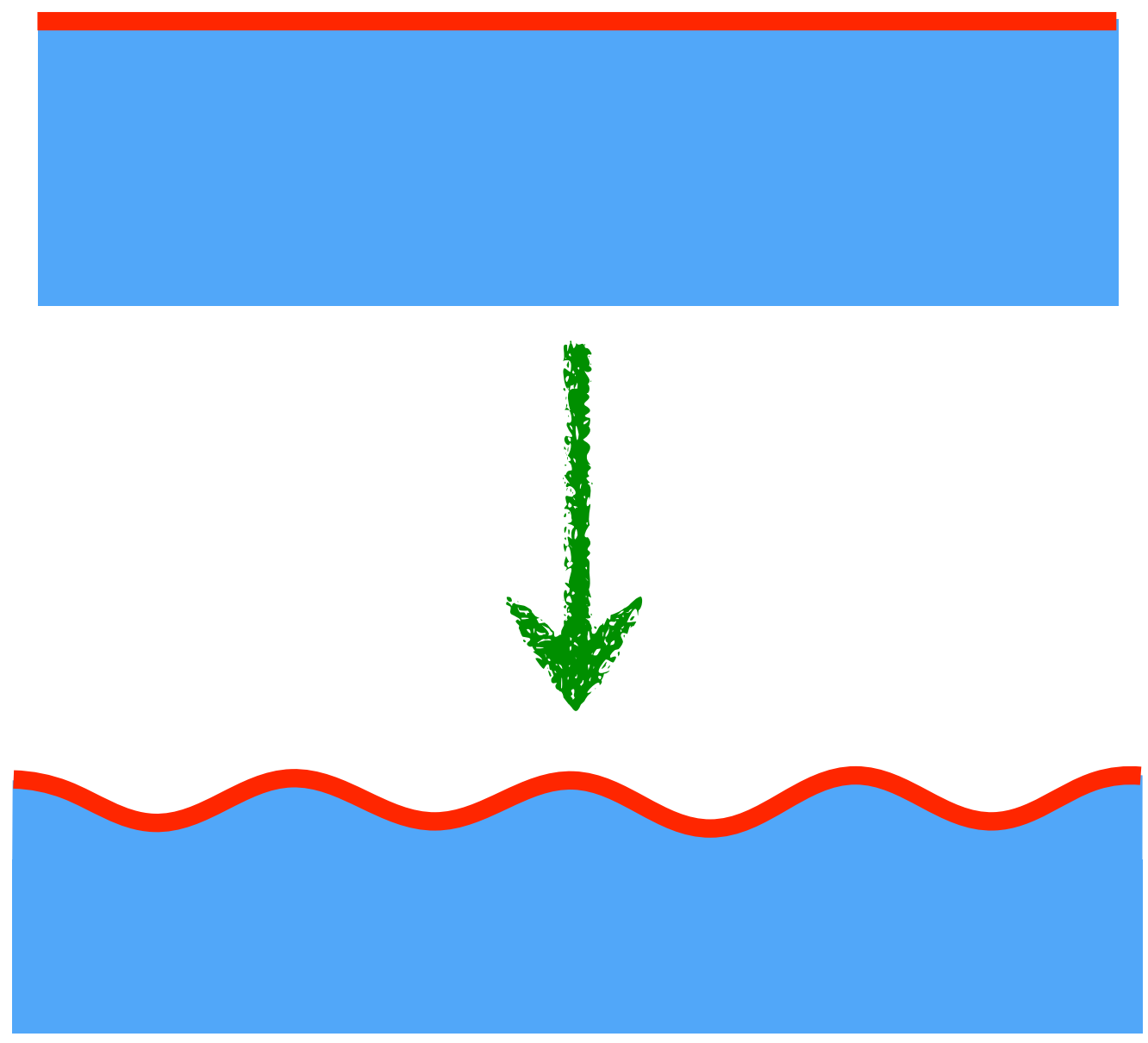
**wrinkling of bacterial biofilm**
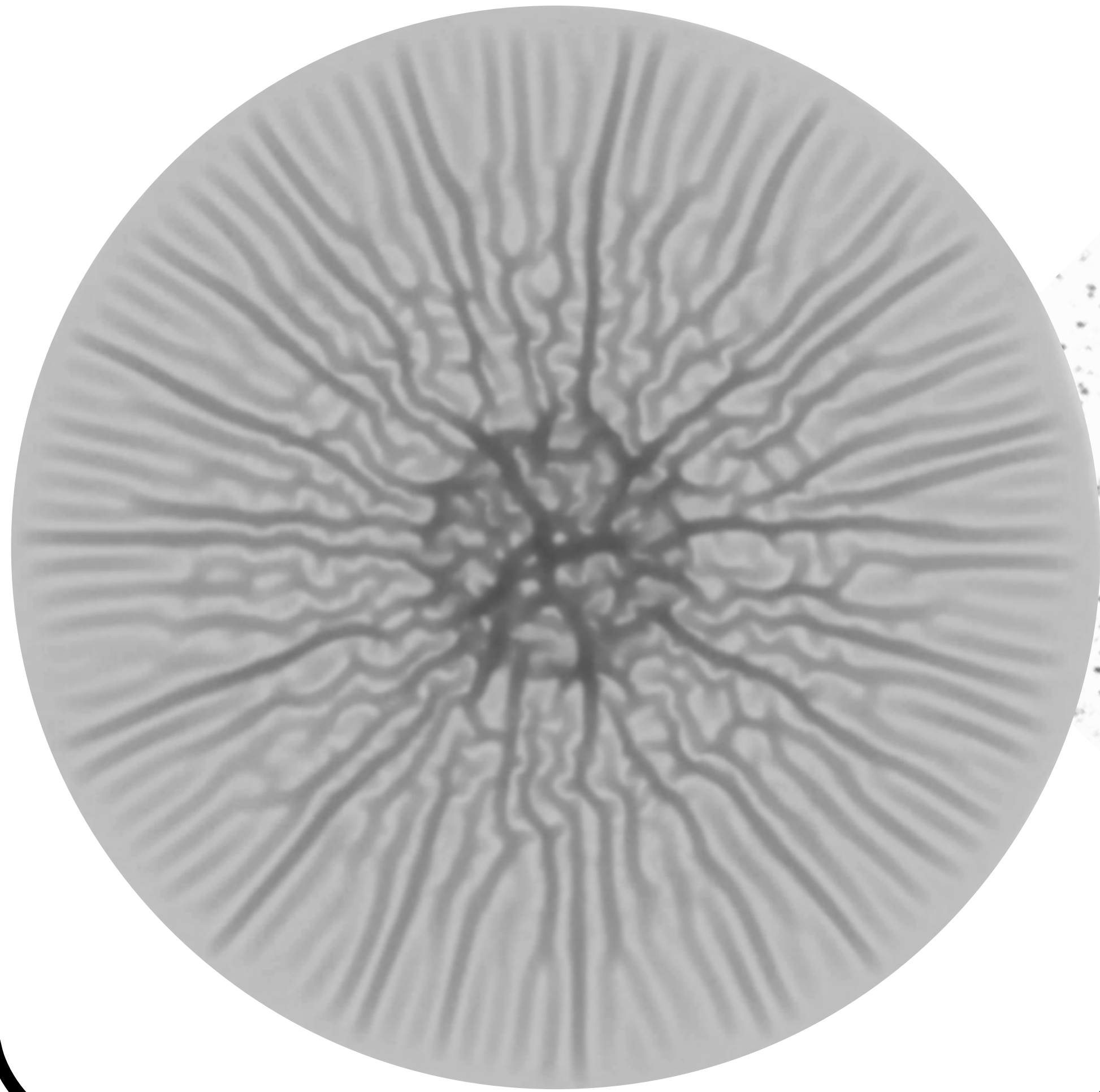
**branching of developing lungs**

# Formation of *Vibrio cholerae* biofilm on agar substrate
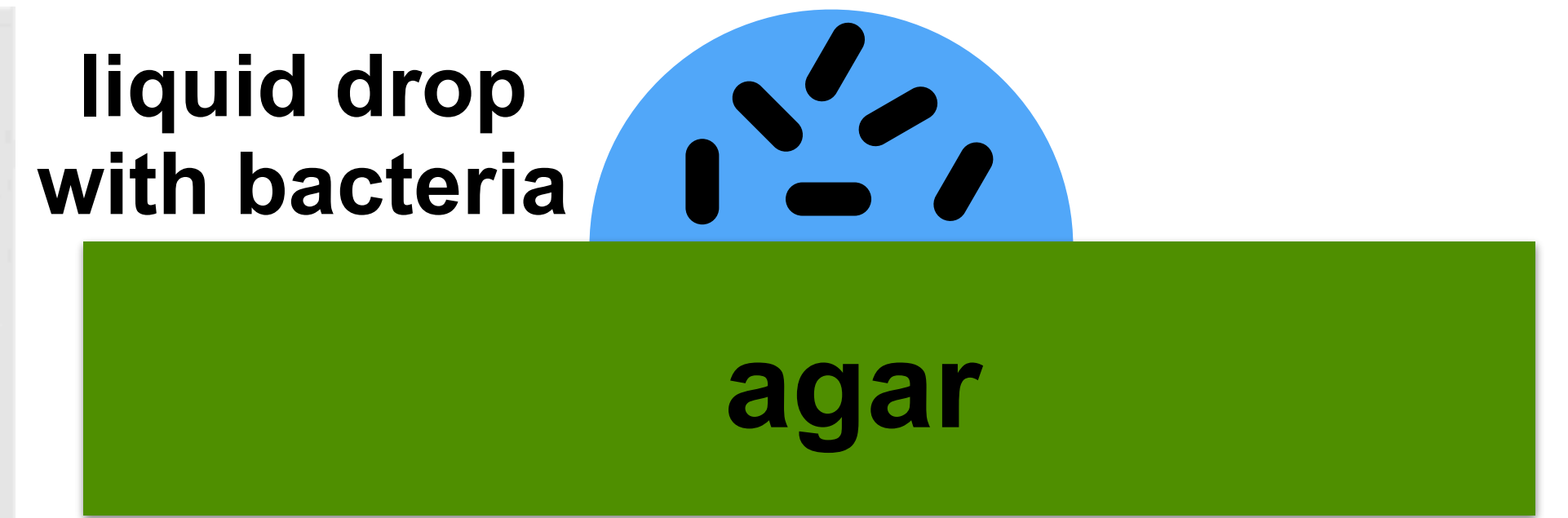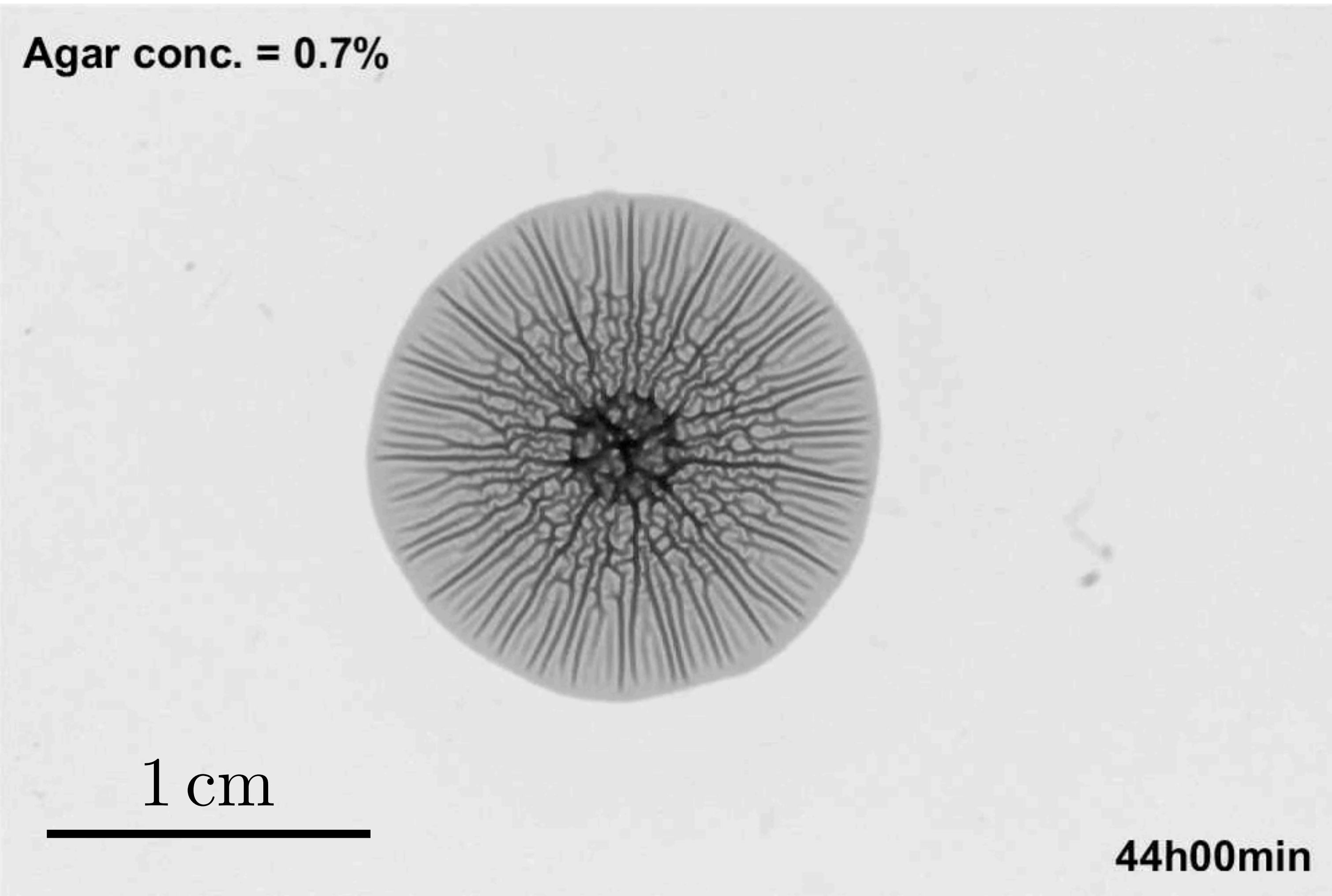
Agar conc. = 0.7%

1 cm

44h00min

liquid drop with bacteria

agar

**biofilm thickness ~** $100\,\mu\mathrm{m}$

J. Yan et al., eLife **8**, e43920 (2018)

# Formation of *Vibrio cholerae* biofilm on agar substrate

Agar conc. = 0.7%

liquid drop
with bacteria

agar

1 cm

44h00min

**biofilm thickness ~** $100\,\mu\mathrm{m}$

J. Yan et al., eLife **8**, e43920 (2018)

# Formation of *Vibrio cholerae* biofilm on agar substrate

Agar conc. = 0.7%

1 cm

44h00min

**biofilm thickness ~** $100\,\mu\mathrm{m}$

J. Yan et al., <u>eLife</u> **8**, e43920 (2018)

**liquid drop with bacteria**

**agar**

**Agar = 0.6%, time=48h**

5 mm

**height profile**

0    100    200    300 $\mu\mathrm{m}$

# Formation of *Vibrio cholerae* biofilm on agar substrate



2 mm

bacteria

extracellular
matrix
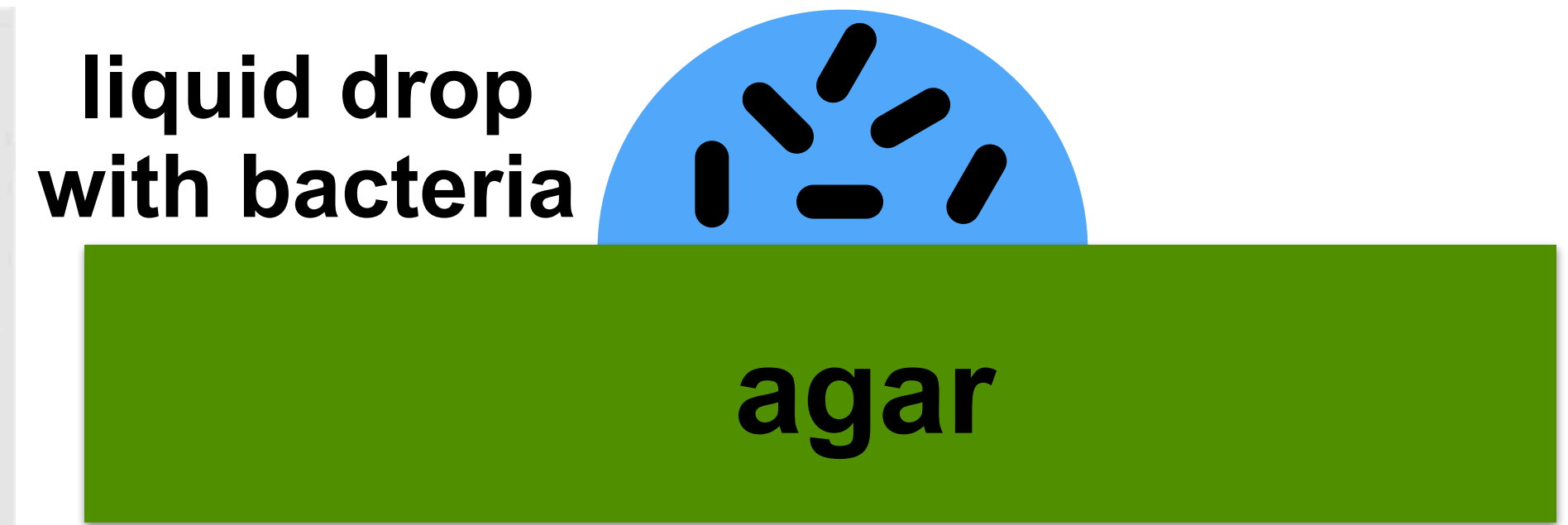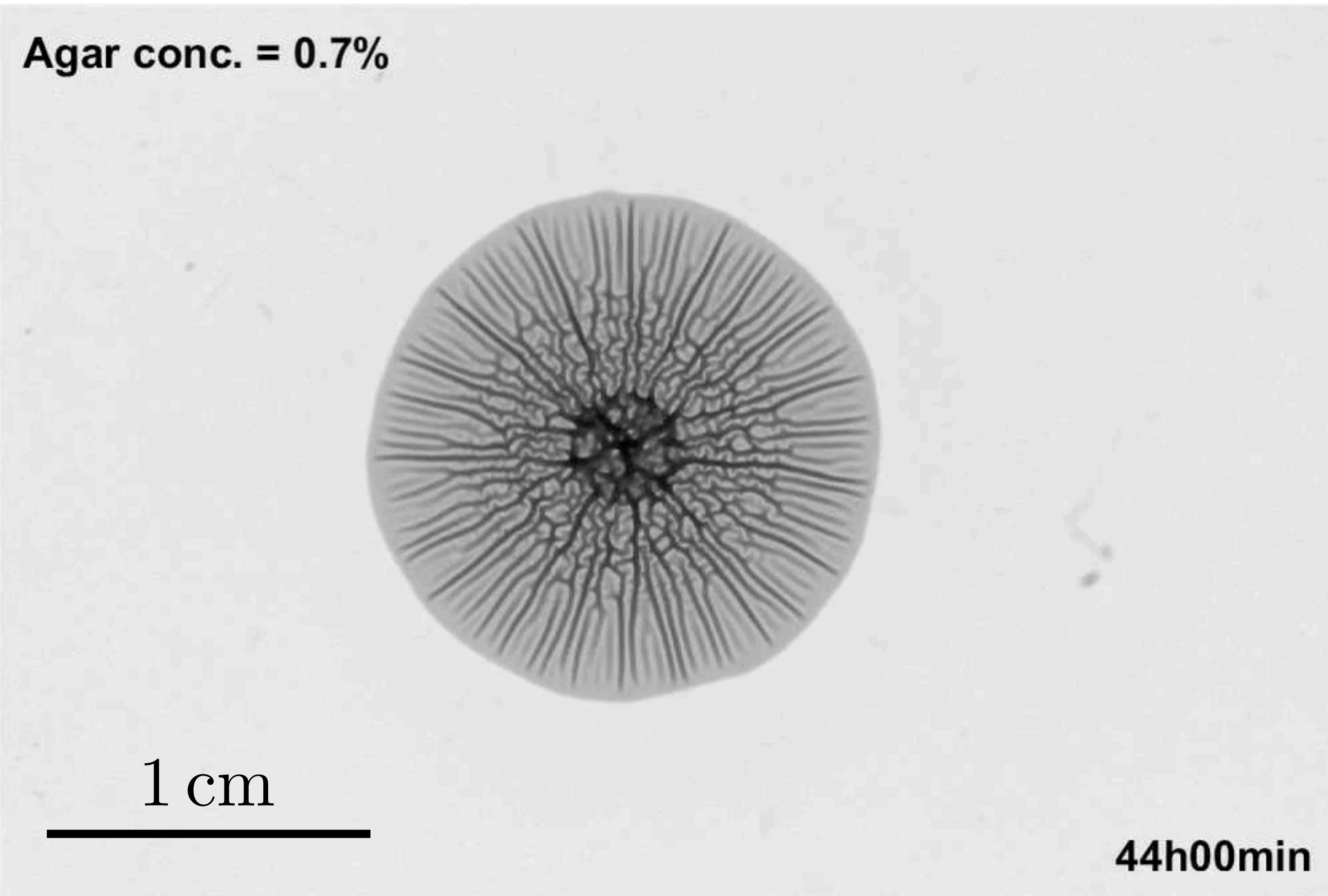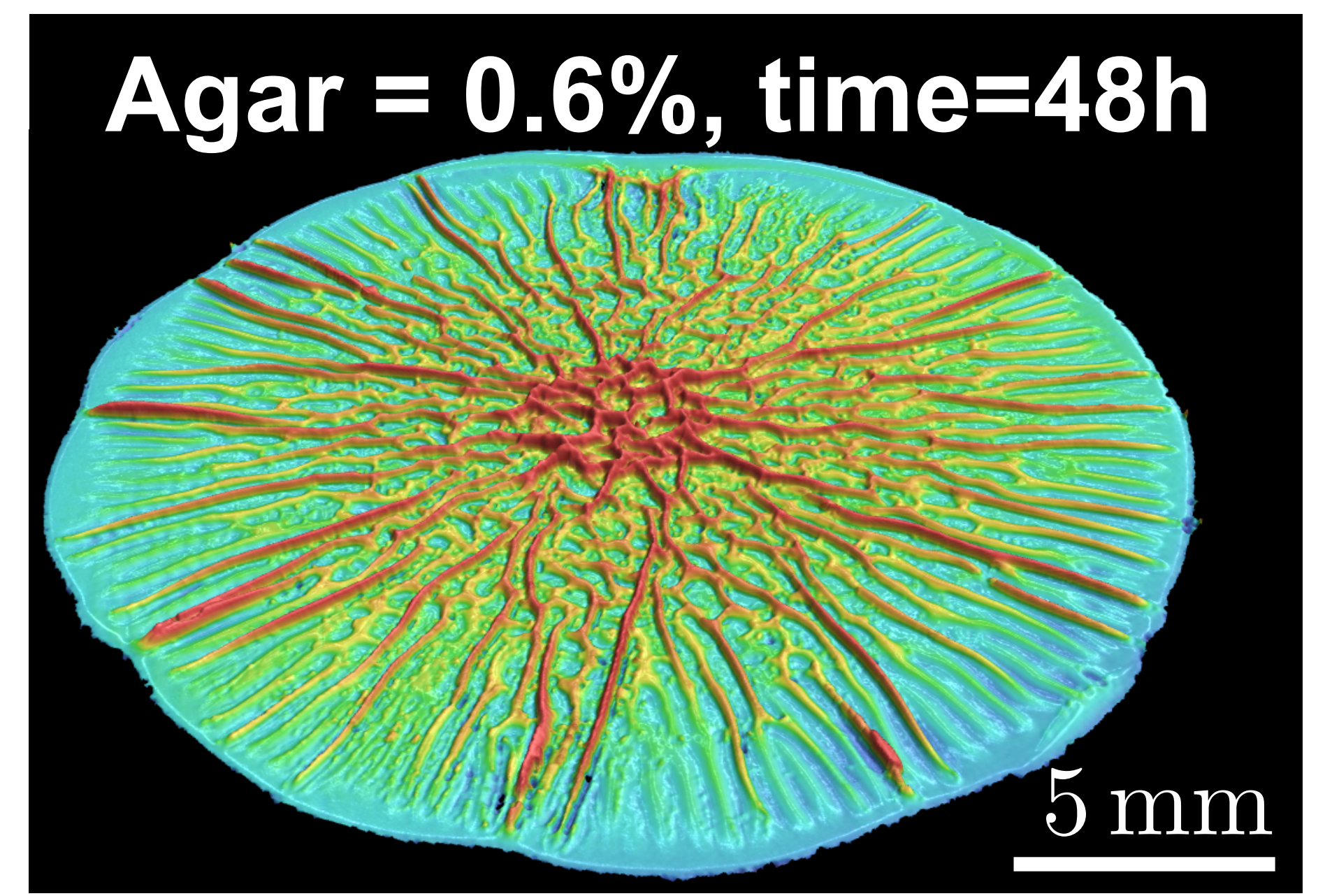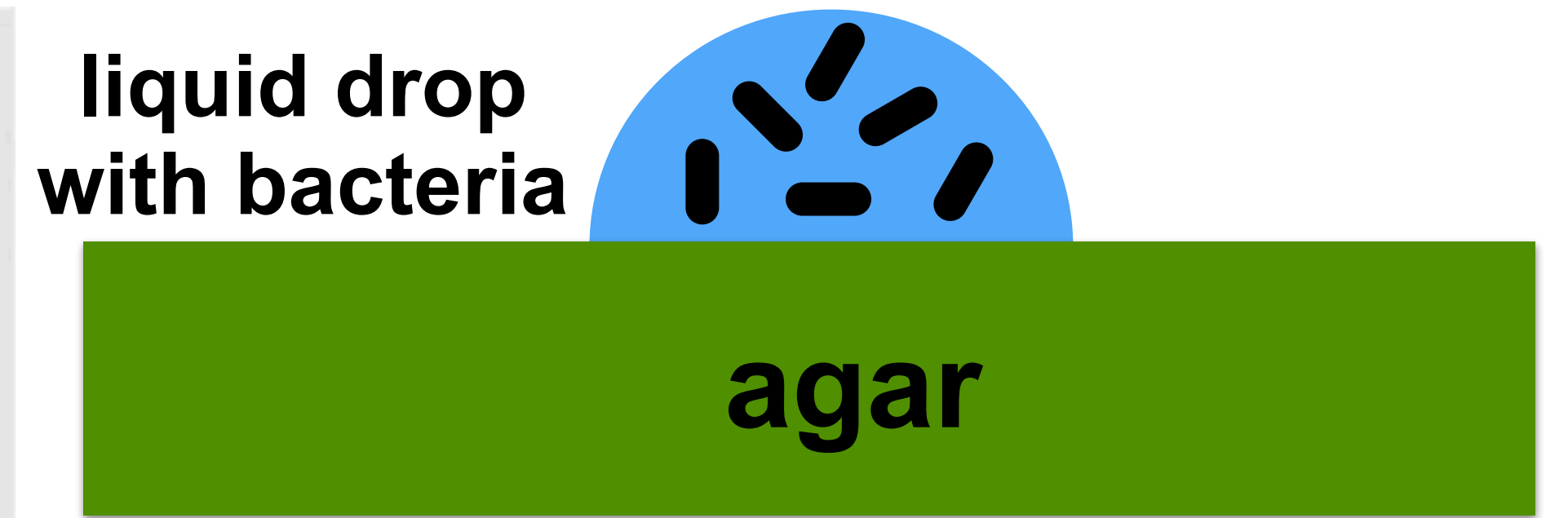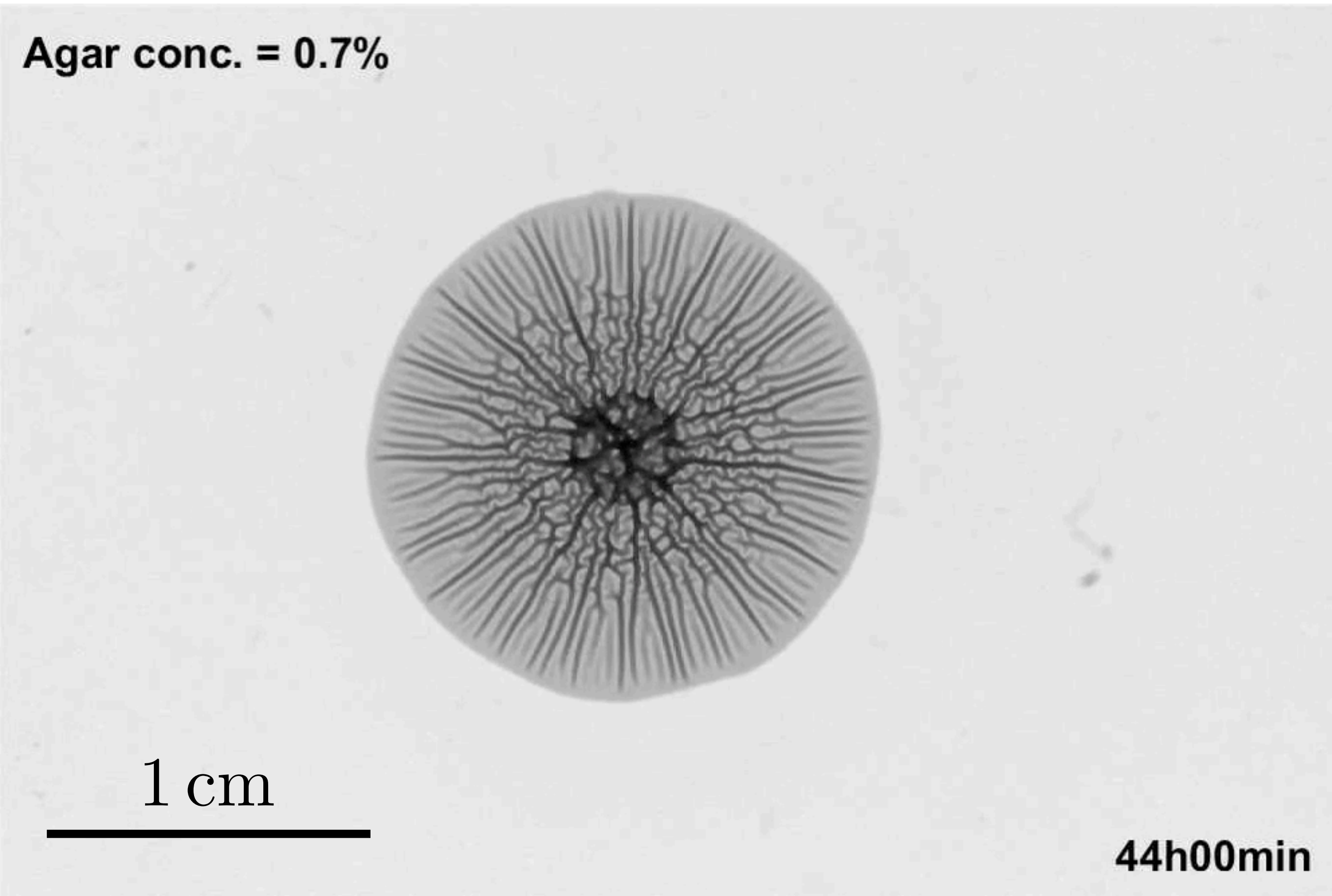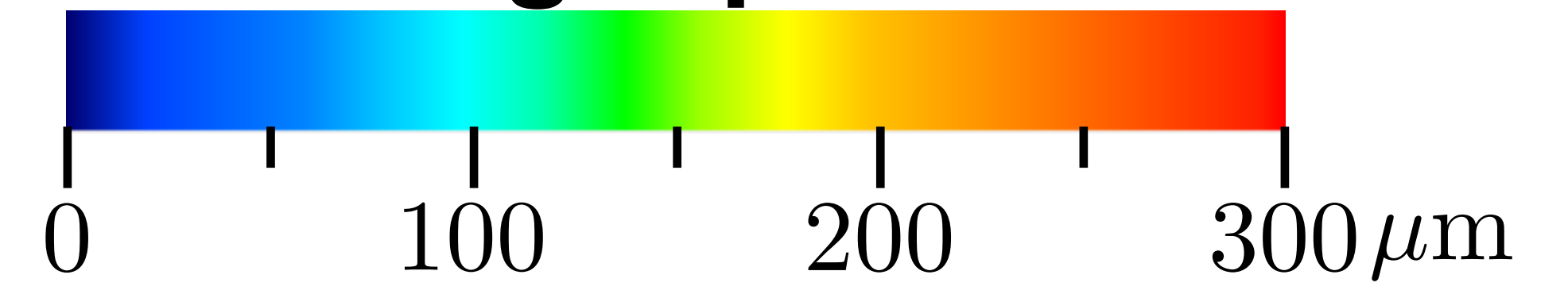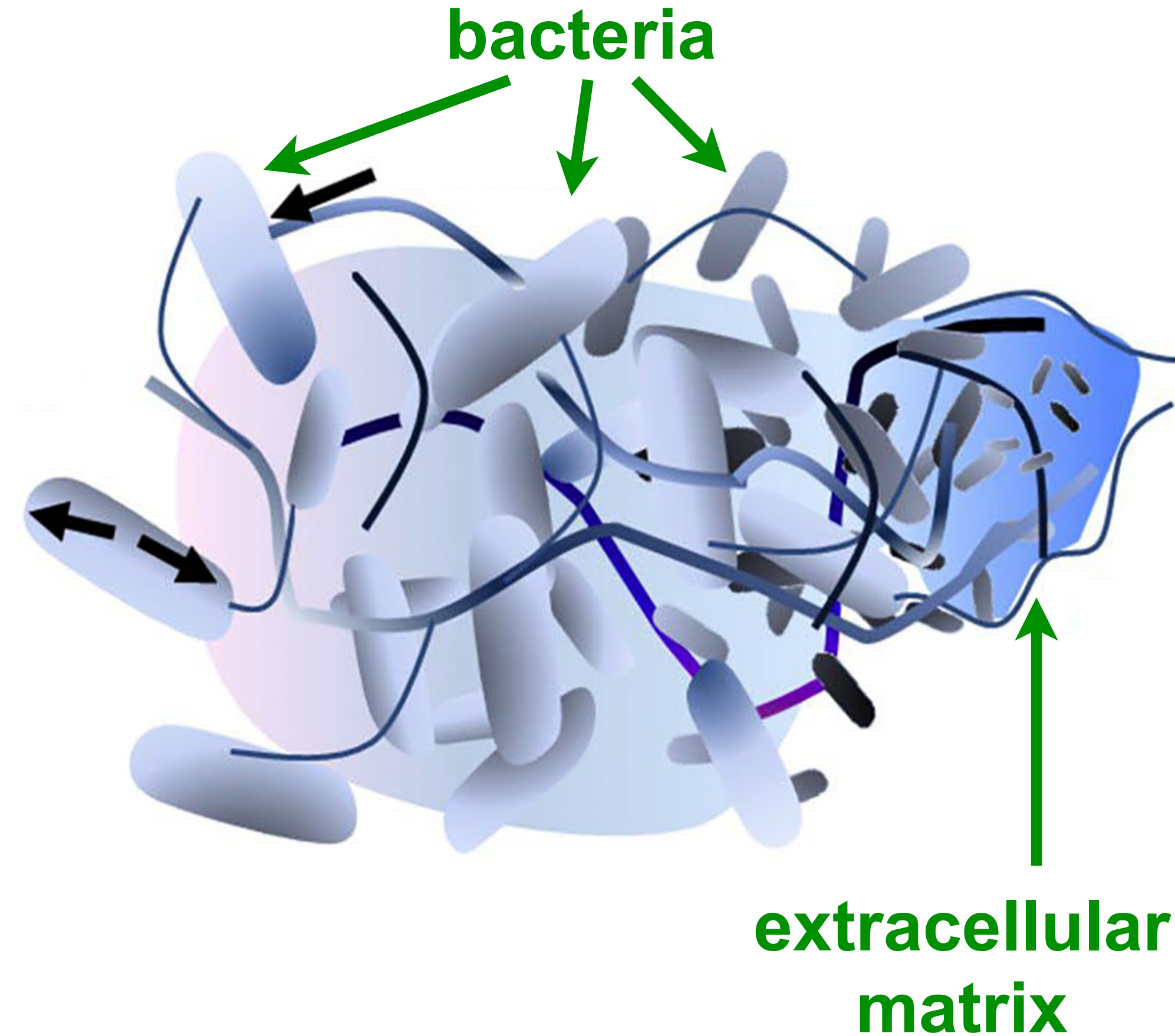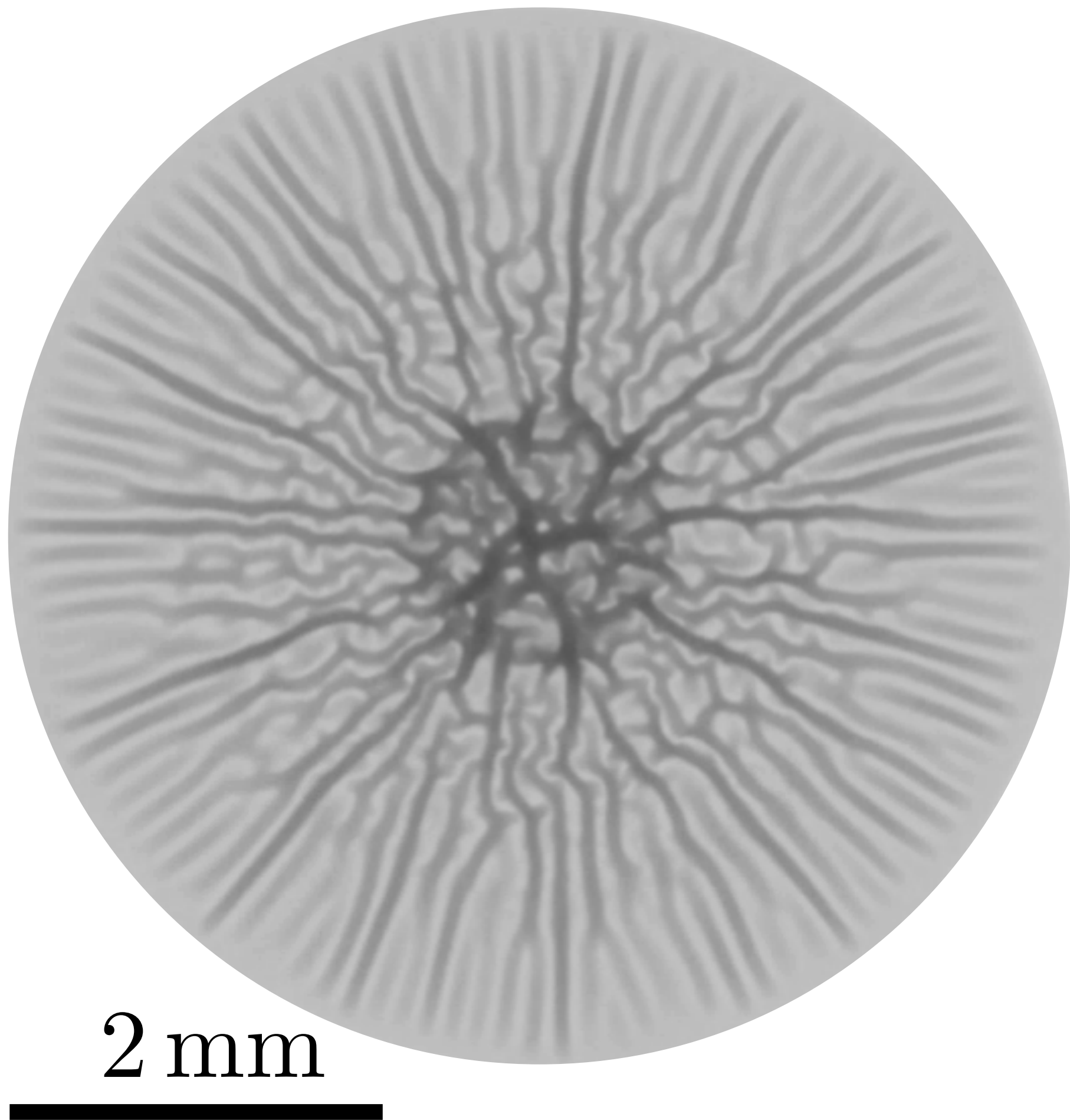
C. Even *et al.*, Adv. Colloid Interface Sci **247**, 573 (2017)

**Are wrinkles the result of mechanical instabilities or are they produced by some active biochemical processes?**

# Wavelength is constant throughout the biofilm

**agar conc. = 0.7%**
**time = 37.5 h**

**wavelength**
$\lambda = 380 \pm 20 \, \mu\text{m}$
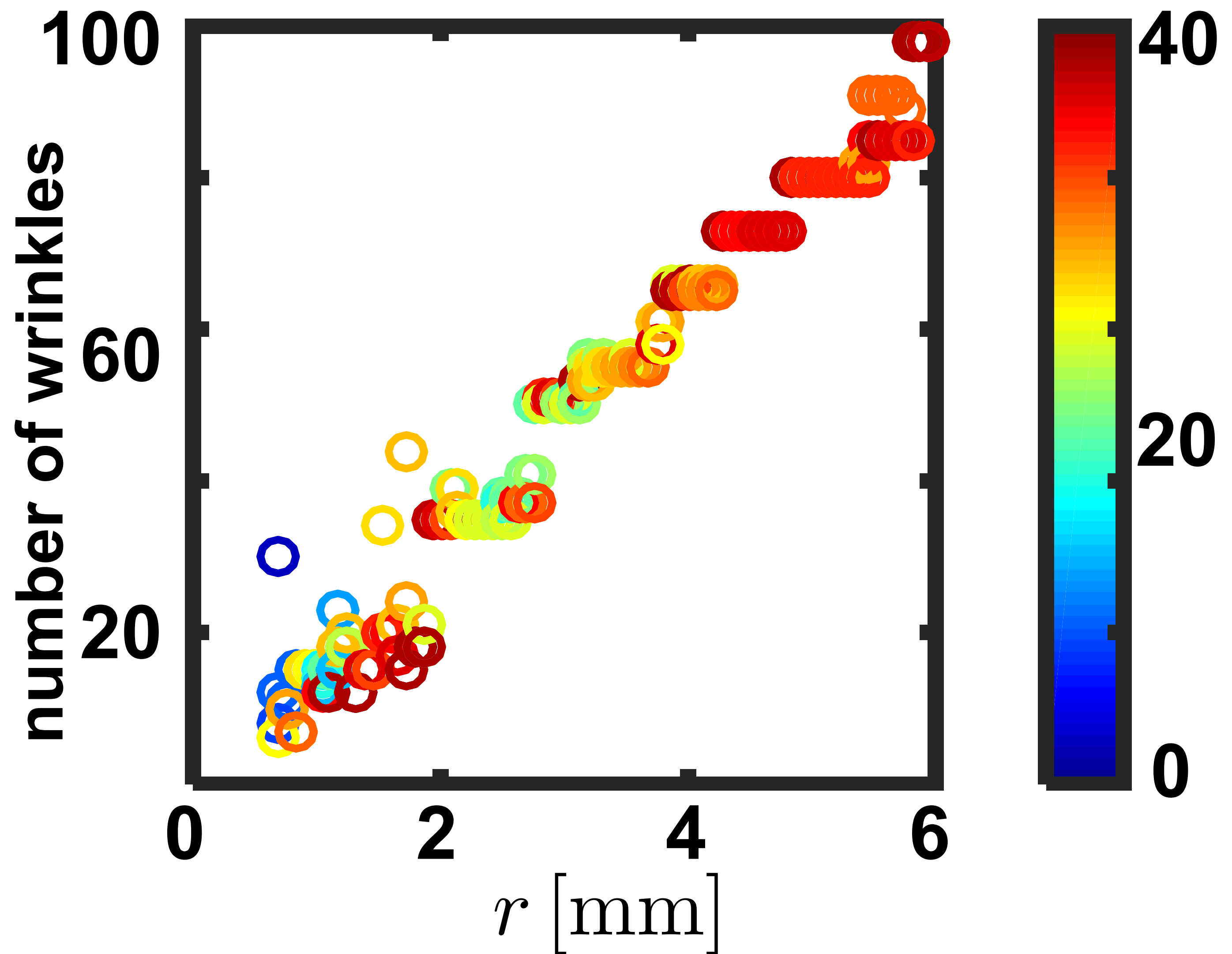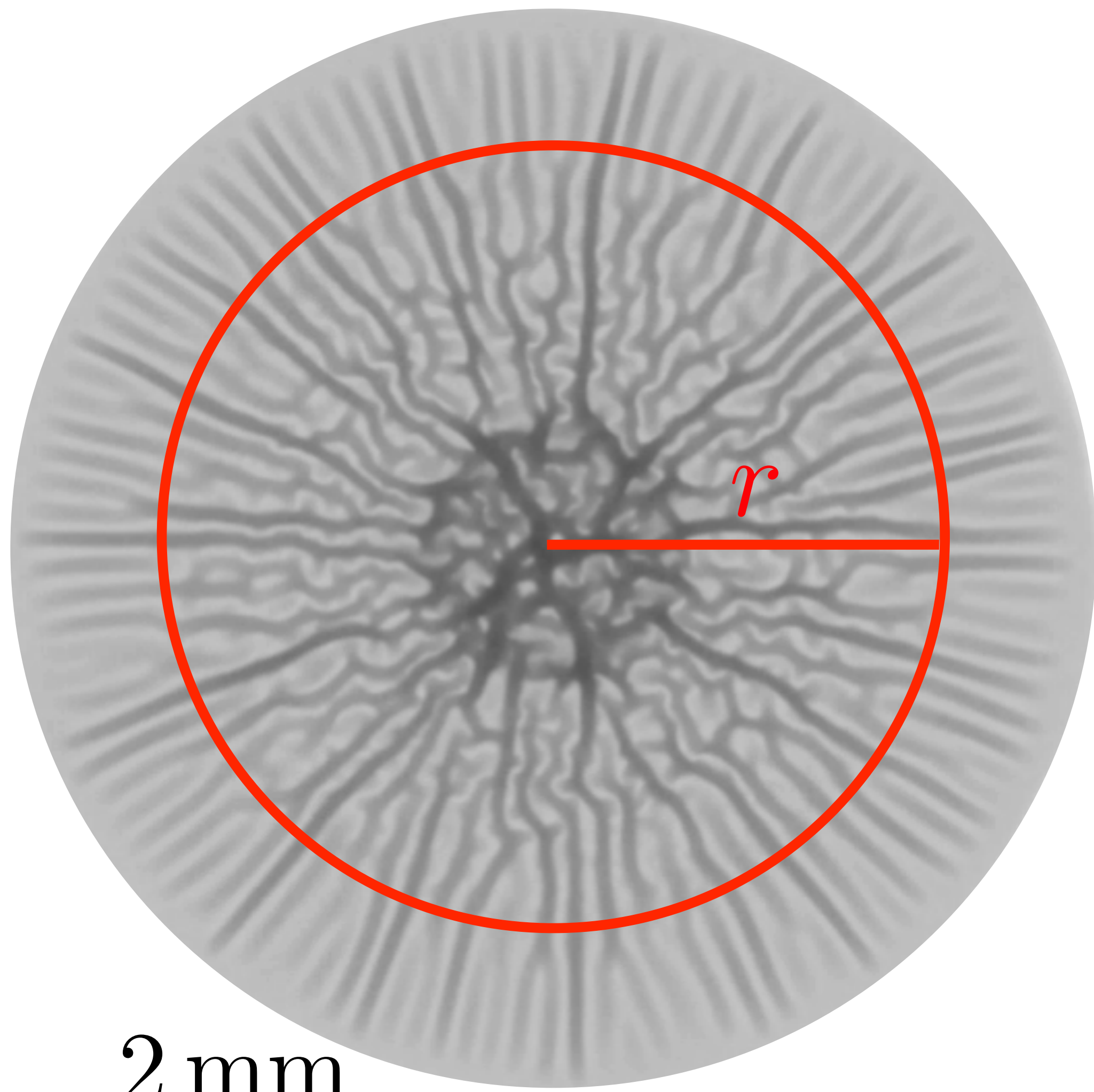


$2 \, \text{mm}$

# Wavelength is independent of biofilm geometry

**formation of straight biofilm (razor blade)**



Agar conc. = 0.5%

1 cm

27h15min

NS

$\lambda \, [\mu m]$

0    200    400

# Wavelength is independent of biofilm geometry

**formation of straight biofilm (razor blade)**



Agar conc. = 0.5%

1 cm

27h15min

NS

$\lambda$ [$\mu$m]

0     200     400
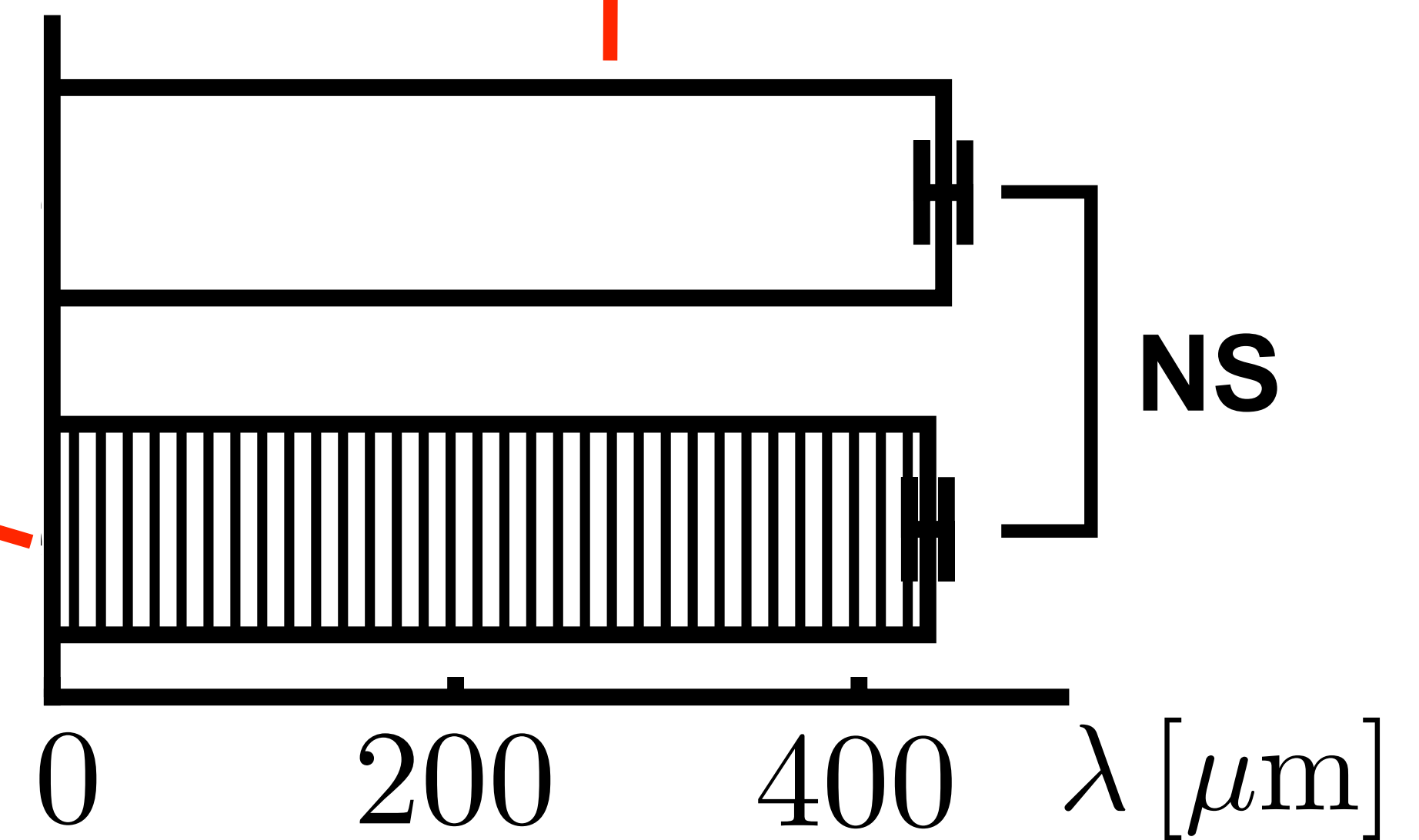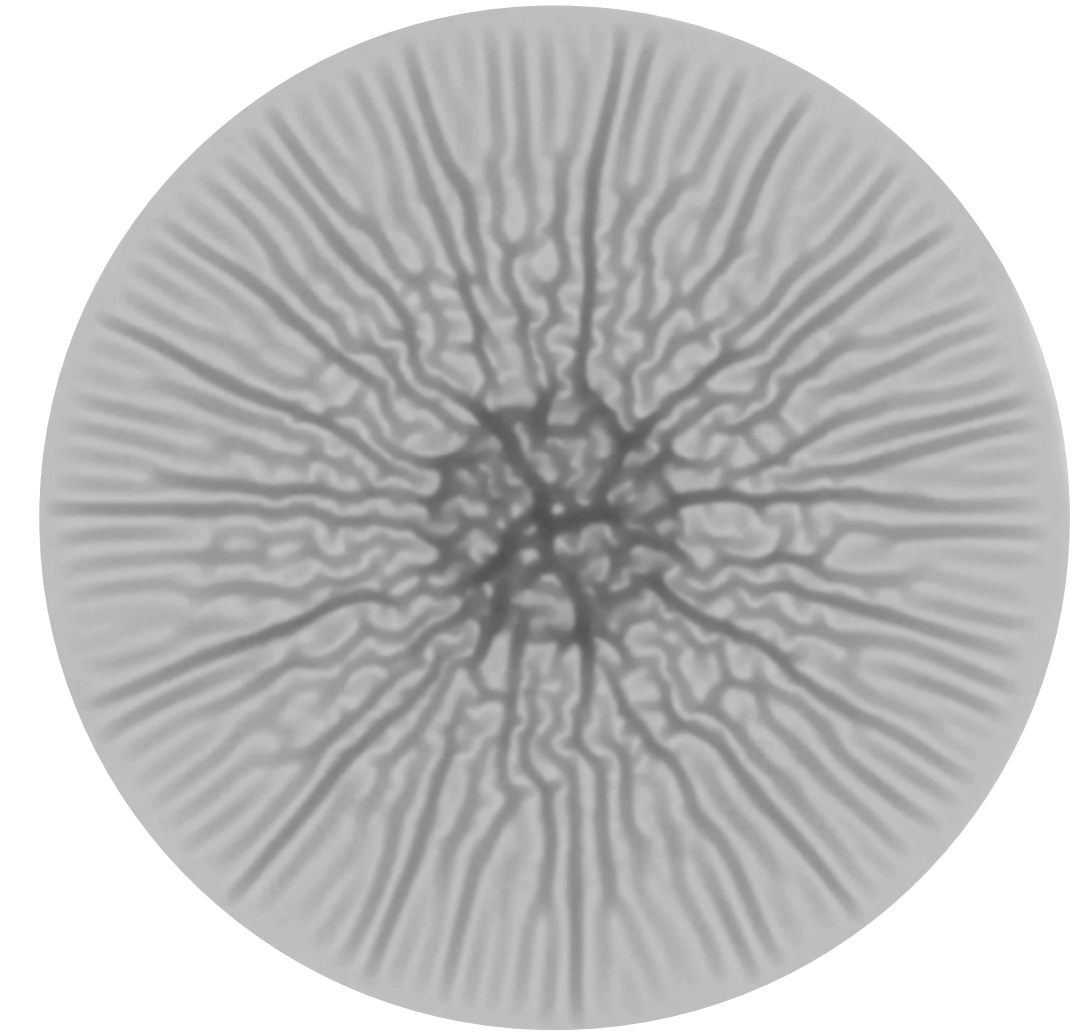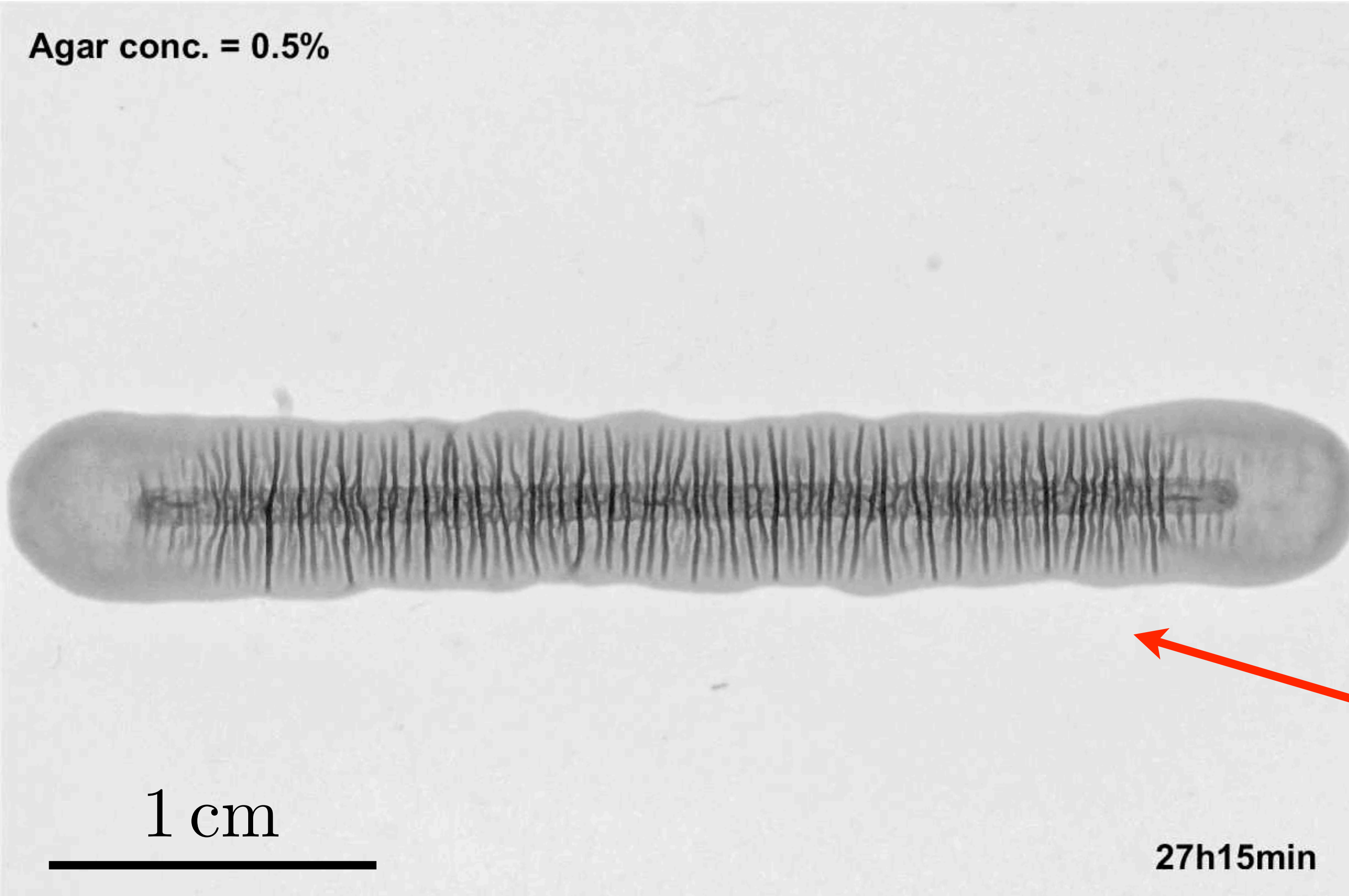
# Measured wavelengths are consistent with wrinkling instabilities of thin films

**agar 0.4%**
$G_{\text{agar}} = 0.14 \pm 0.02\,\text{kPa}$

**agar 0.6%**
$G_{\text{agar}} = 0.51 \pm 0.08\,\text{kPa}$

**agar 0.8%**
$G_{\text{agar}} = 1.9 \pm 0.2\,\text{kPa}$

**agar 1.0%**
$G_{\text{agar}} = 3.8 \pm 1.1\,\text{kPa}$
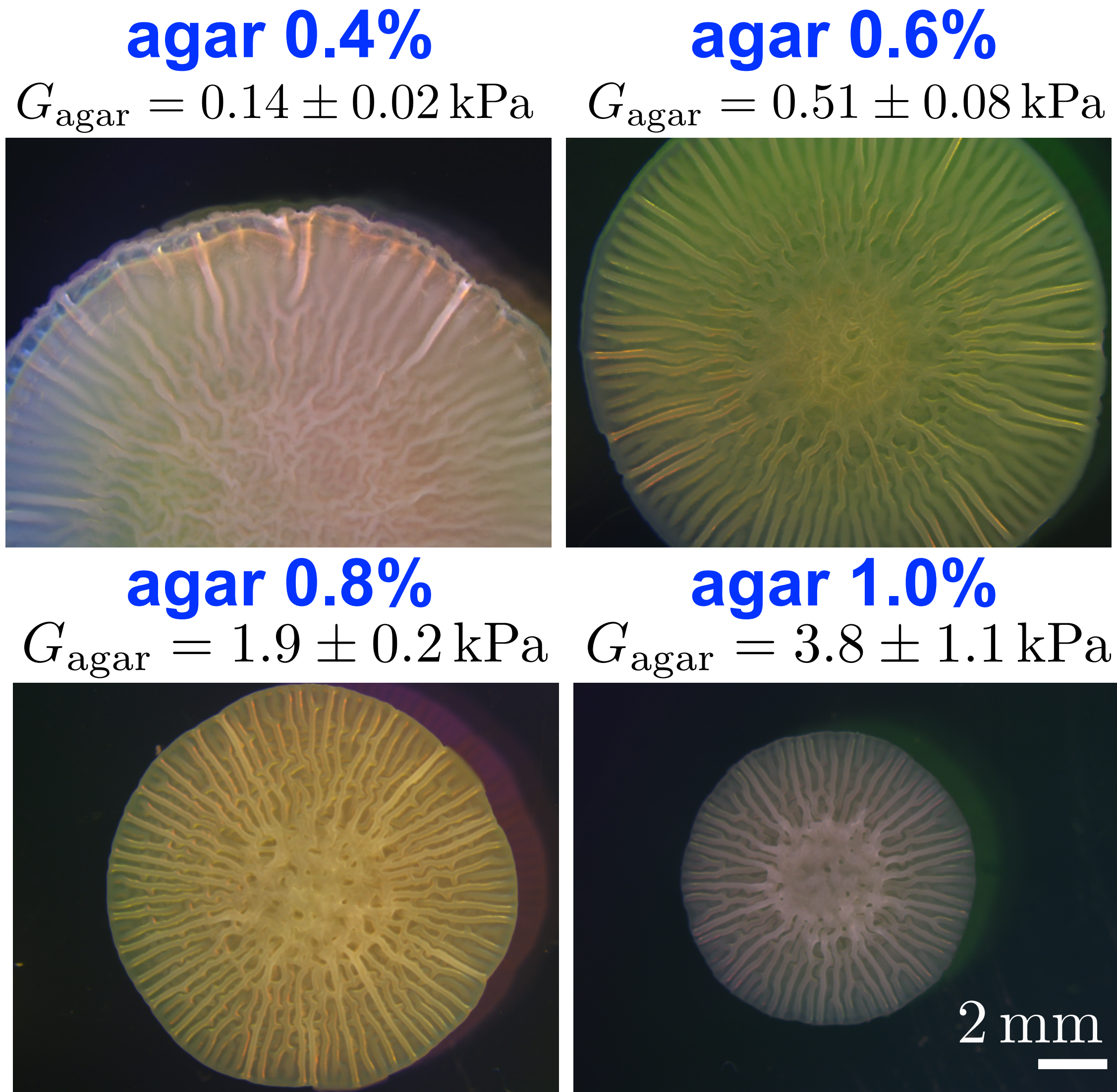
**biofilm thickness** $\quad h \sim 50\text{-}120\,\mu\text{m}$

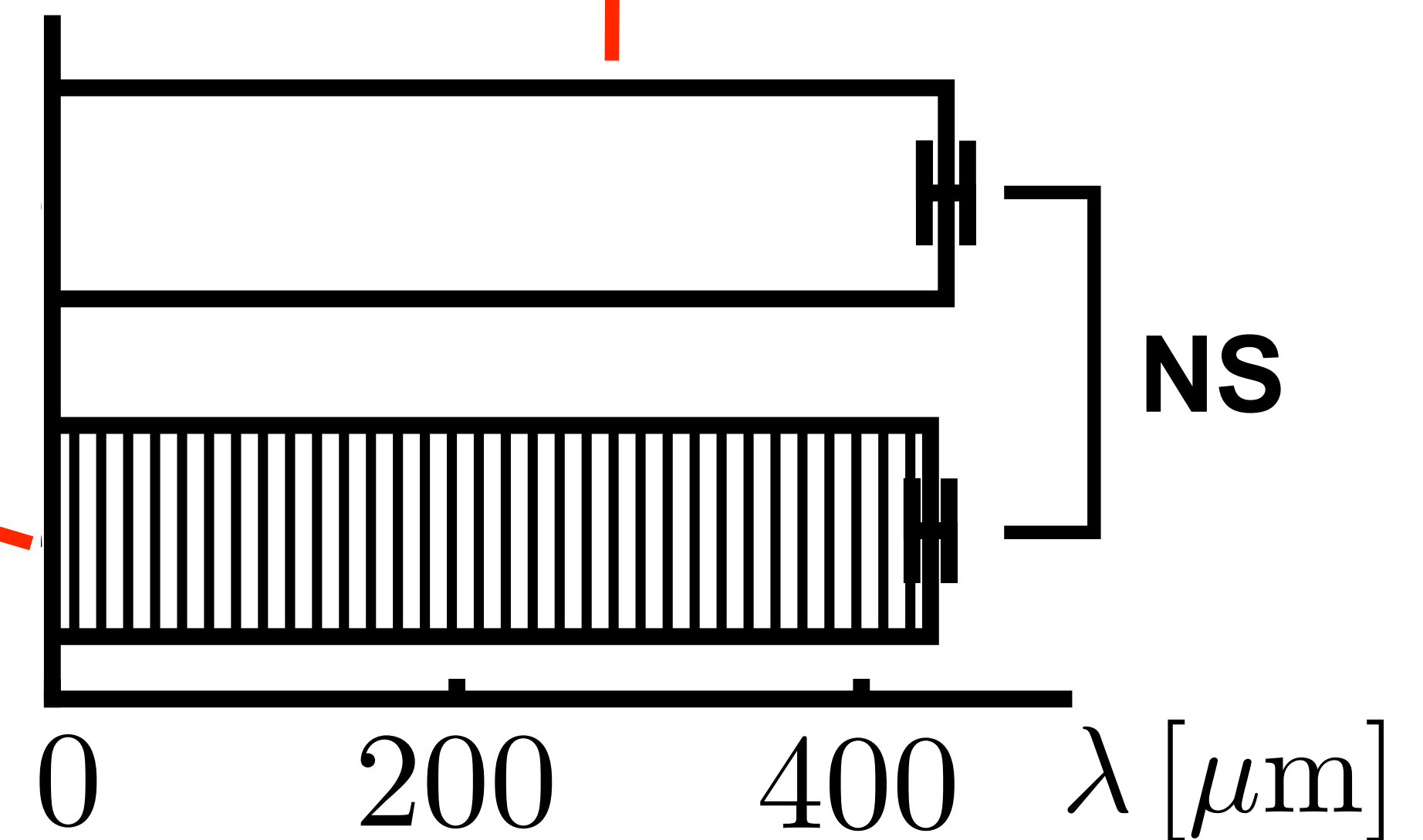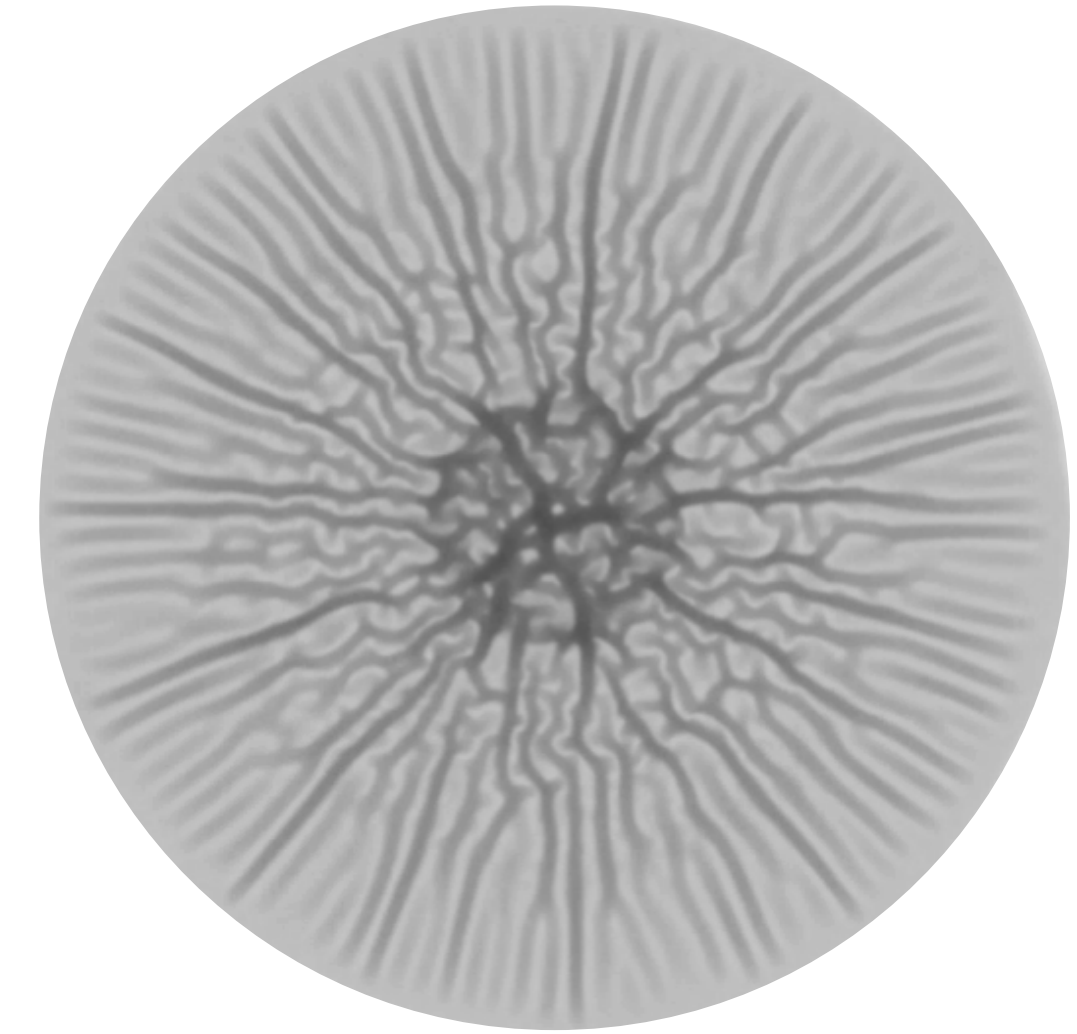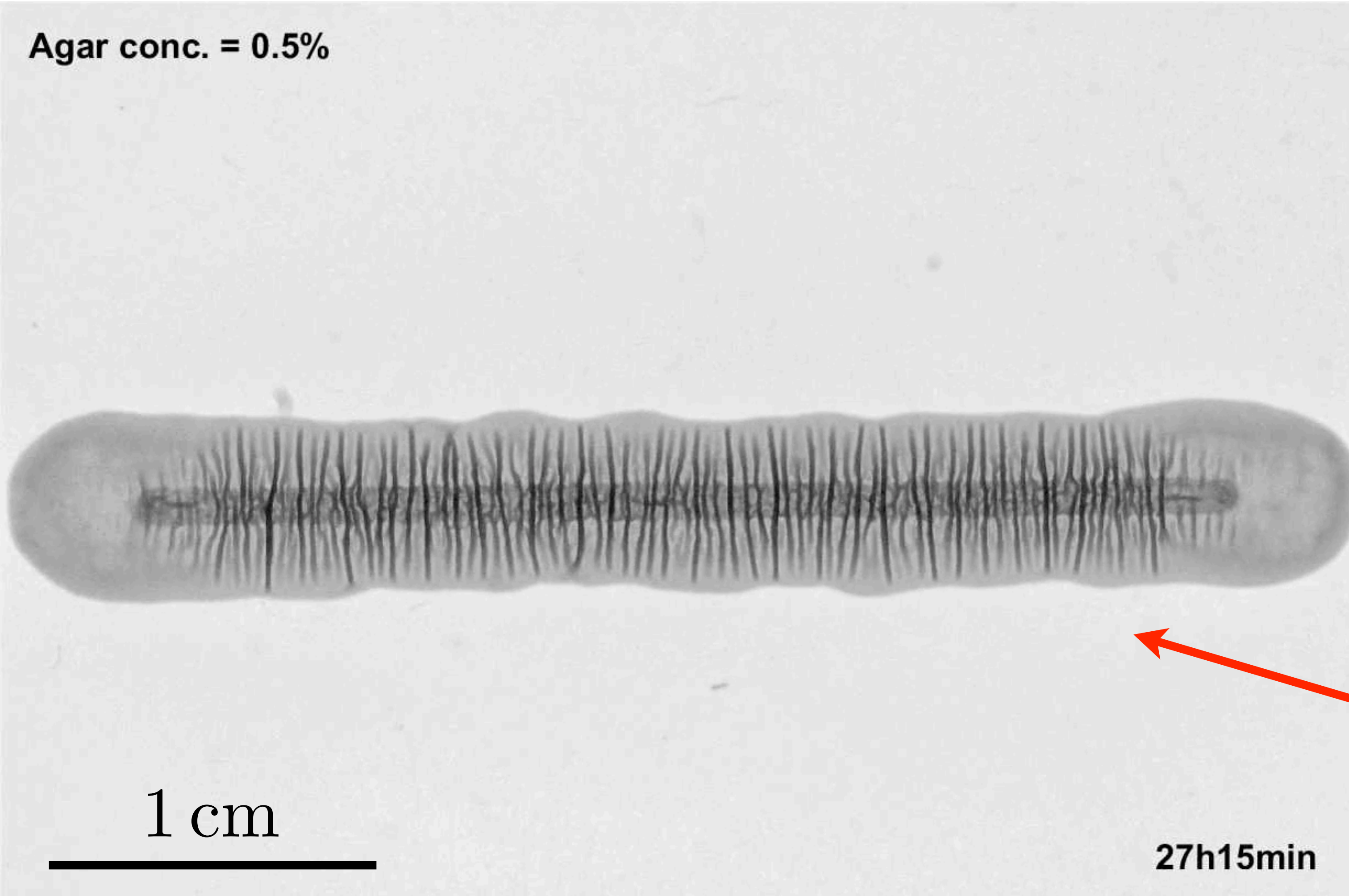**biofilm shear modulus** $G_{\text{film}} \sim 1\text{-}2\,\text{kPa}$

**wavelength of wrinkles**
$\lambda \sim 300\text{-}500\,\mu\text{m}$

$2\,\text{mm}$

J. Yan et al., <u>eLife</u> **8**, e43920 (2018)

**measured wavelength** $\lambda/h$

**predicted wavelength** $2\pi\left(G_{\text{film}}/3G_{\text{agar}}\right)^{1/3}$
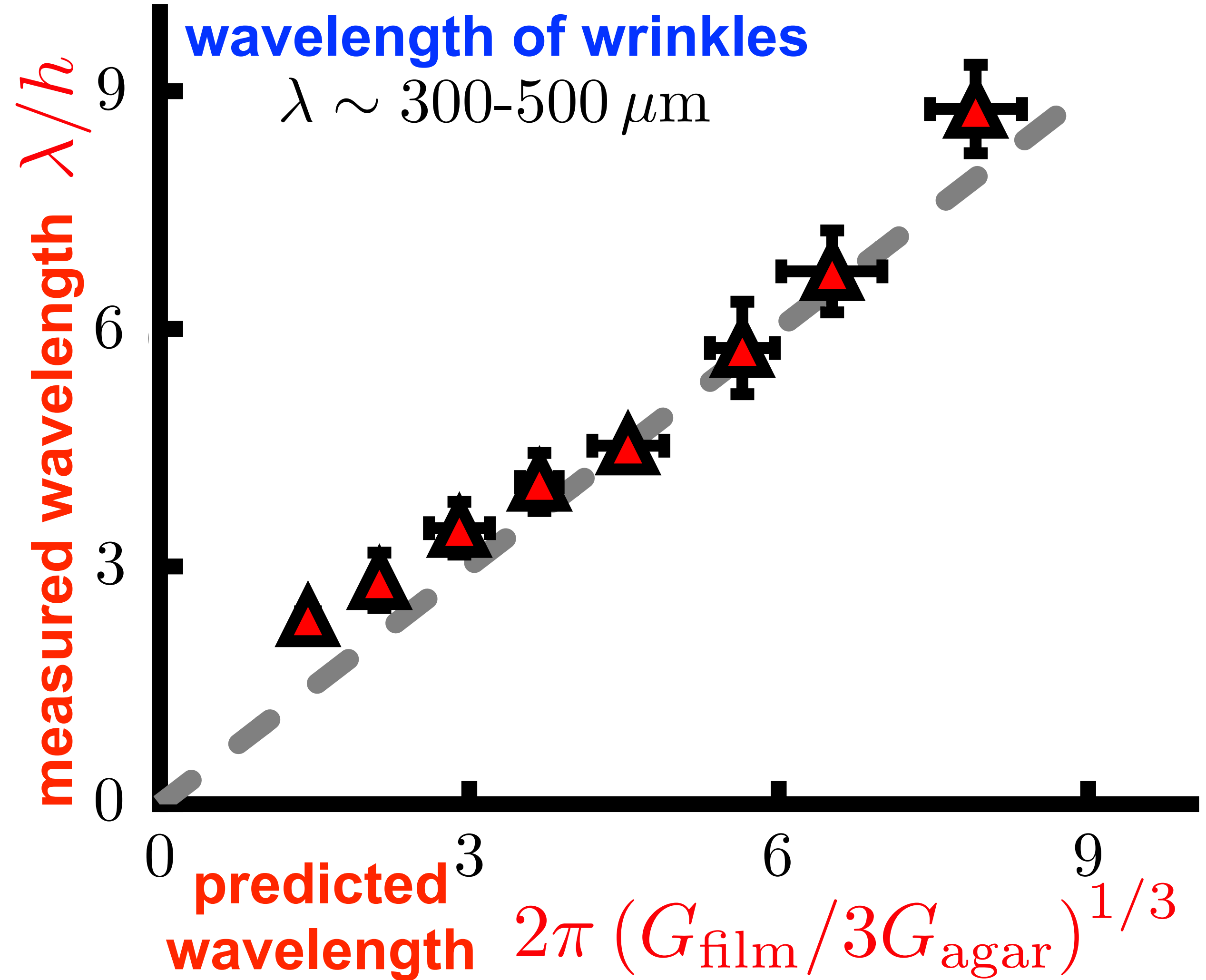
# Morphology of wrinkled patterns
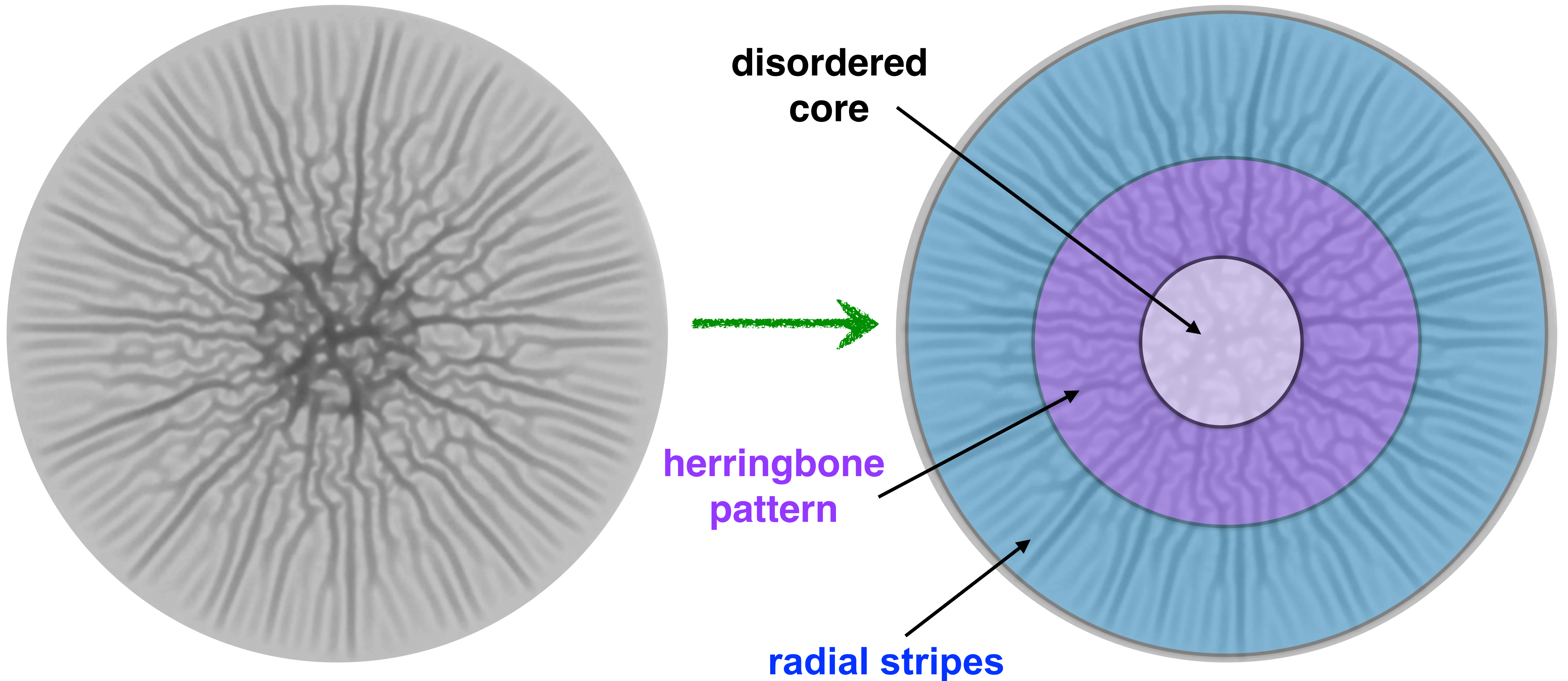


disordered core

herringbone pattern

radial stripes

# Time evolution of wrinkled patterns



Agar conc. = 0.7%

45h15min



disordered core

herringbone pattern

radial stripes



agar 0.8%

20 h

31 h

40 h

$t$ (h)

$r$ (mm)

# Time evolution of wrinkled patterns

Agar conc. = 0.7%

45h15min

disordered core

herringbone pattern

radial stripes

agar 0.8%

20 h

31 h

40 h

$t$ (h)

$r$ (mm)
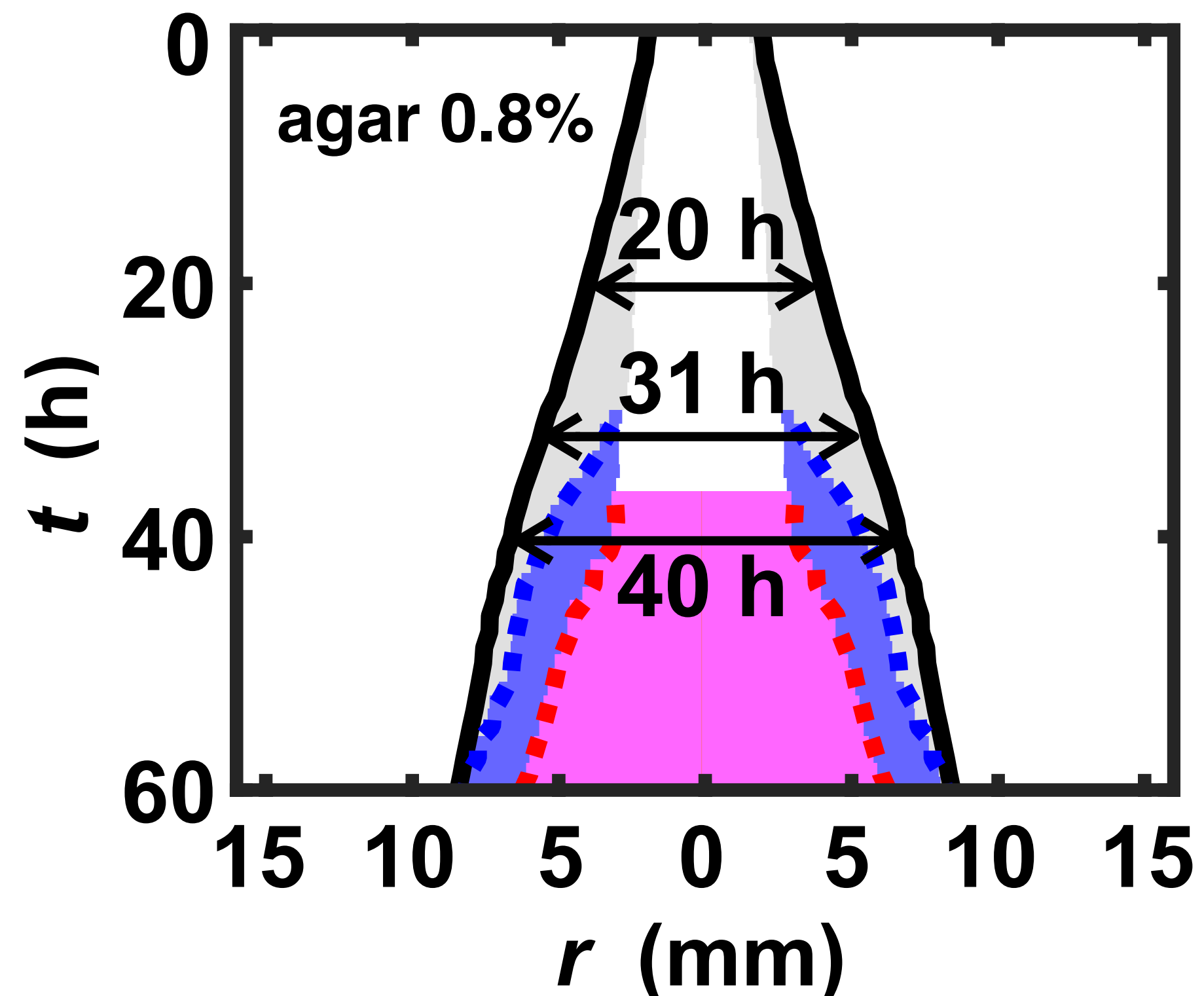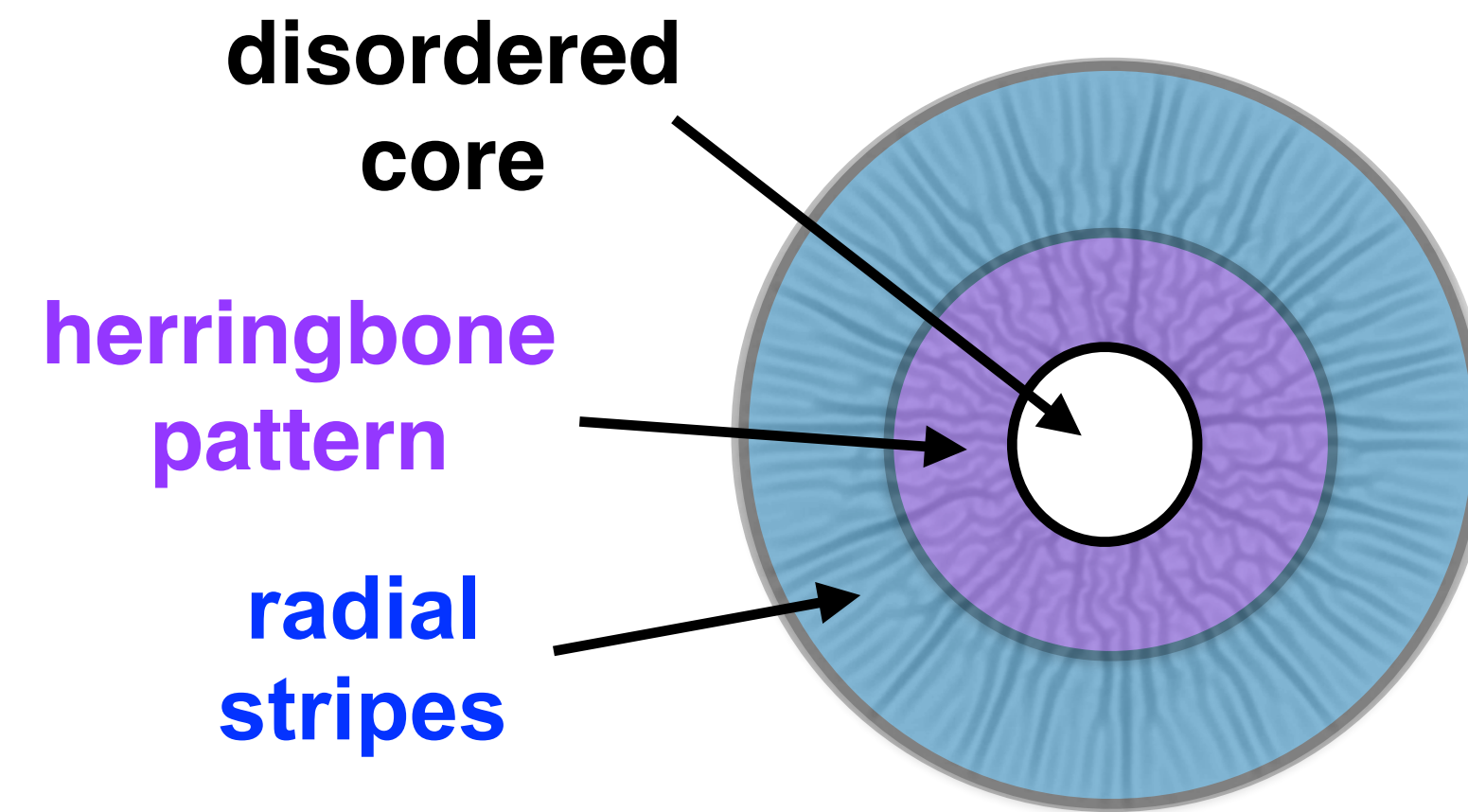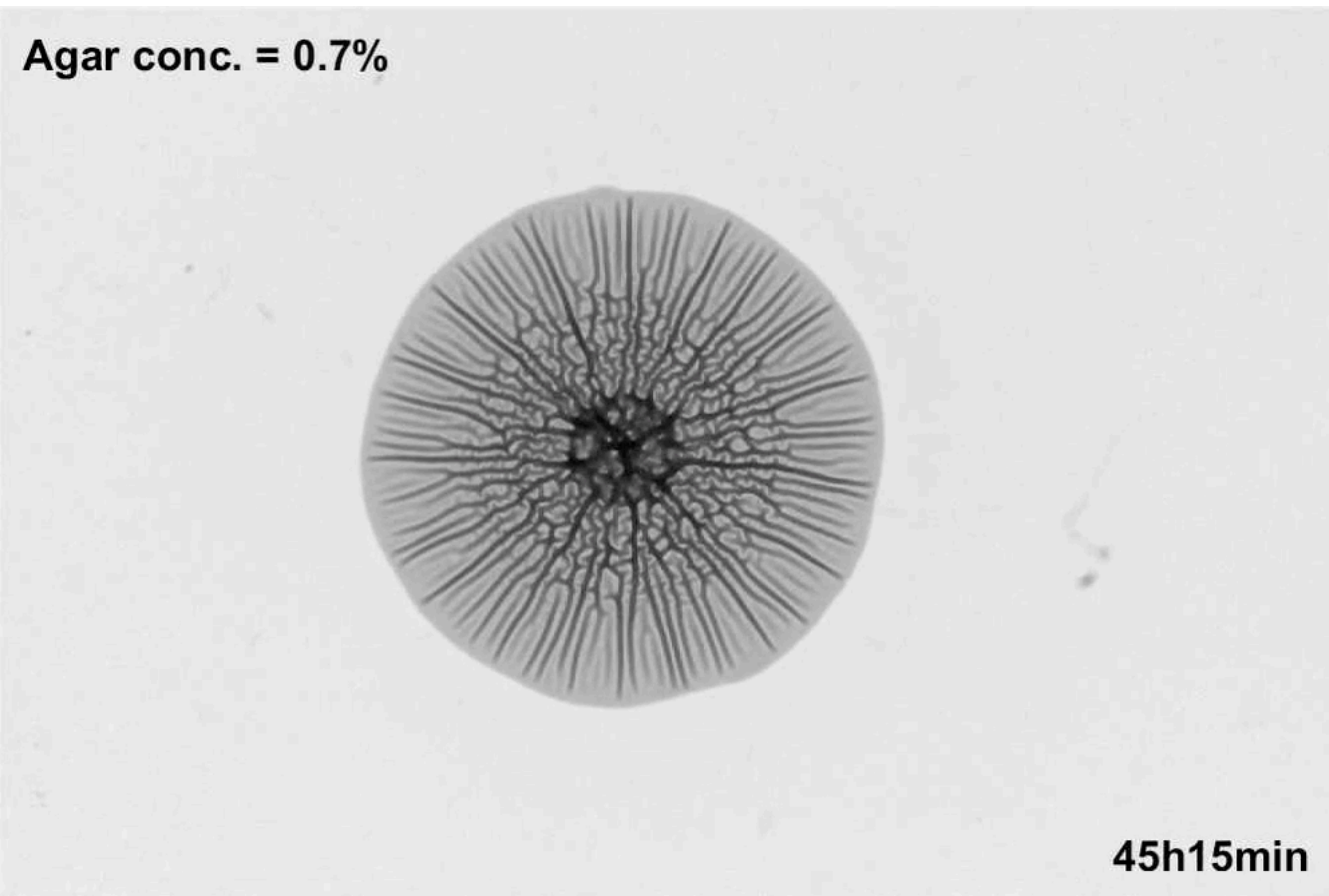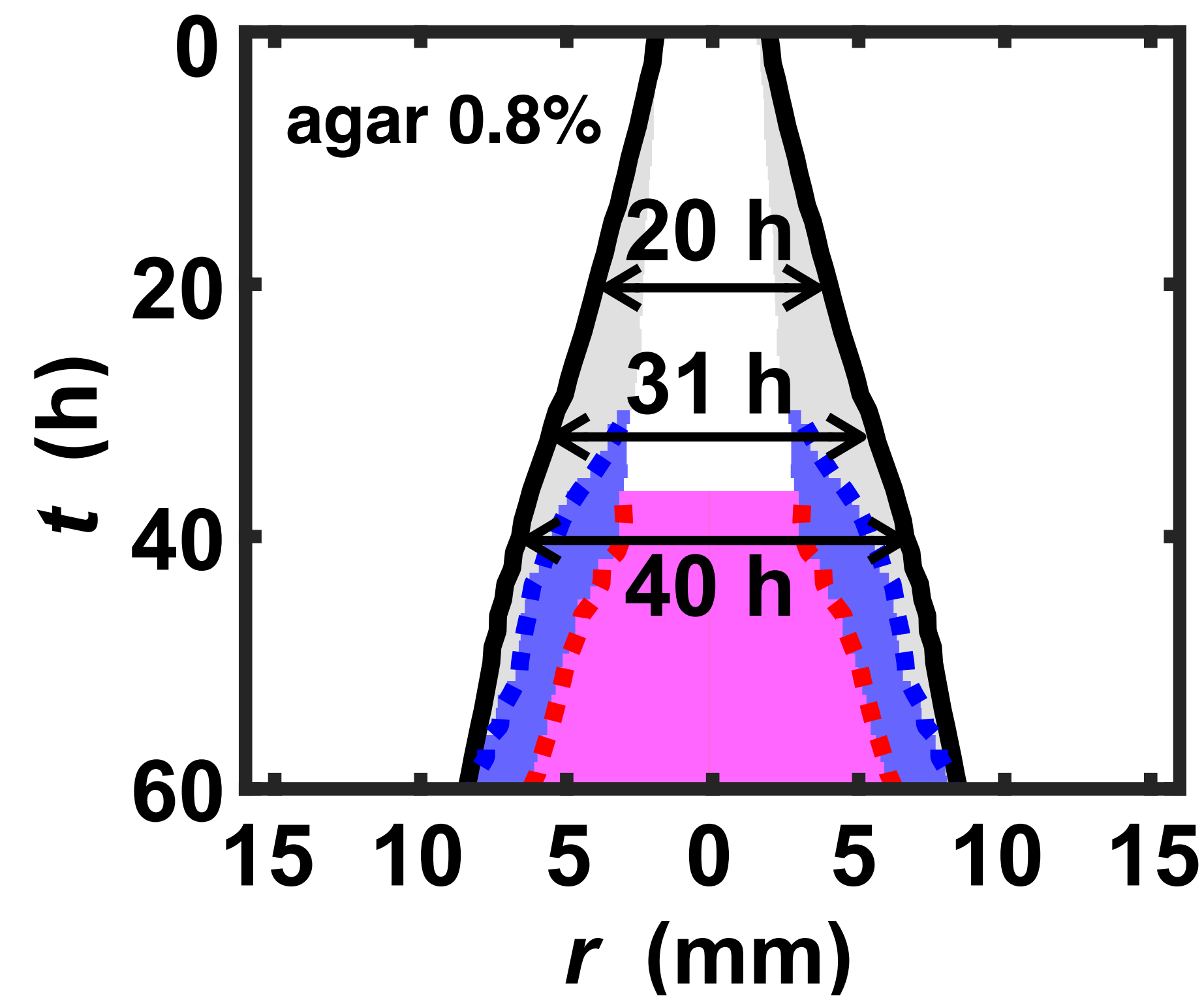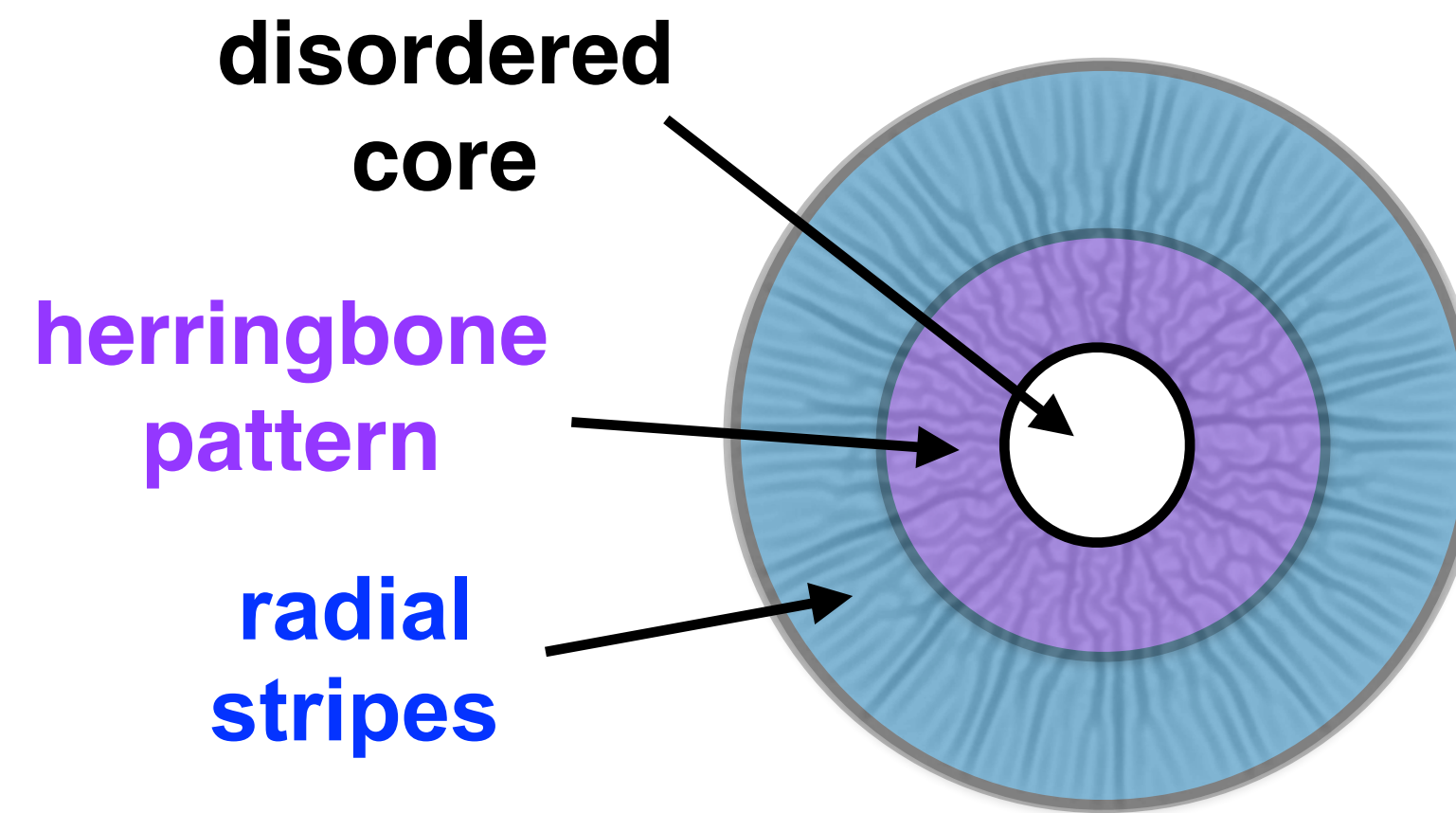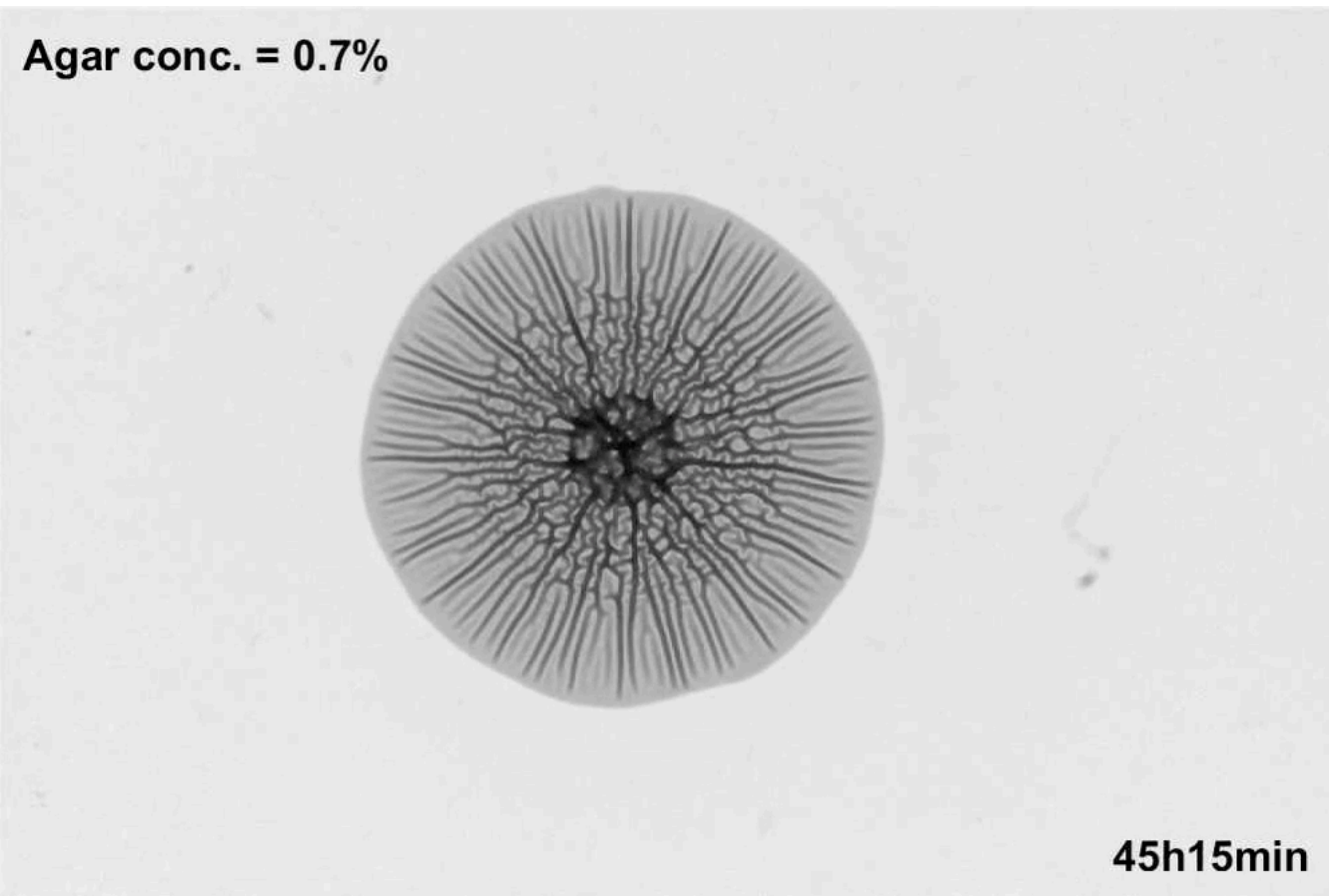
# Time evolution of wrinkled patterns

## agar 0.4% (wrinkles emerge from the outer edge)
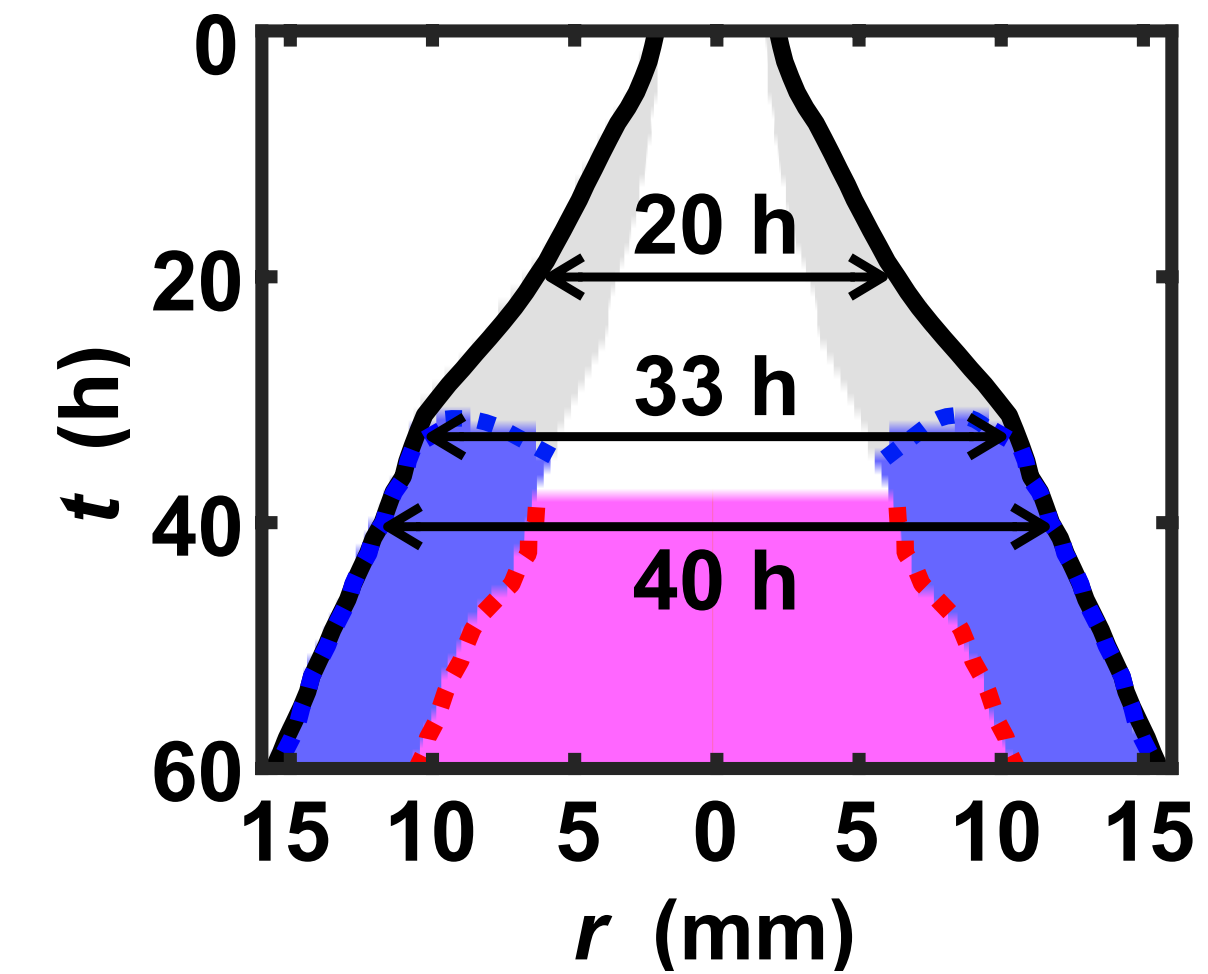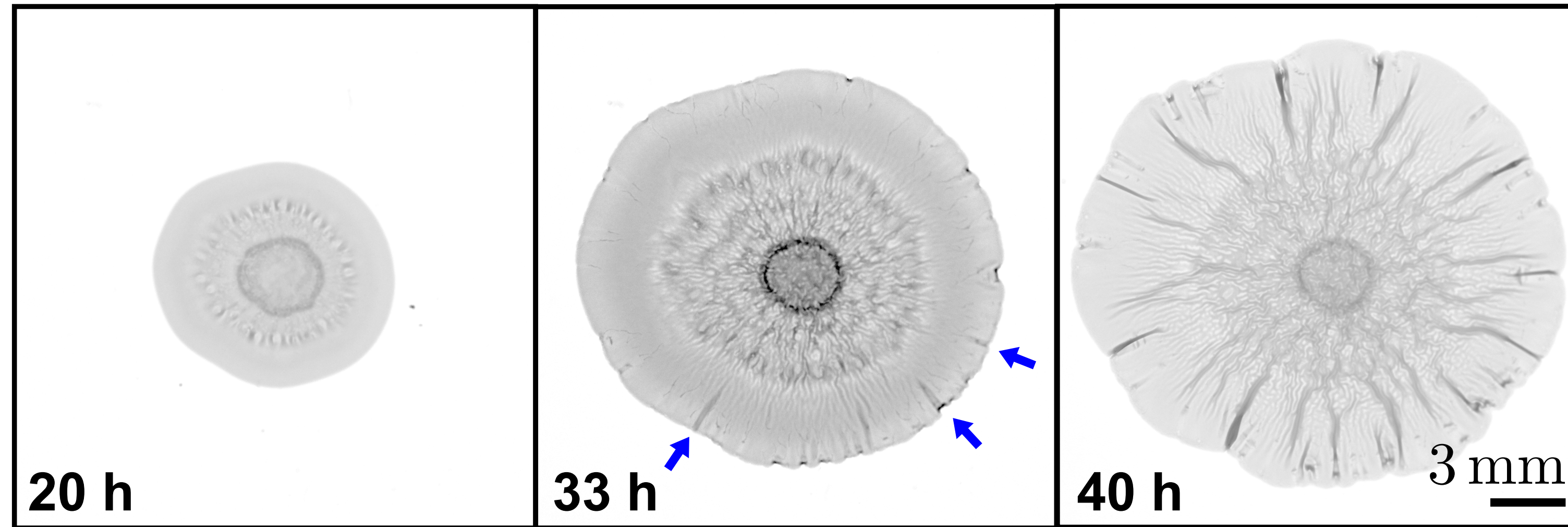


## agar 0.8% (wrinkles emerge from the central region)

# Model of biofilm growth

Growth zone



$z$

**Nutrient uptake**

**Diffusion**

$r$
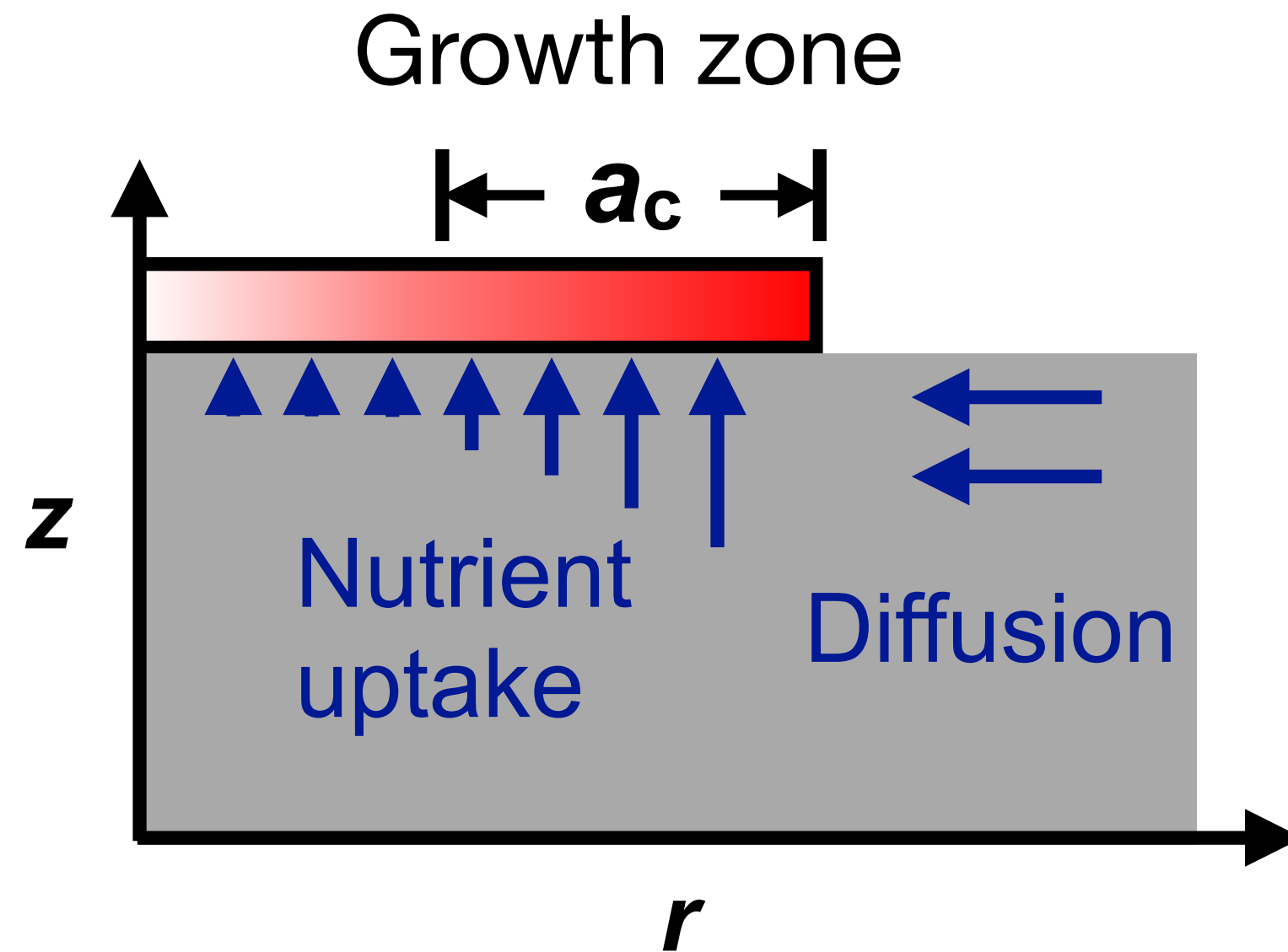
**diffusion and uptake of nutrients**

$$\frac{\partial c}{\partial t} = D\nabla^2 c - \frac{Qc}{(K+c)}$$

**nutrient limited growth**

$$\frac{\partial \epsilon_g}{\partial t} = \frac{k_g c}{(K+c)}$$

**friction between expanding biofilm and substrate**
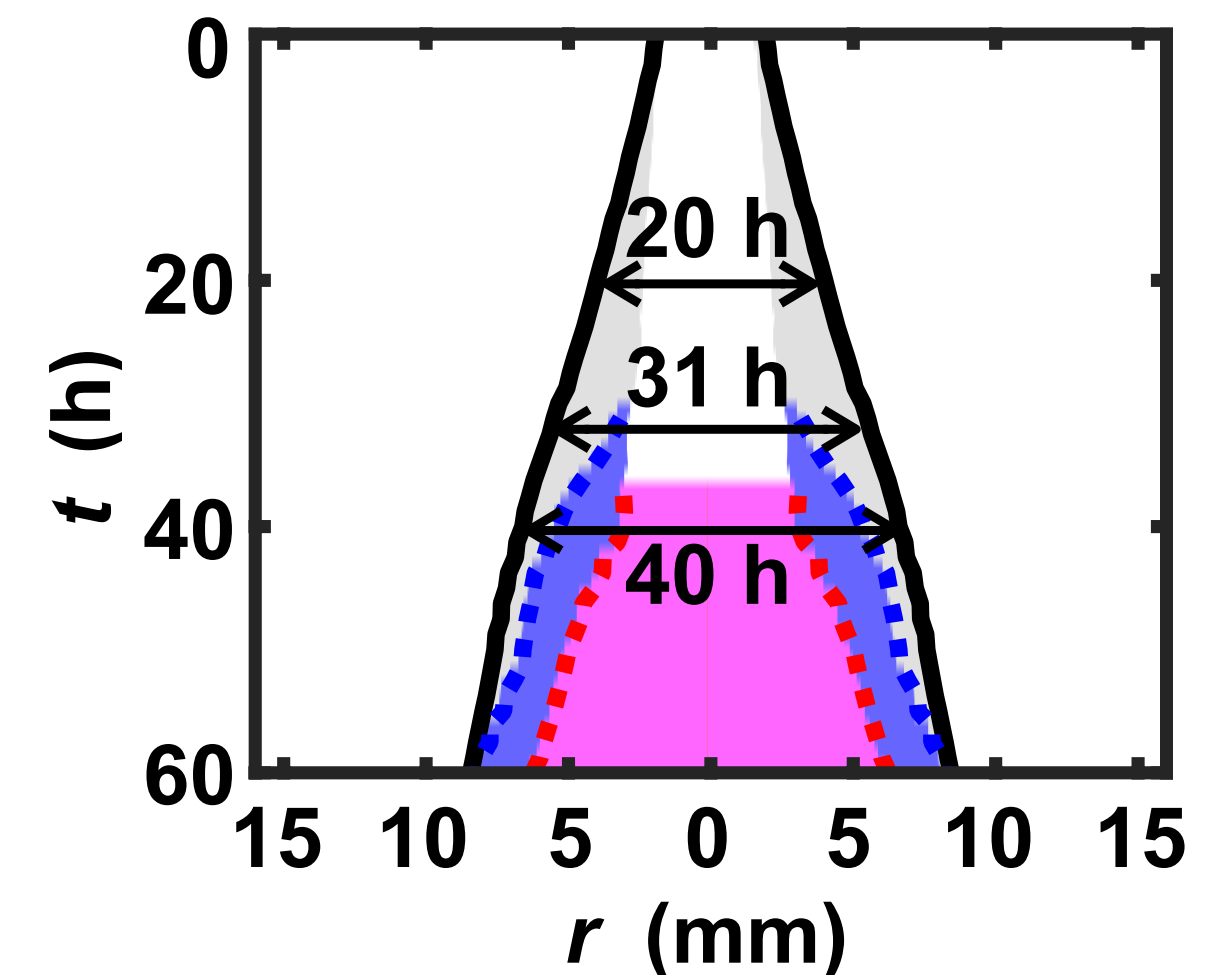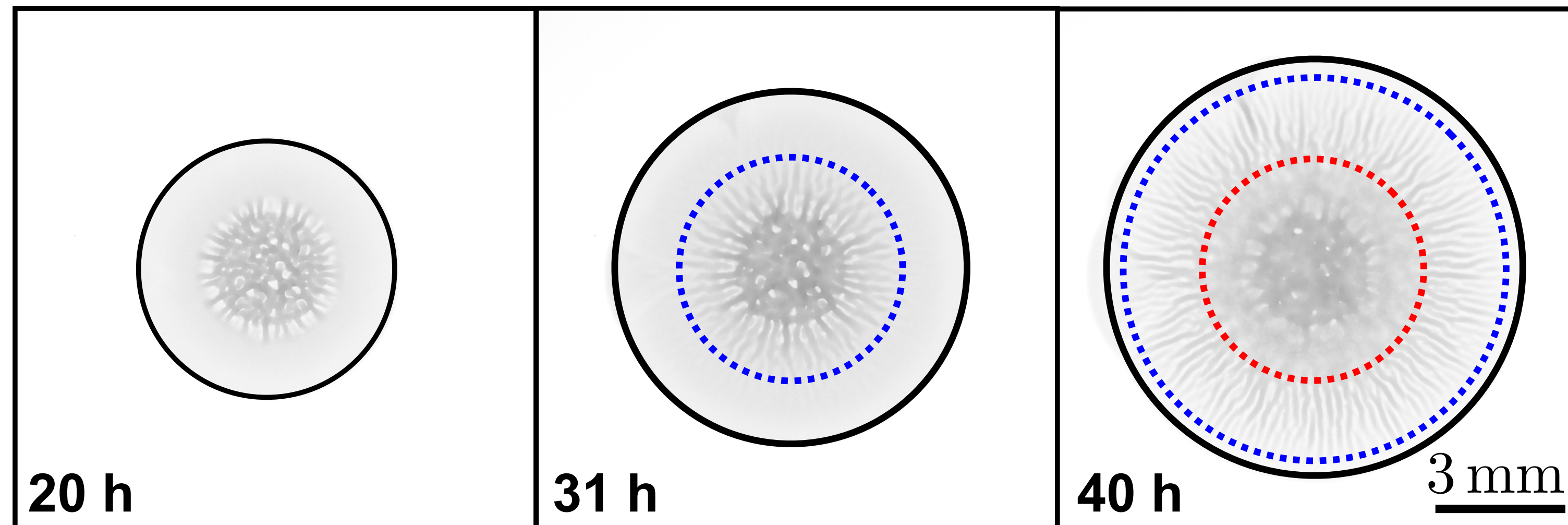


$$f = -\eta v$$
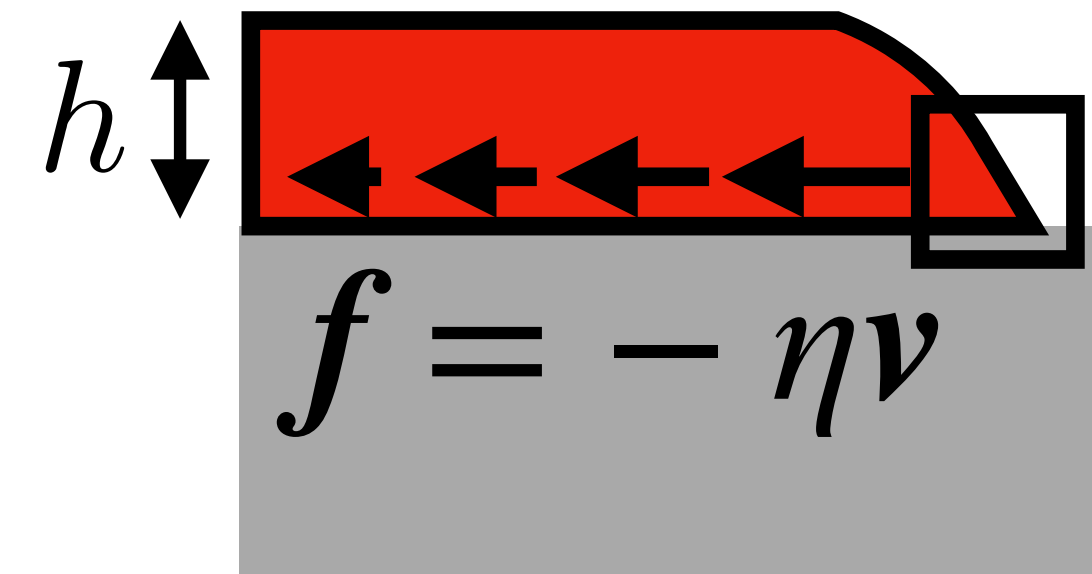
**force balance**

$$\nabla \cdot (h\boldsymbol{\sigma}) - \eta \mathbf{v} = 0$$

**mechanics of biofilm is modeled as a plane stress thin plate made from a nearly incompressible neo-Hookean material**
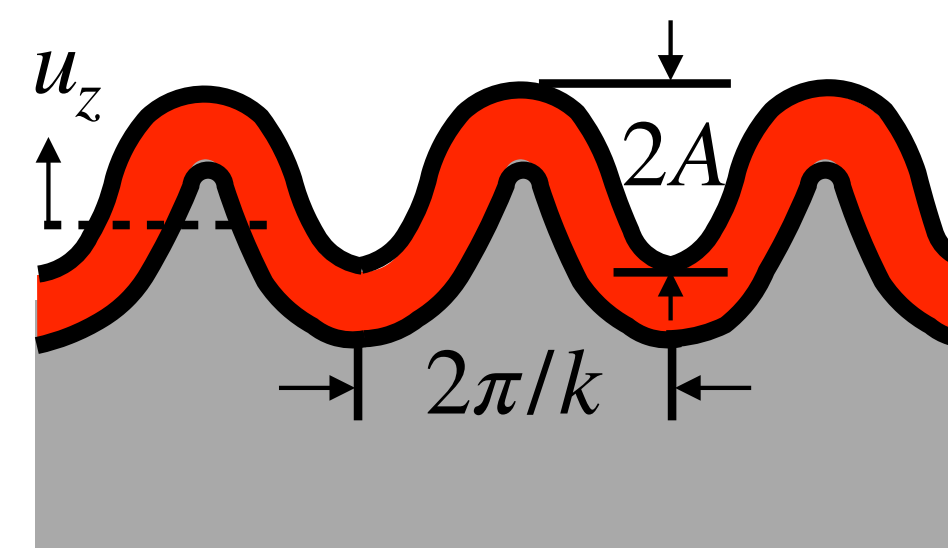


**wrinkling instability occurs once a critical compressive stress is reached inside biofilm**

# Model of biofilm growth
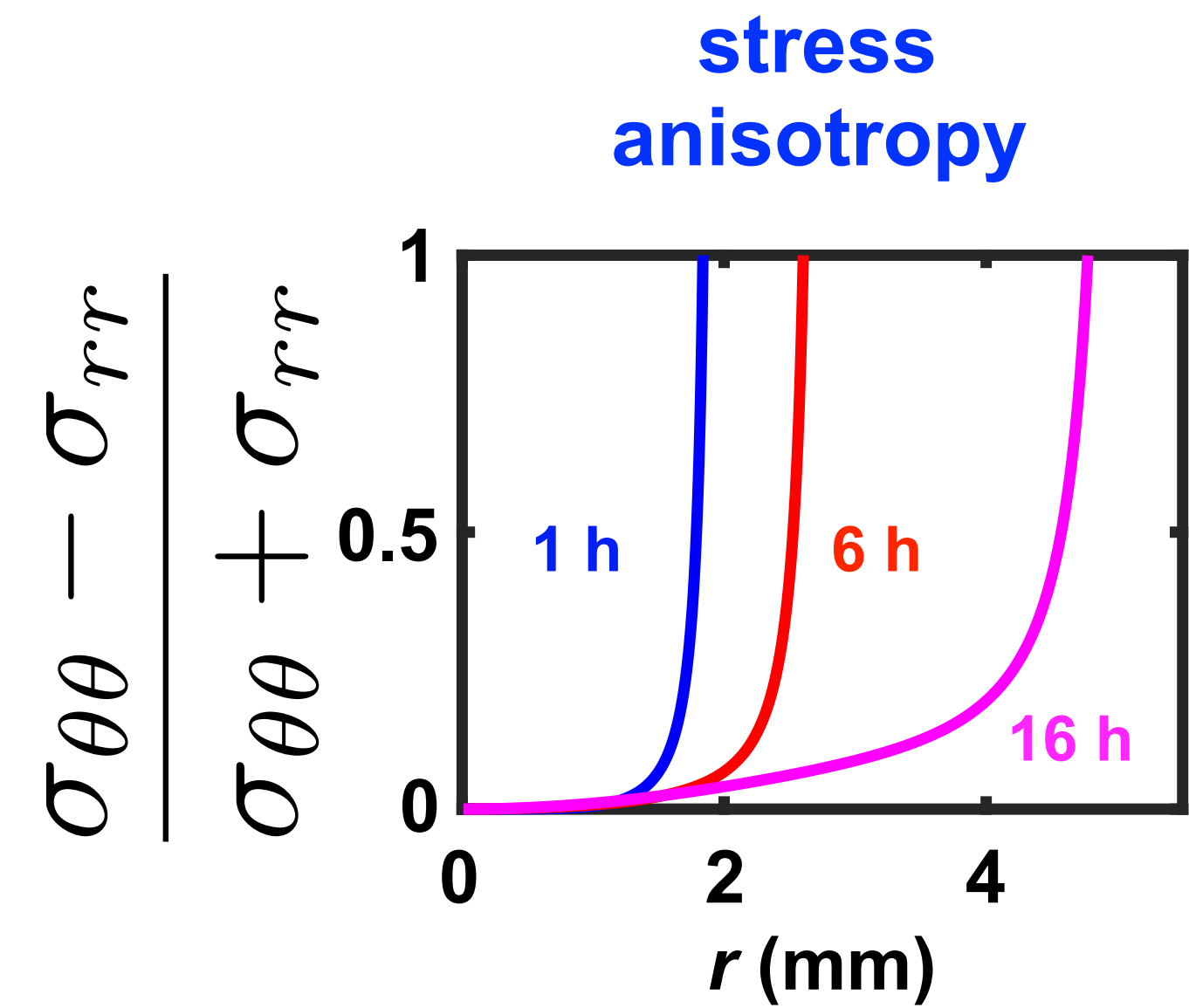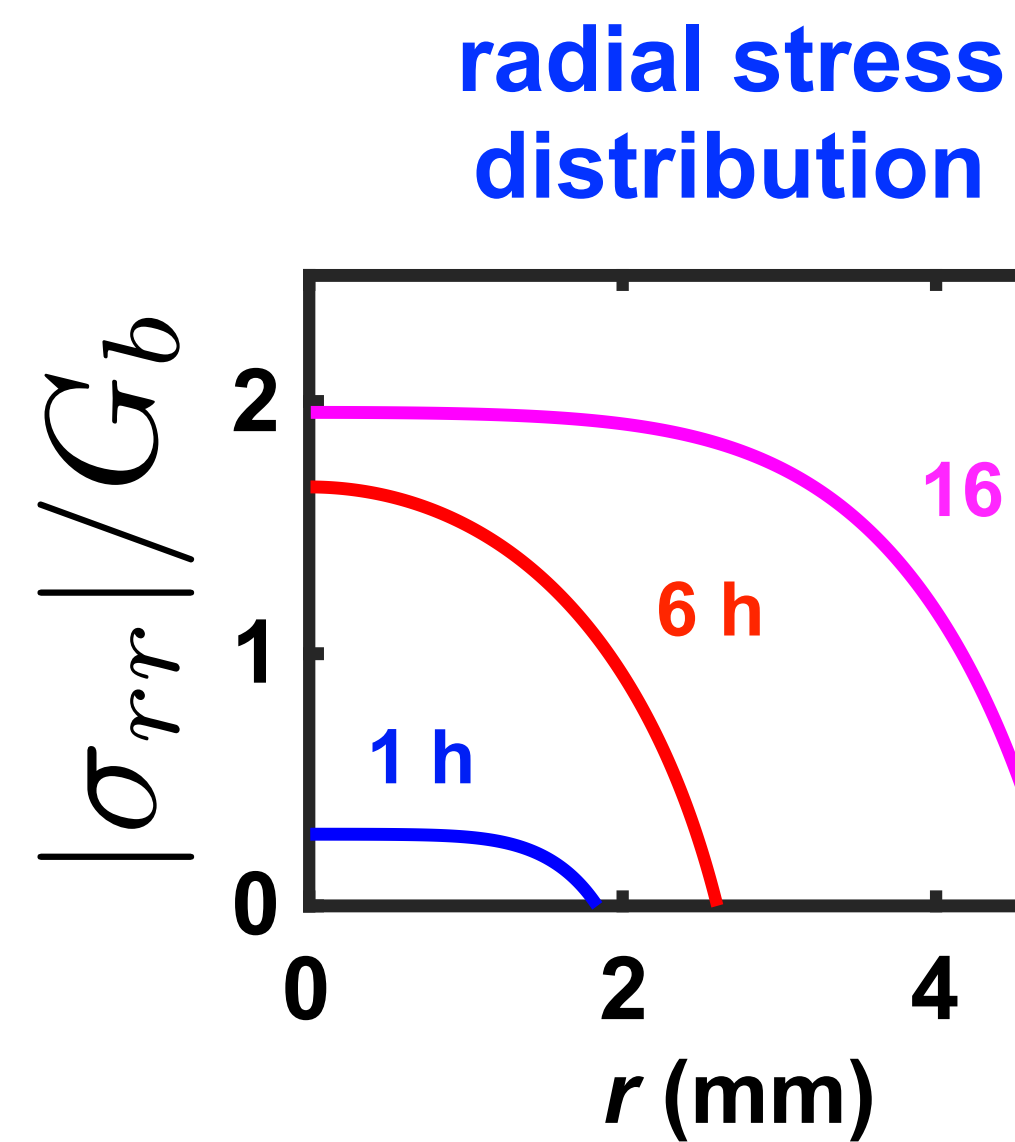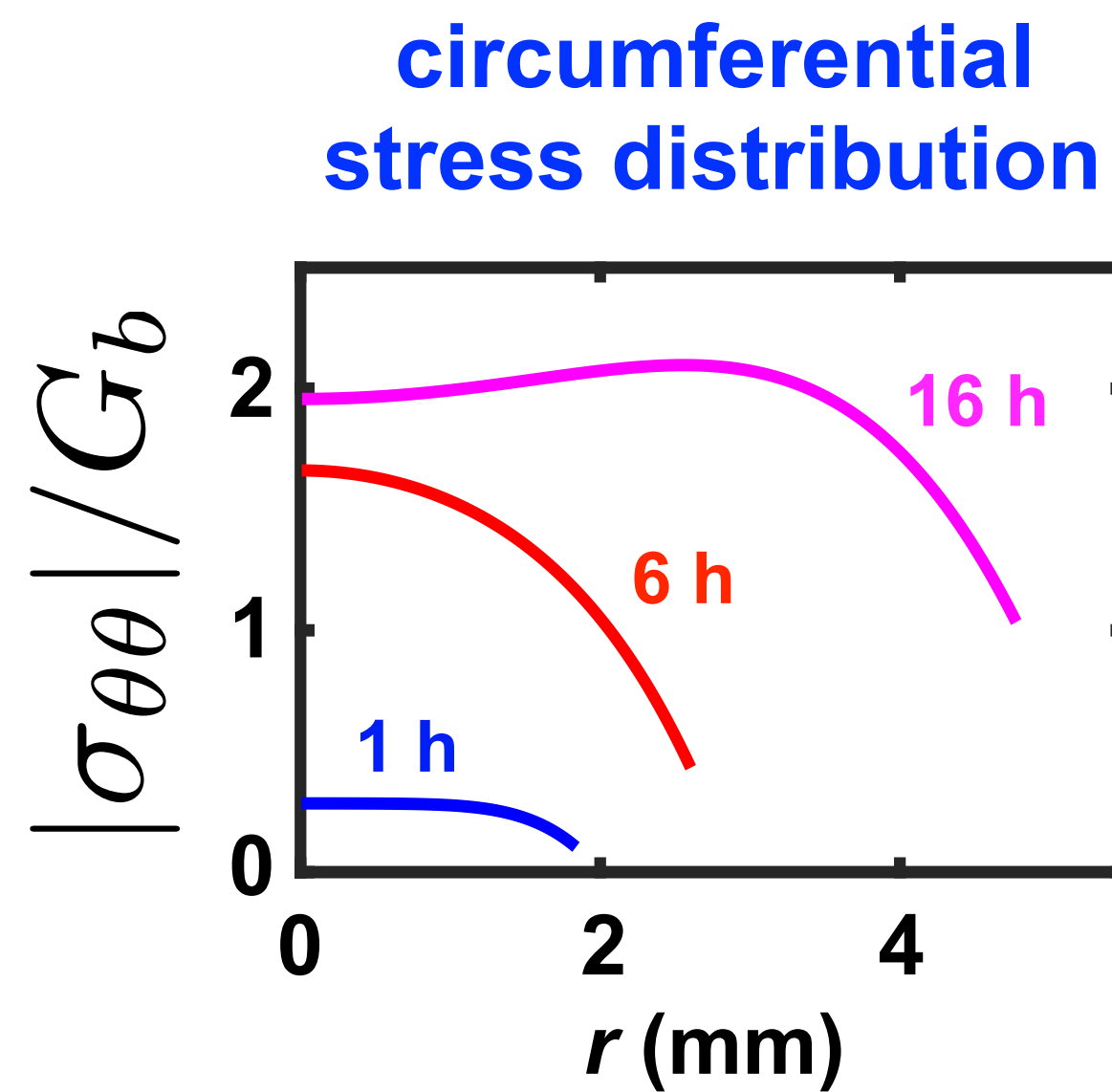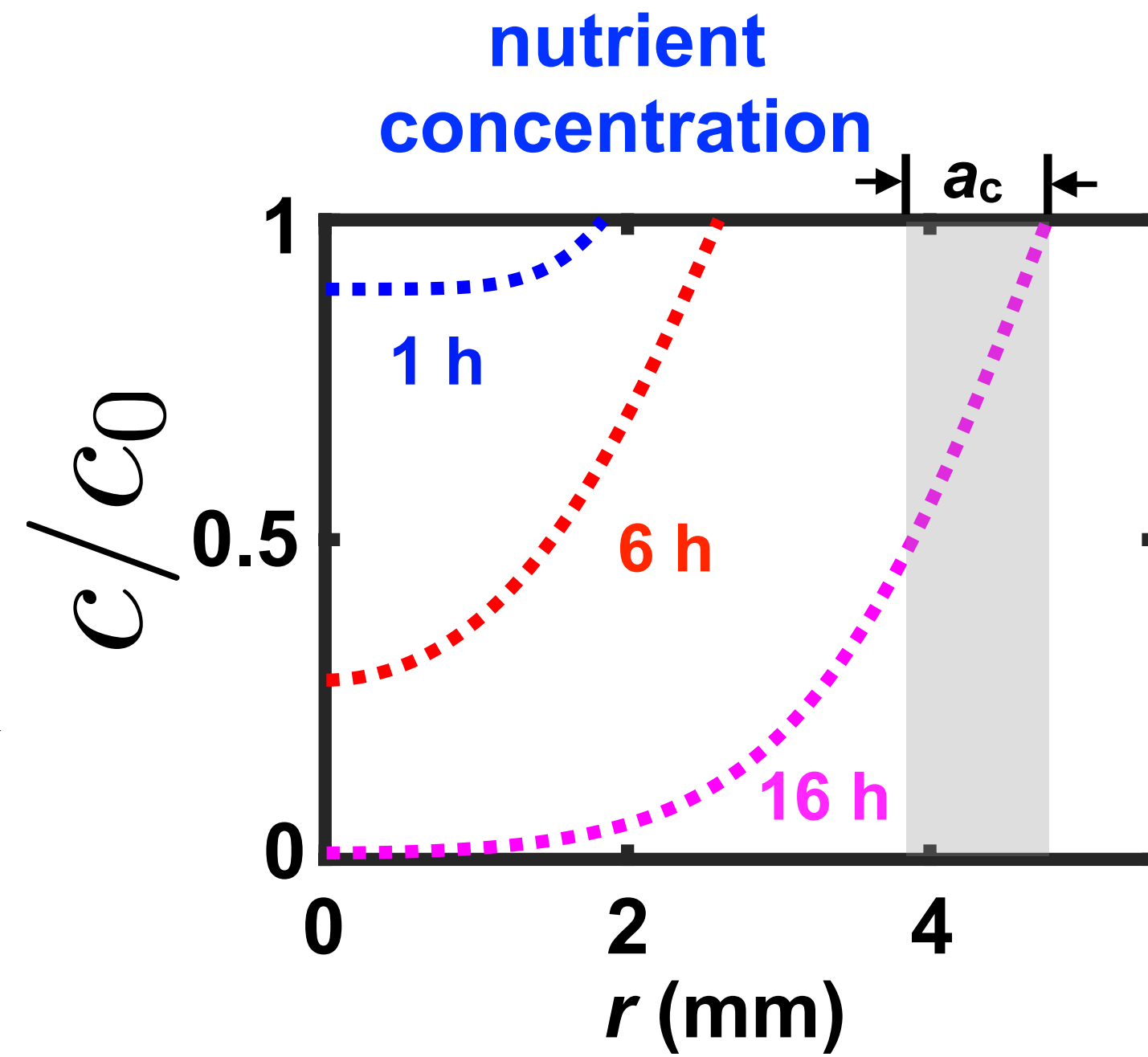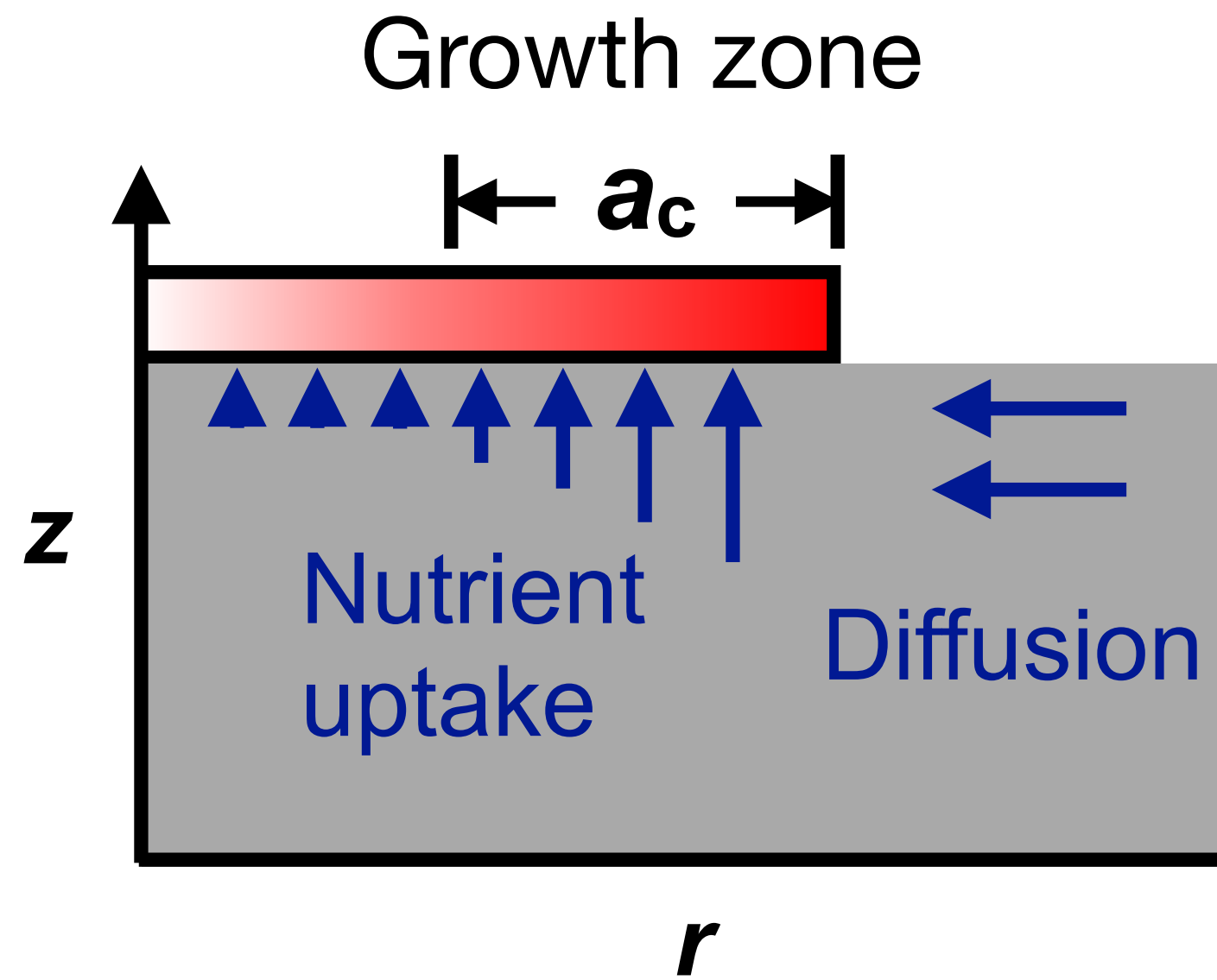
# Stress distribution dictates the morphology of wrinkling patterns
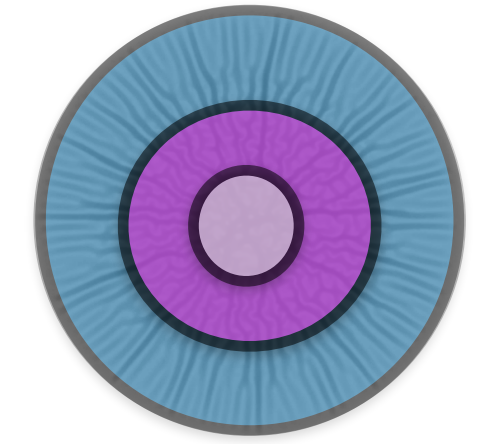
**circumferential stress distribution**

$$\sigma_{\theta\theta}/\sigma_{\theta\theta}^0$$

$r^*$

$r/r_b$

**stress anisotropy**

$$\frac{\sigma_{\theta\theta}-\sigma_{rr}}{\sigma_{\theta\theta}+\sigma_{rr}}$$

$r/r_b$

**friction (agar concentration)**

**Section view**

$u_z$

$2A$

$2\pi/k$

**uniaxial stress**  **Top view**  **biaxial stress**

$u_z/A$

$e_r$

$e_\theta$

$S=0$: Stripe        $S>0$: Zigzag
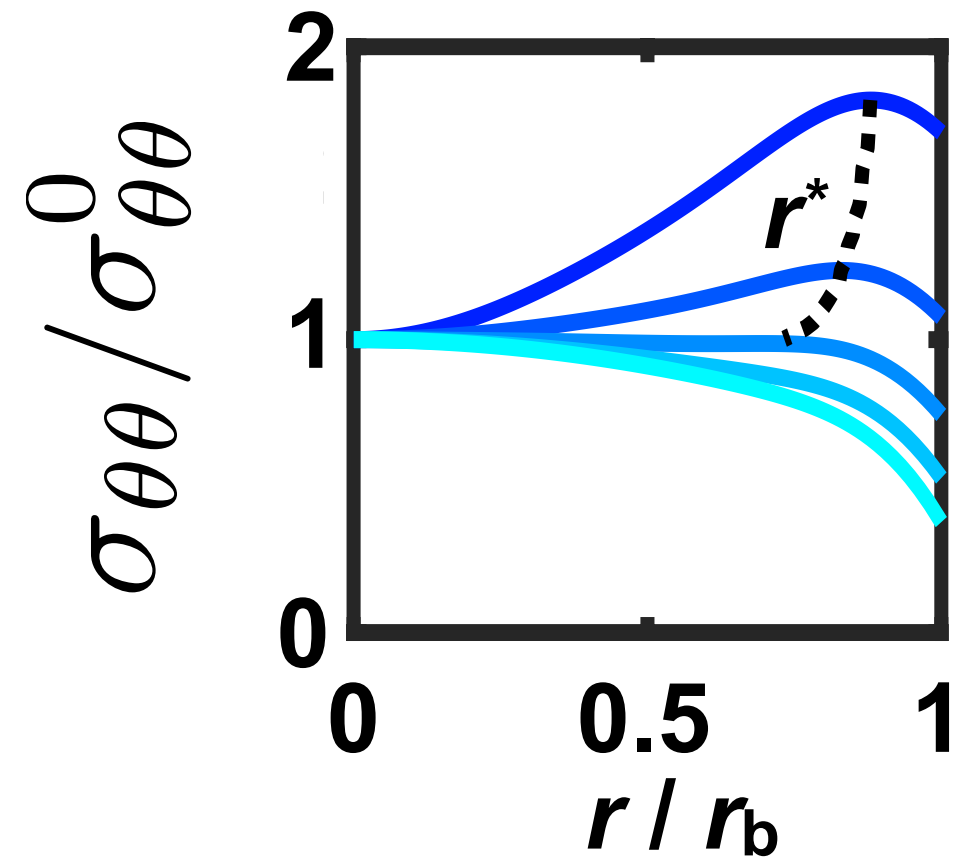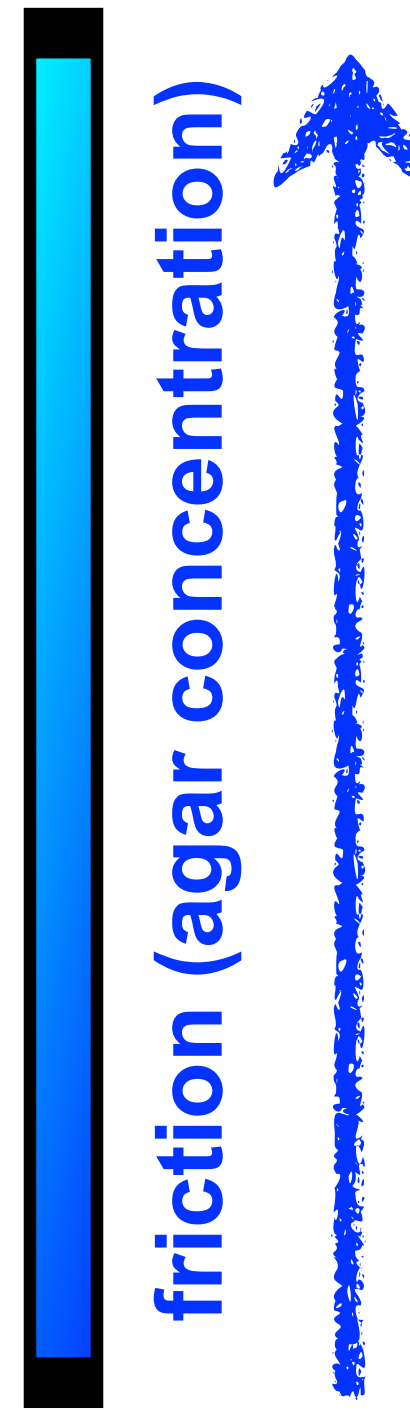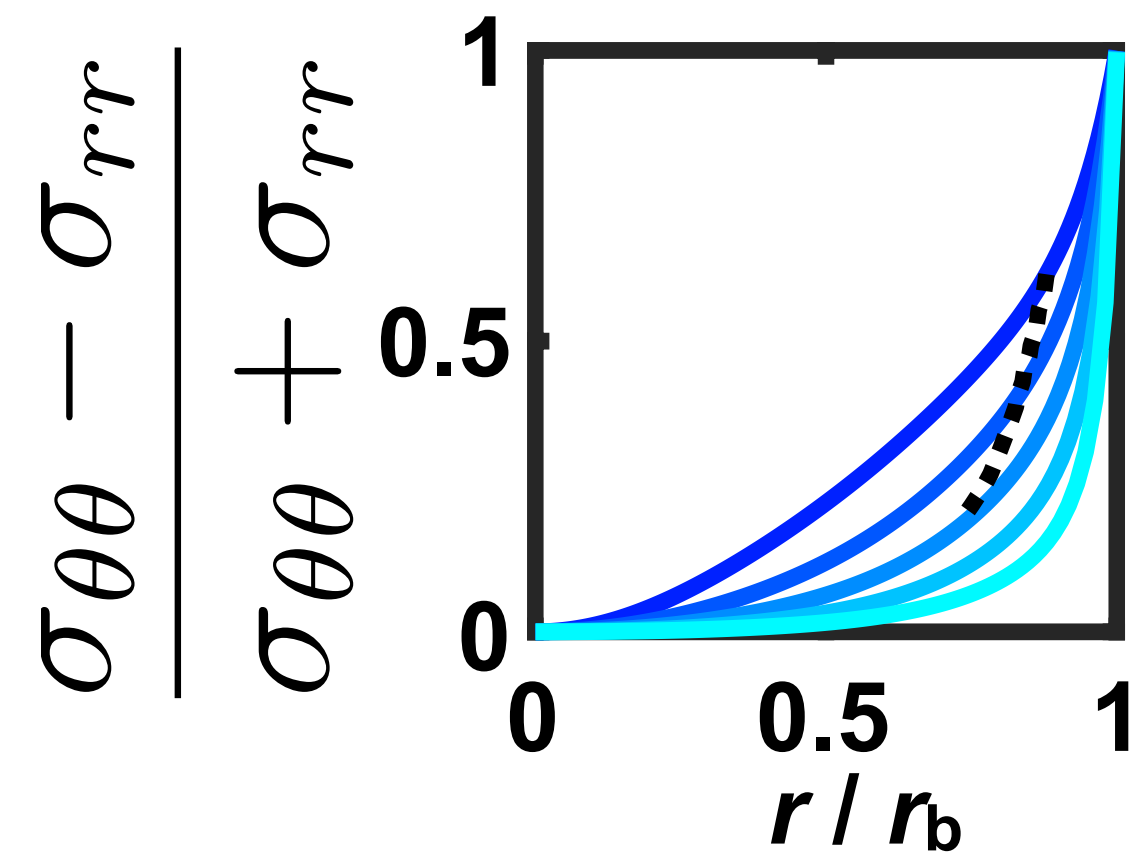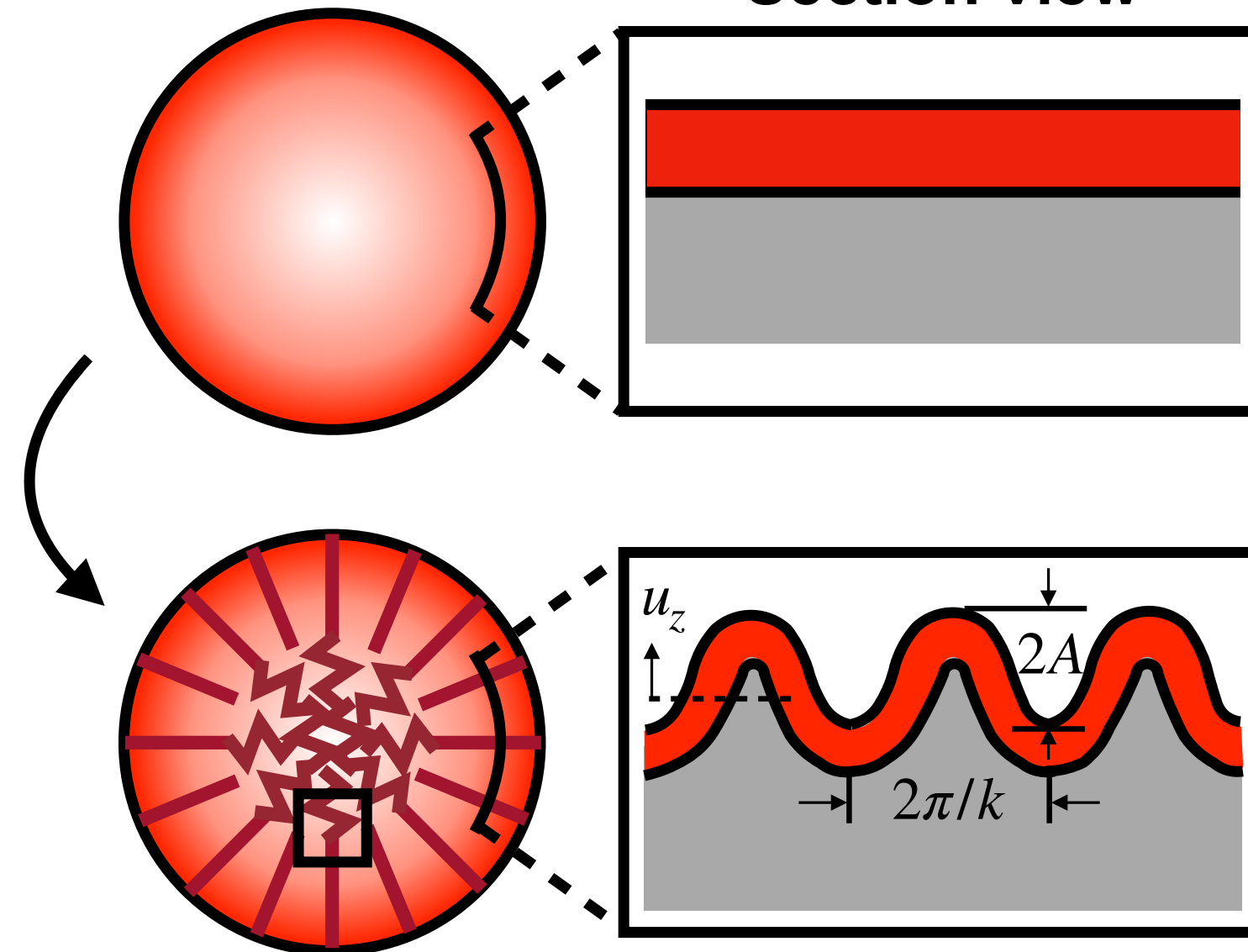
**evolution of patterns (low agar concentration)**

$t$ (h)

No pattern

Zigzag        Stripe
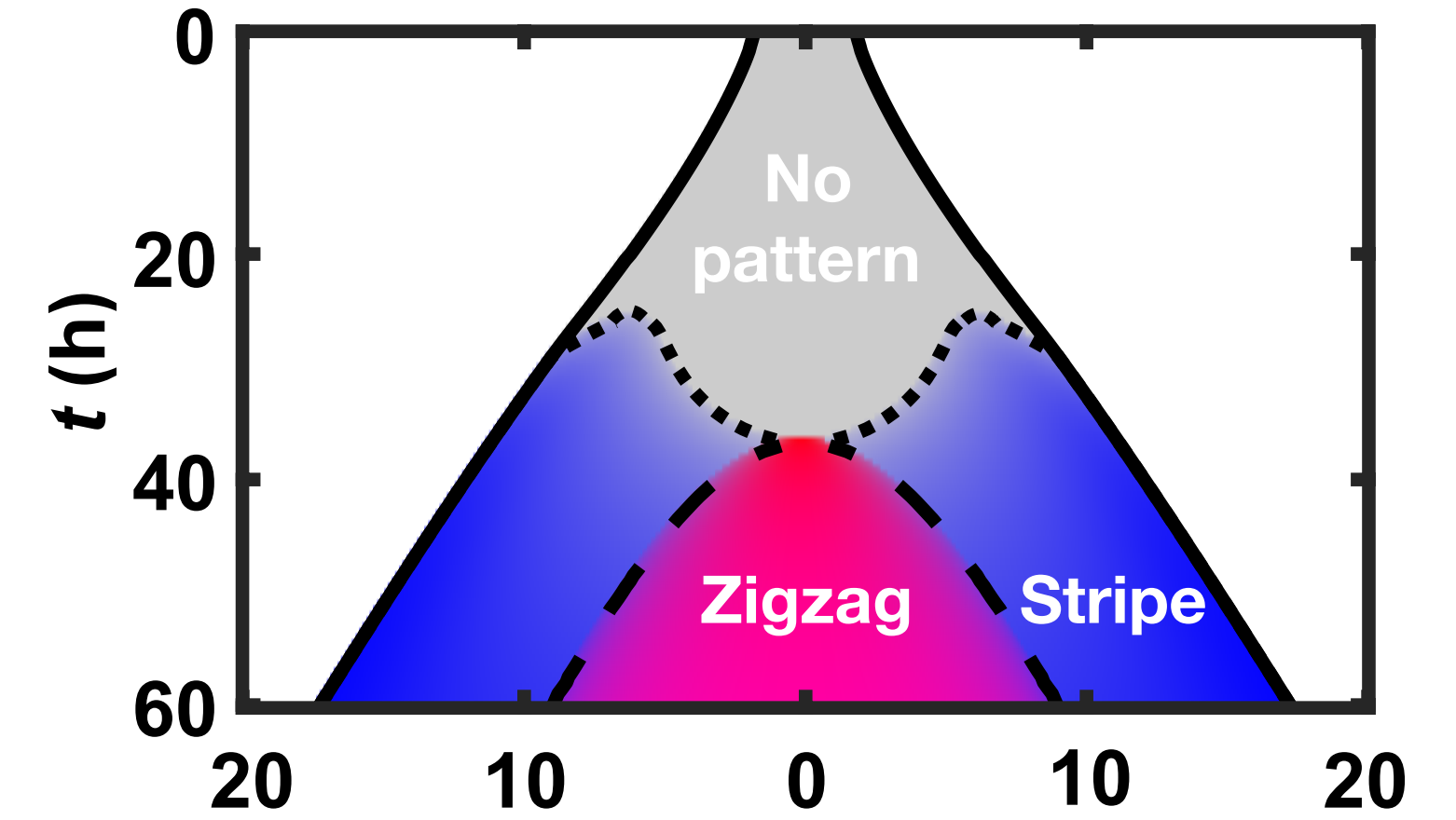
**evolution of patterns (high agar concentration)**
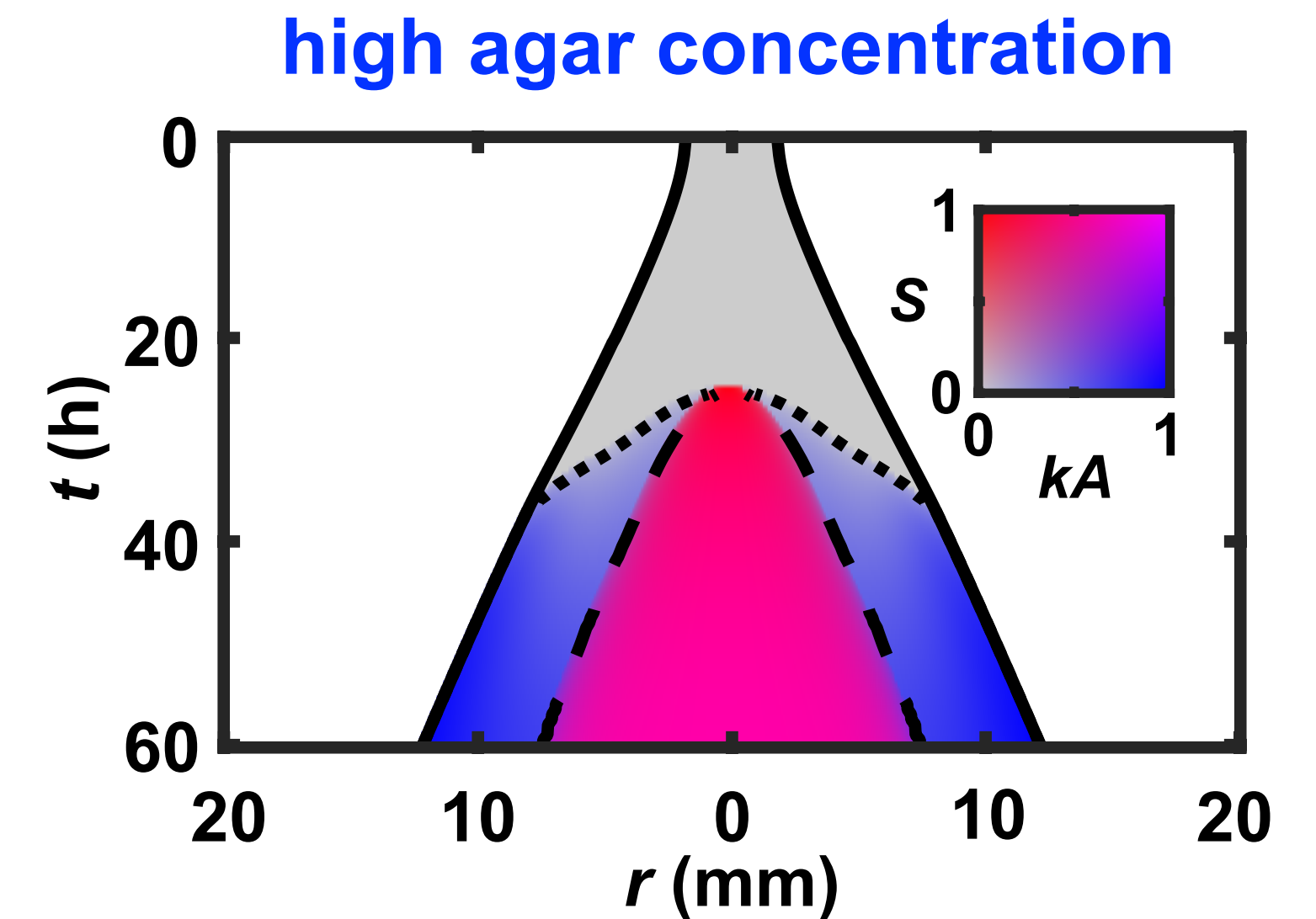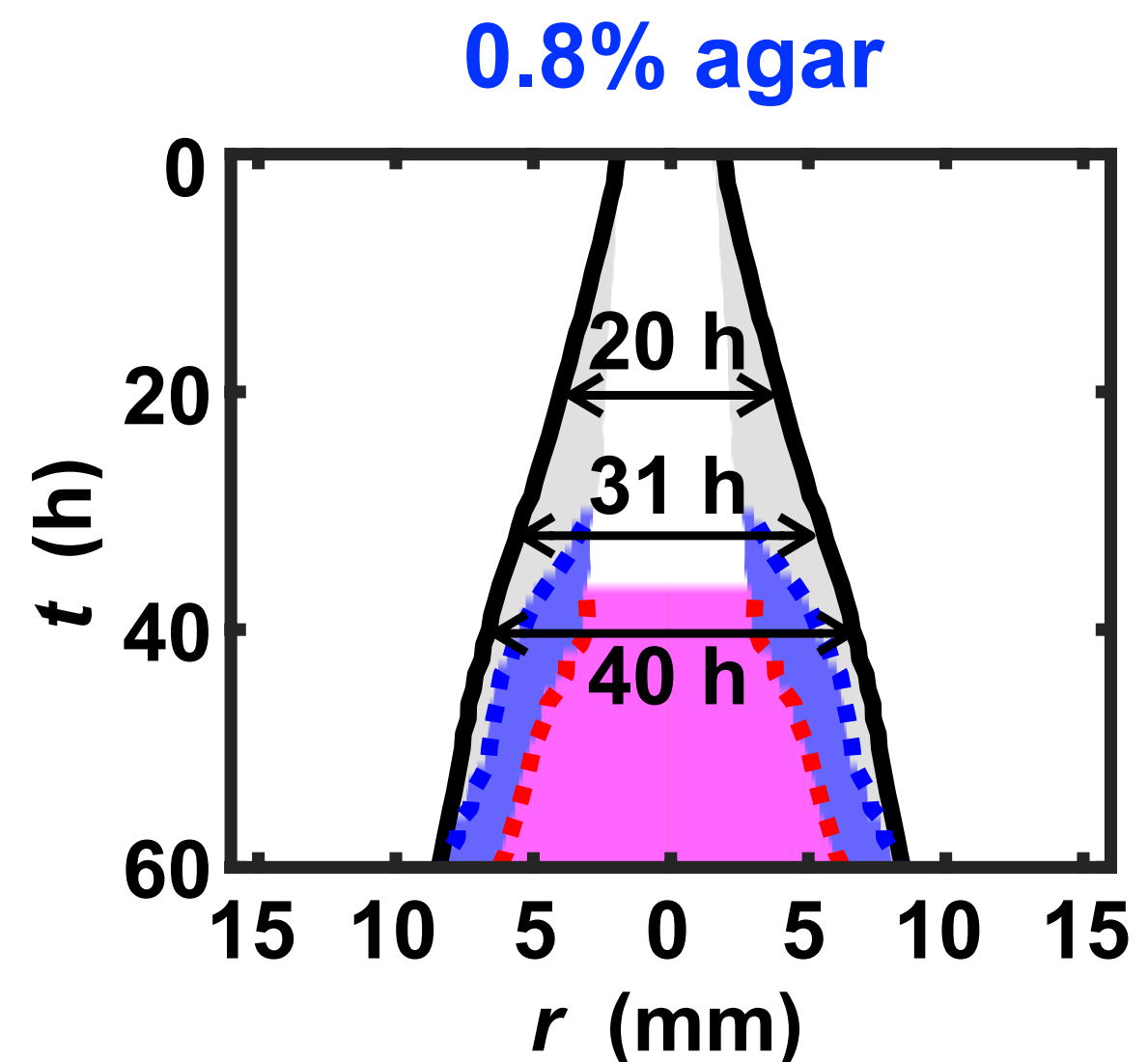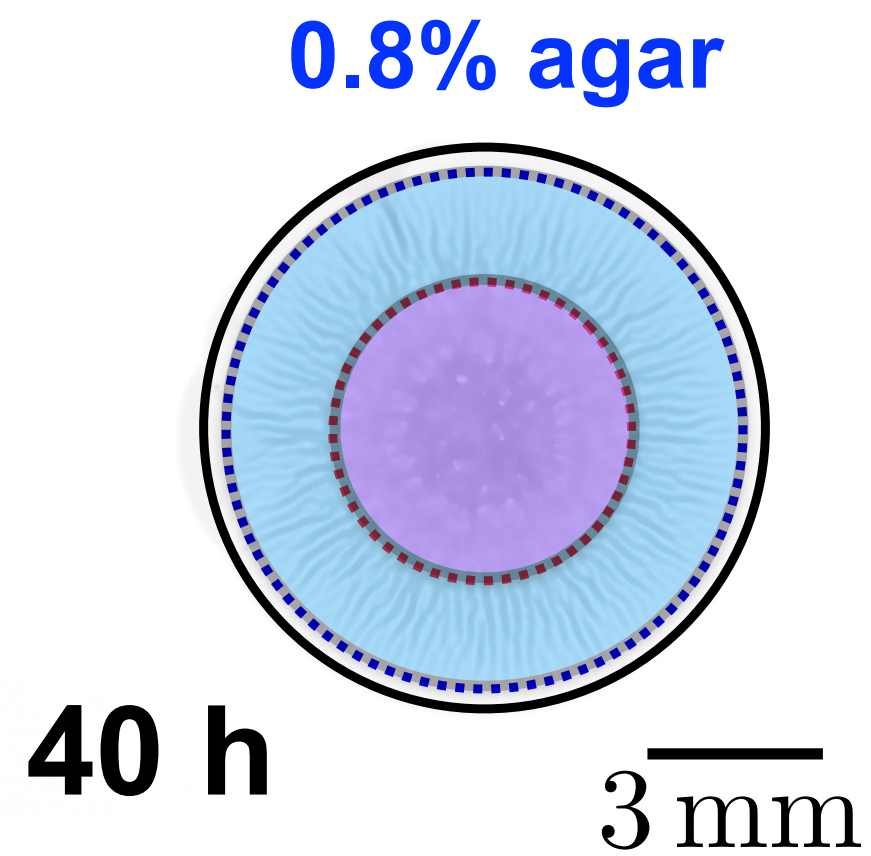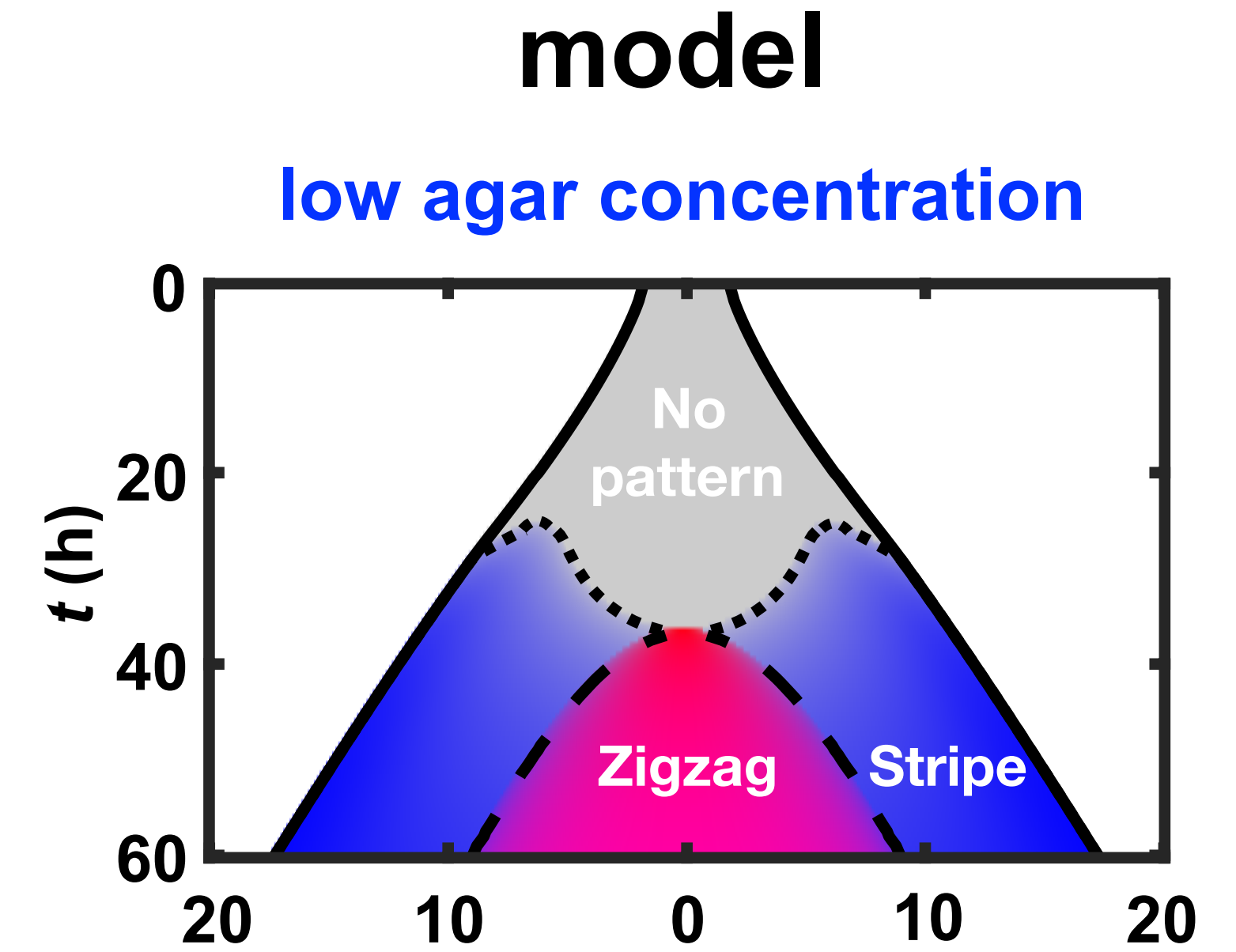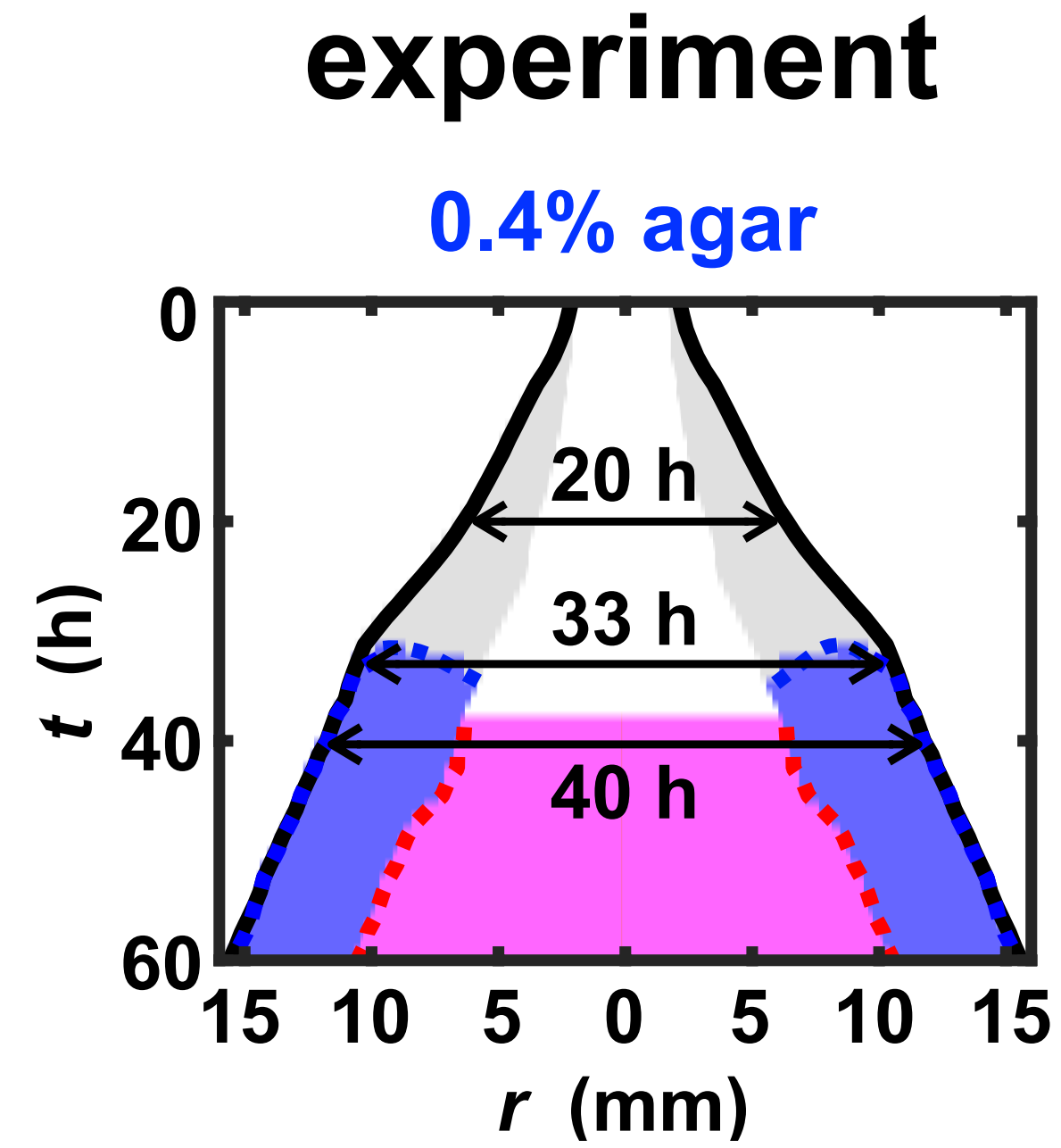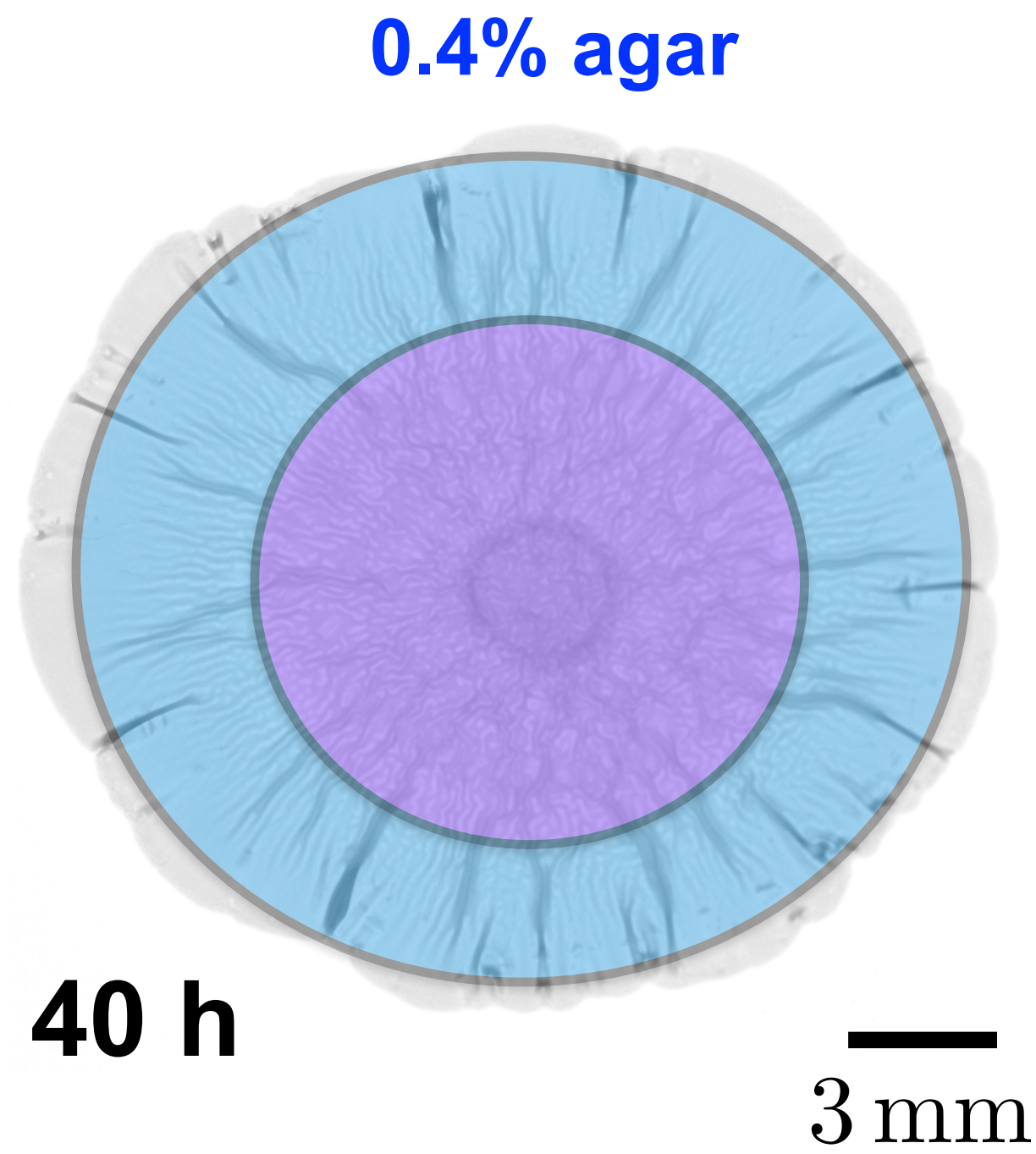
$t$ (h)

$S$

$kA$

$r$ (mm)

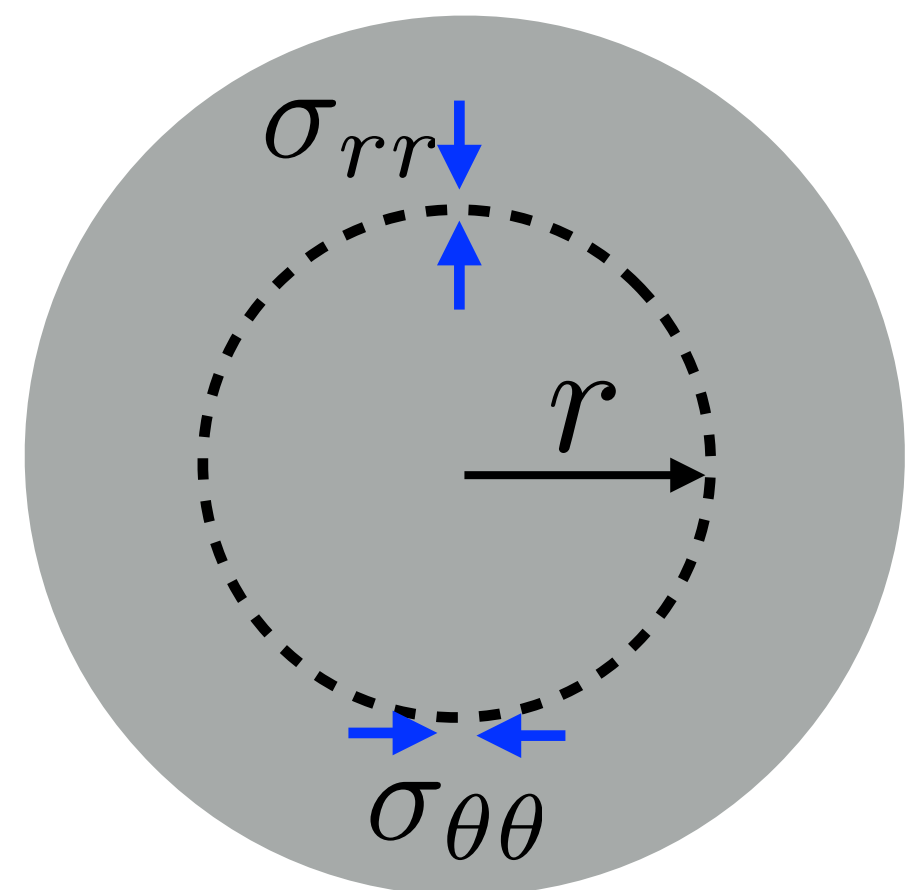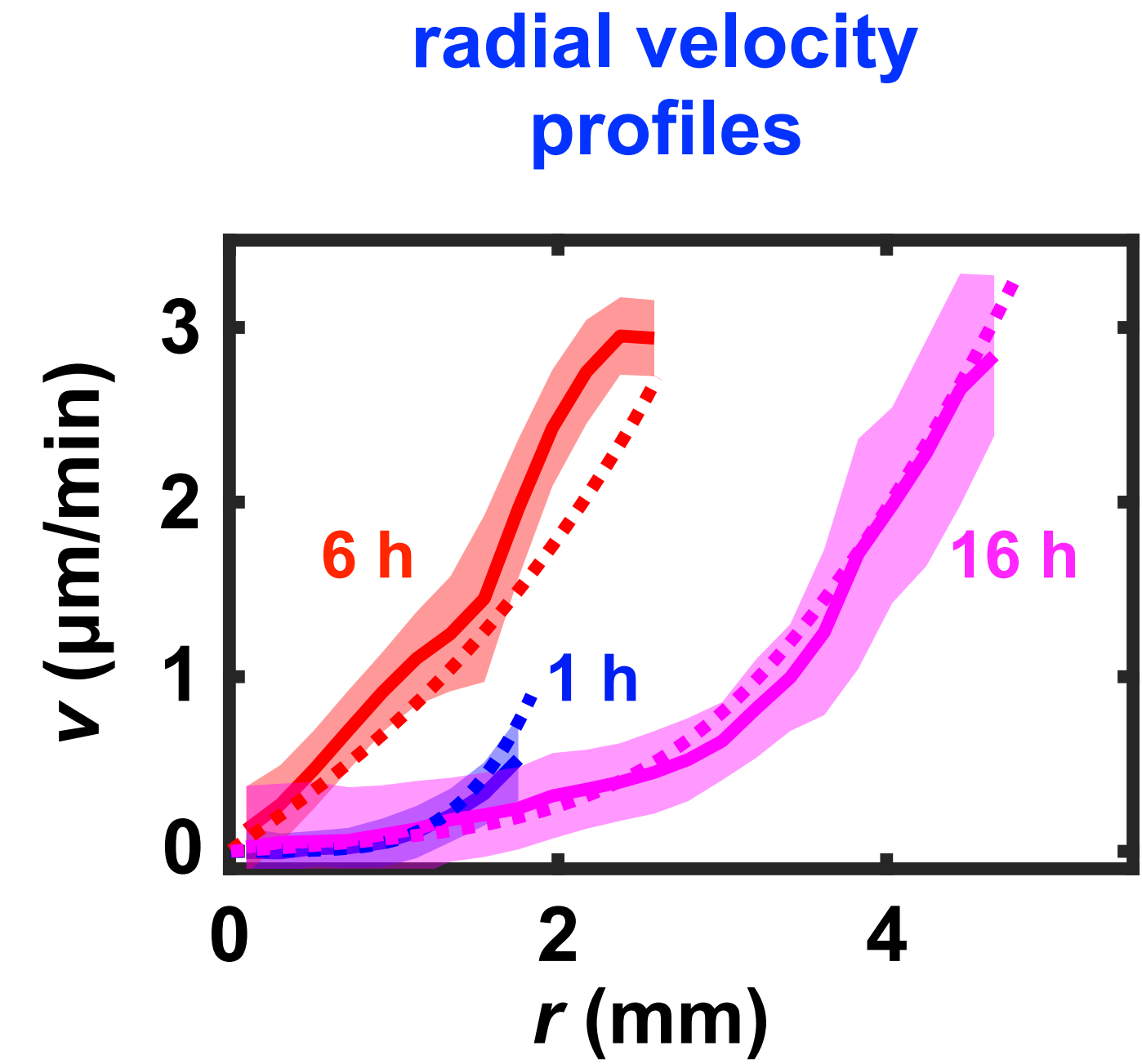# Time evolution of wrinkled patterns

# Outline

**wrinkling instability of growing films**
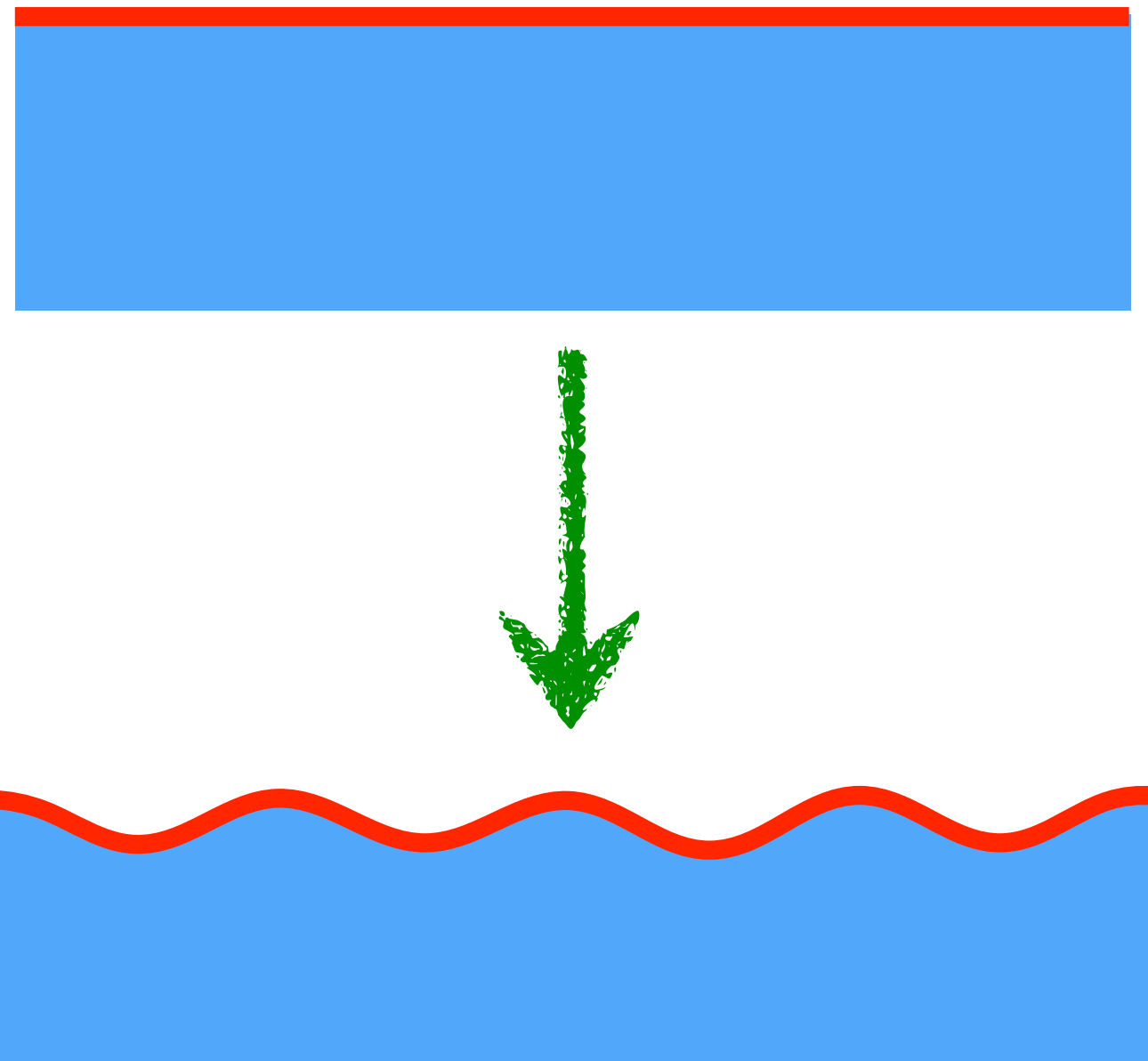
**wrinkling of bacterial biofilm**
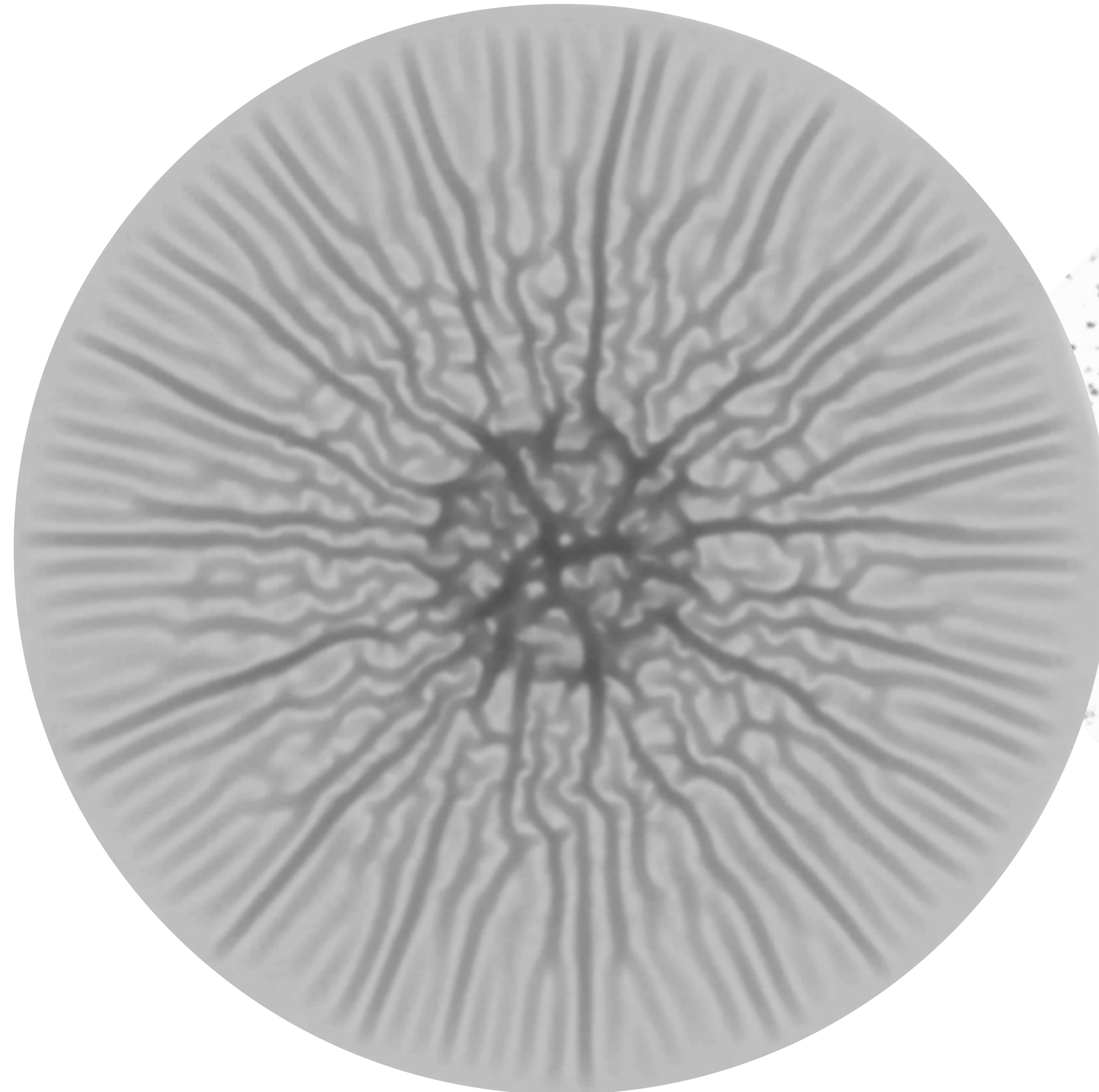
**branching of developing lungs**

# Branching morphogenesis of the mouse lung

**Exx = age of mouse embryo in days**

E11
E12
E13
E14
E15
E16

RCr
RMd
RAc
RCd
L
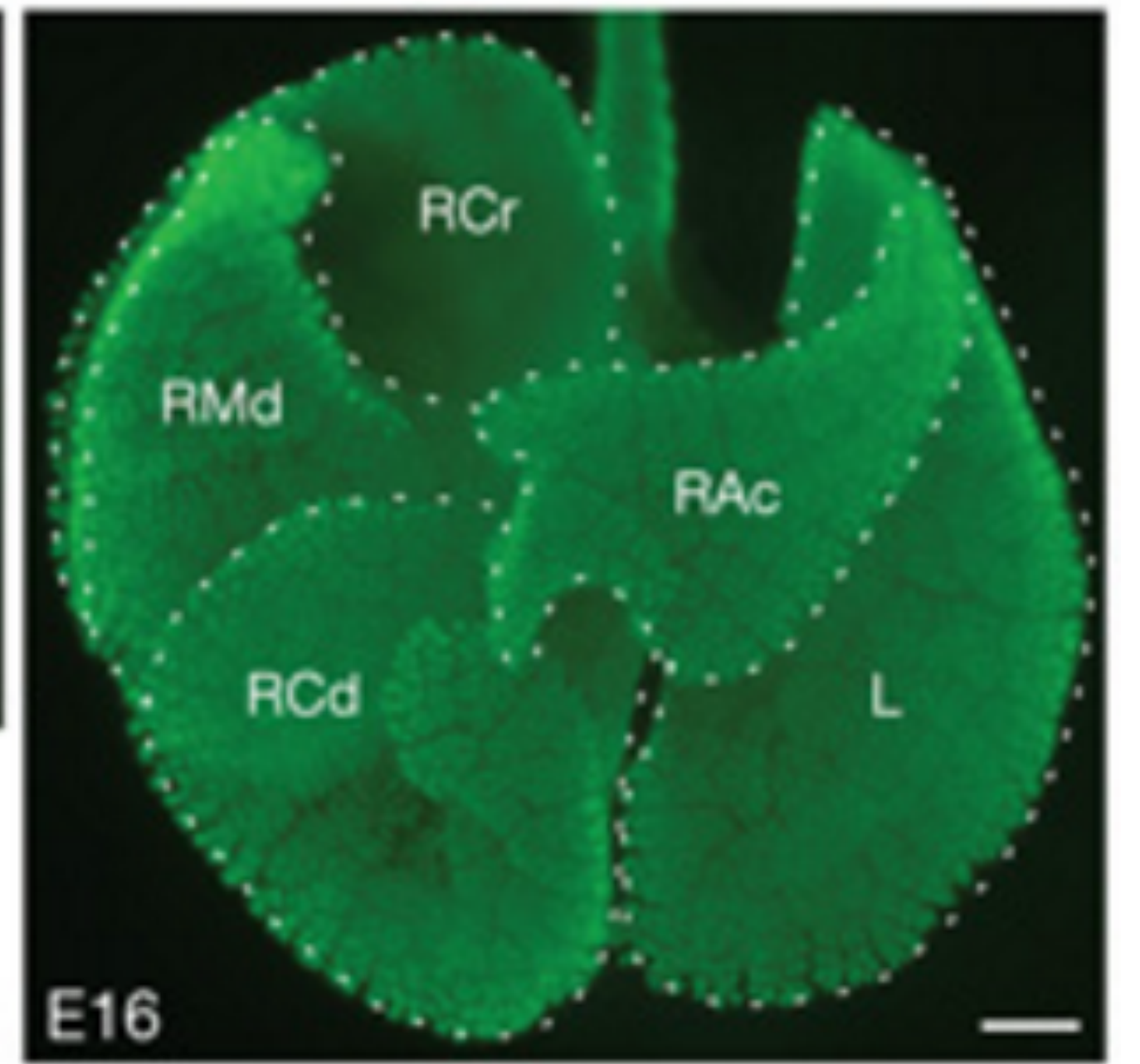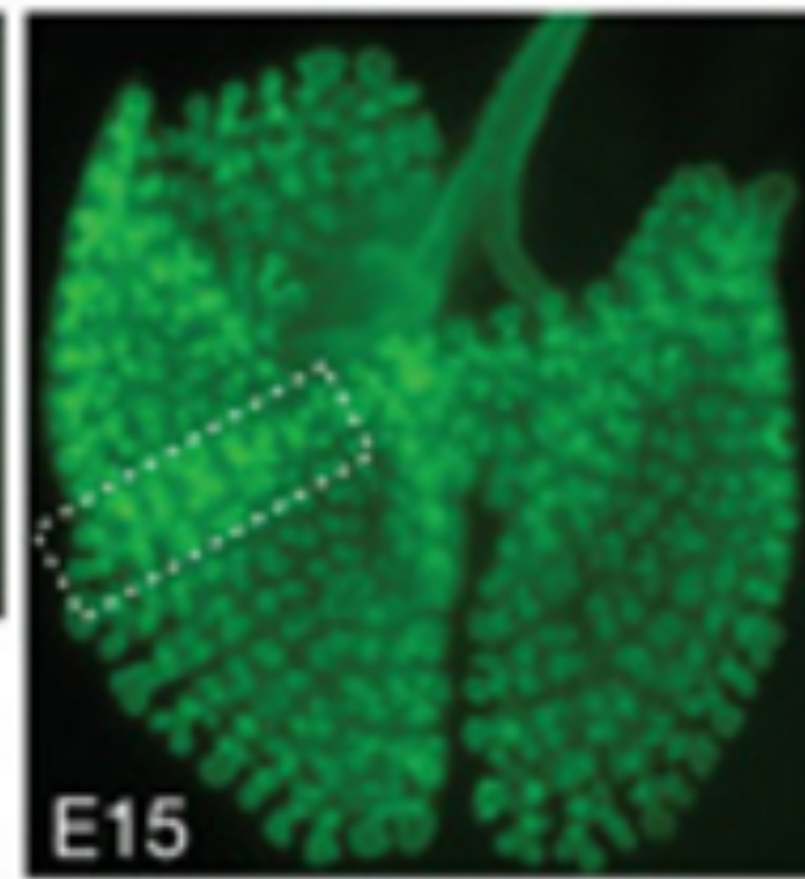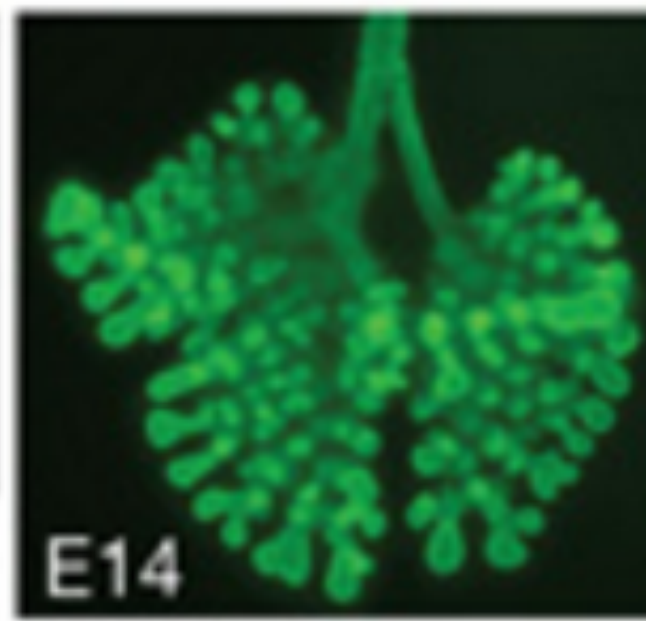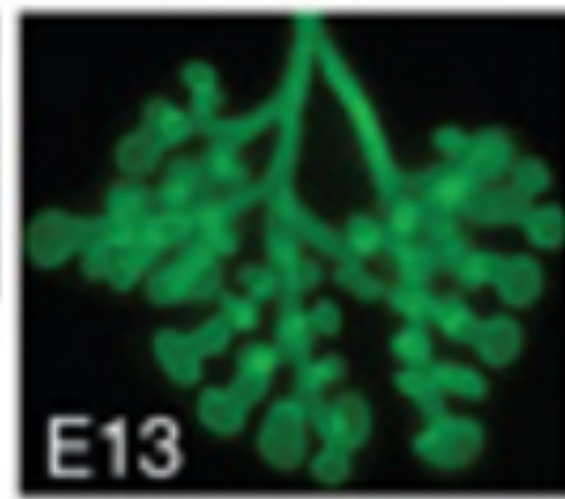
$500\,\mu\mathrm{m}$

**bifurcations**
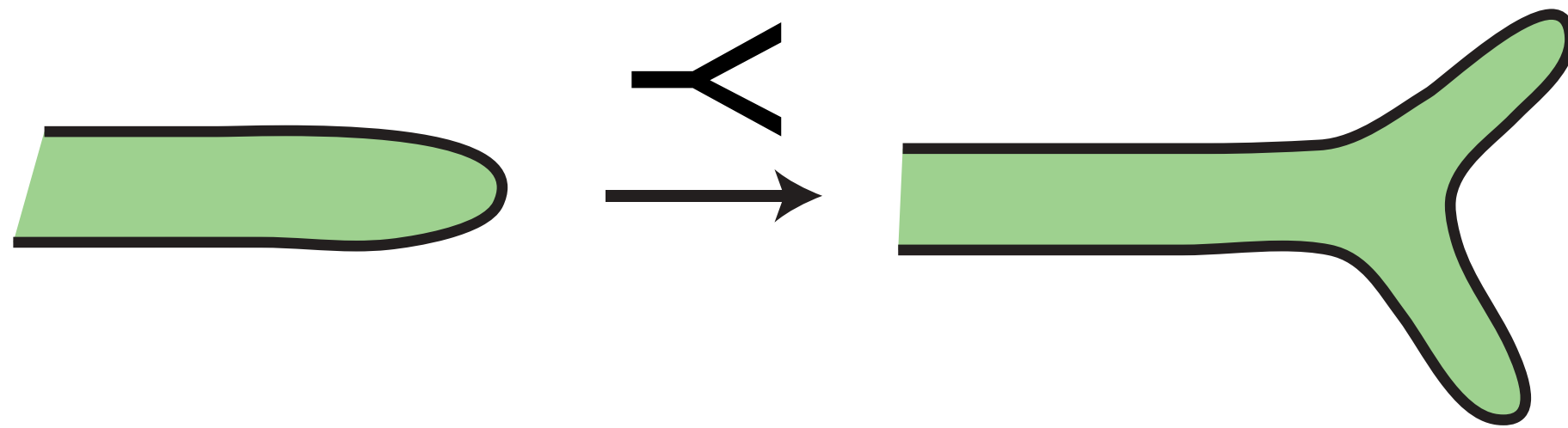


**domain branching**
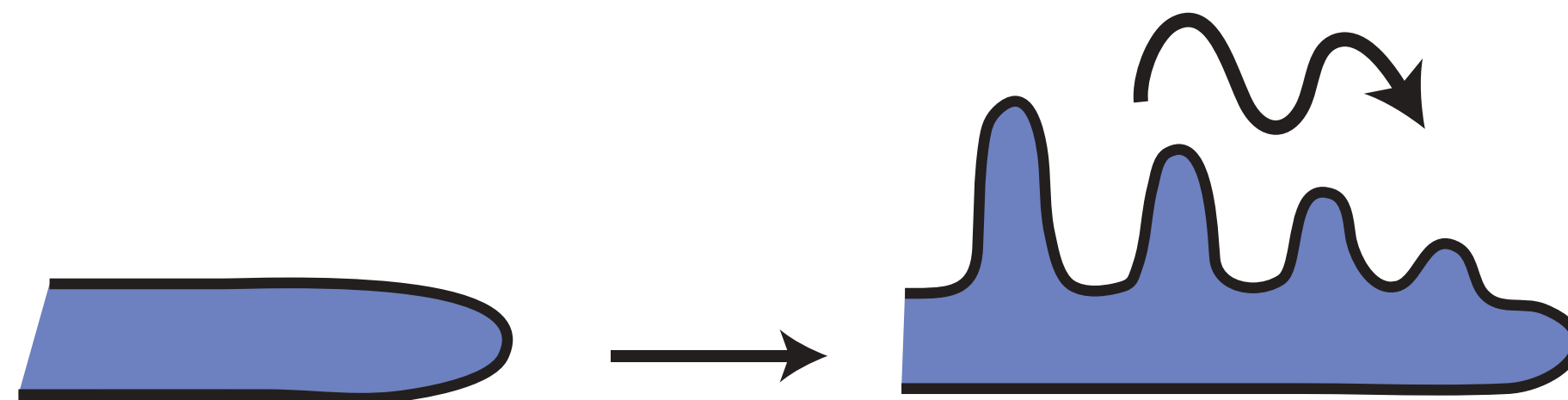


**Formation of new branches is highly reproducible in both space and time.**

J. Metzger *et al.*, Nature **453**, 745 (2008)

# Branching morphogenesis of the mouse lung



E11

E12

E13

E14

E15

E16

RCr

RMd

RAc

RCd

L
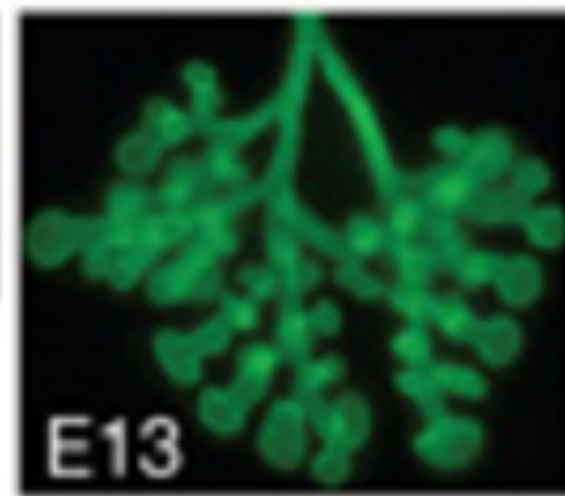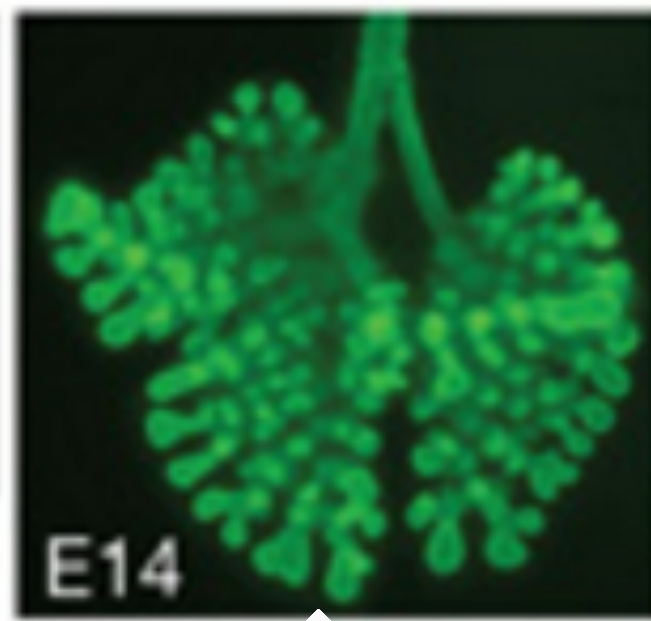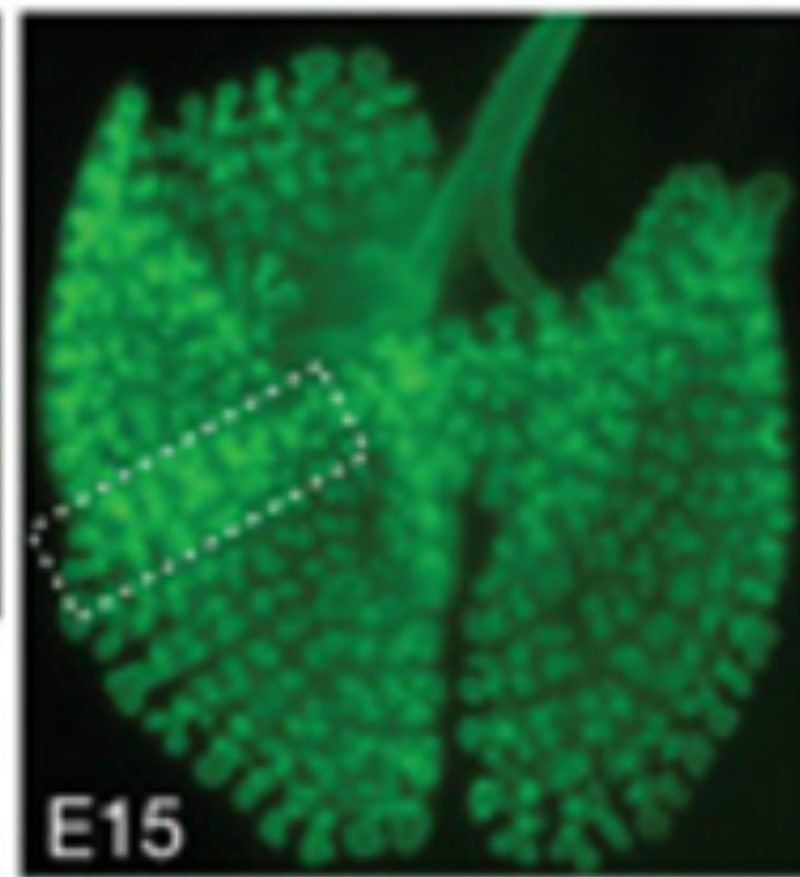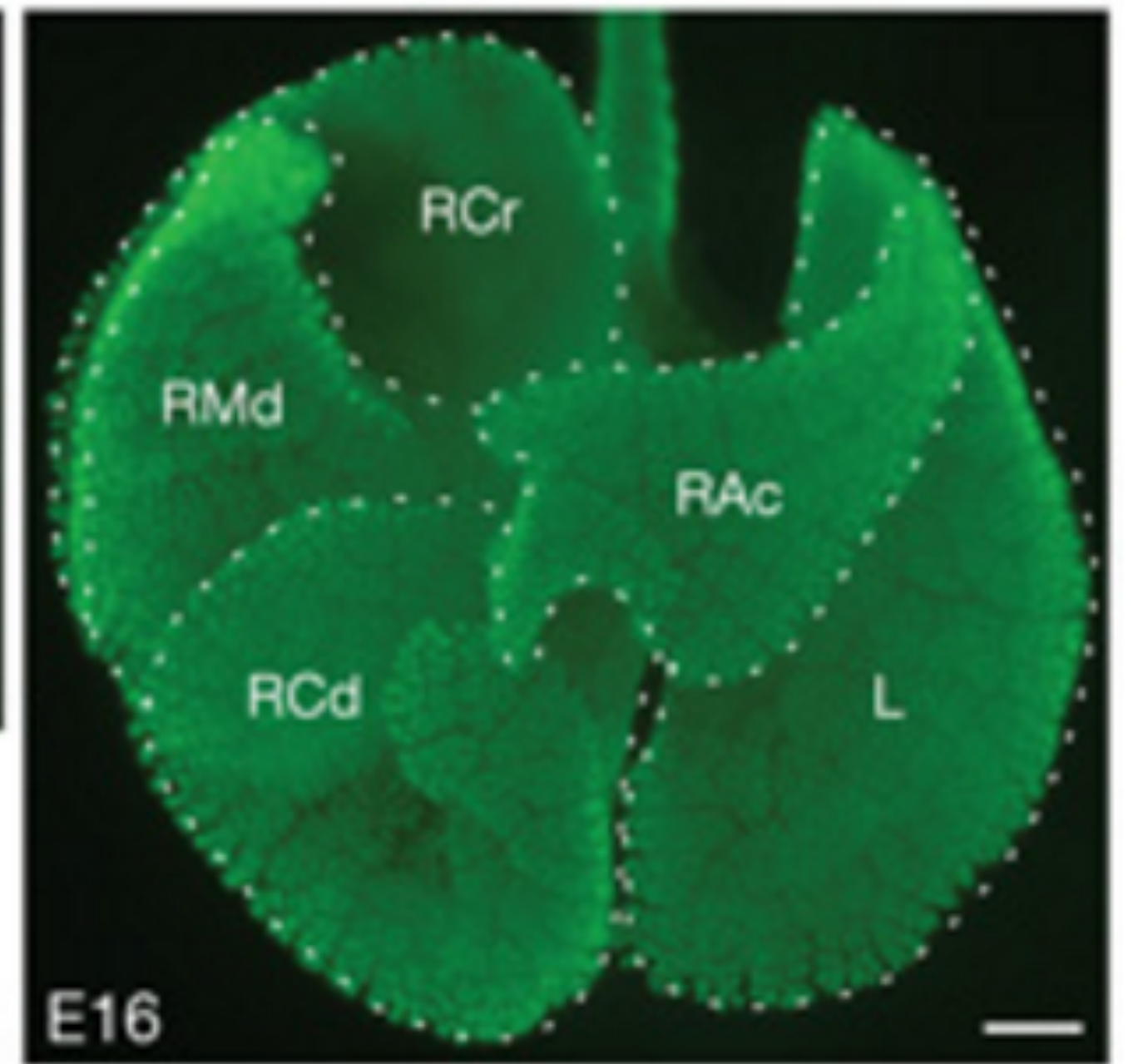
$500\,\mu\mathrm{m}$

**Exx = age of mouse embryo in days**

**Surgically removed lungs from mouse embryo at E12 and cultured for 24 hours**

**(K. Goodwin)**

$200\,\mu\mathrm{m}$

J. Metzger *et al.*, <u>Nature</u> **453**, 745 (2008)

# Branching morphogenesis of the mouse lung

**Exx = age of mouse embryo in days**

**Surgically removed lungs from mouse embryo at E12 and cultured for 24 hours**

**(K. Goodwin)**

$500\,\mu$m
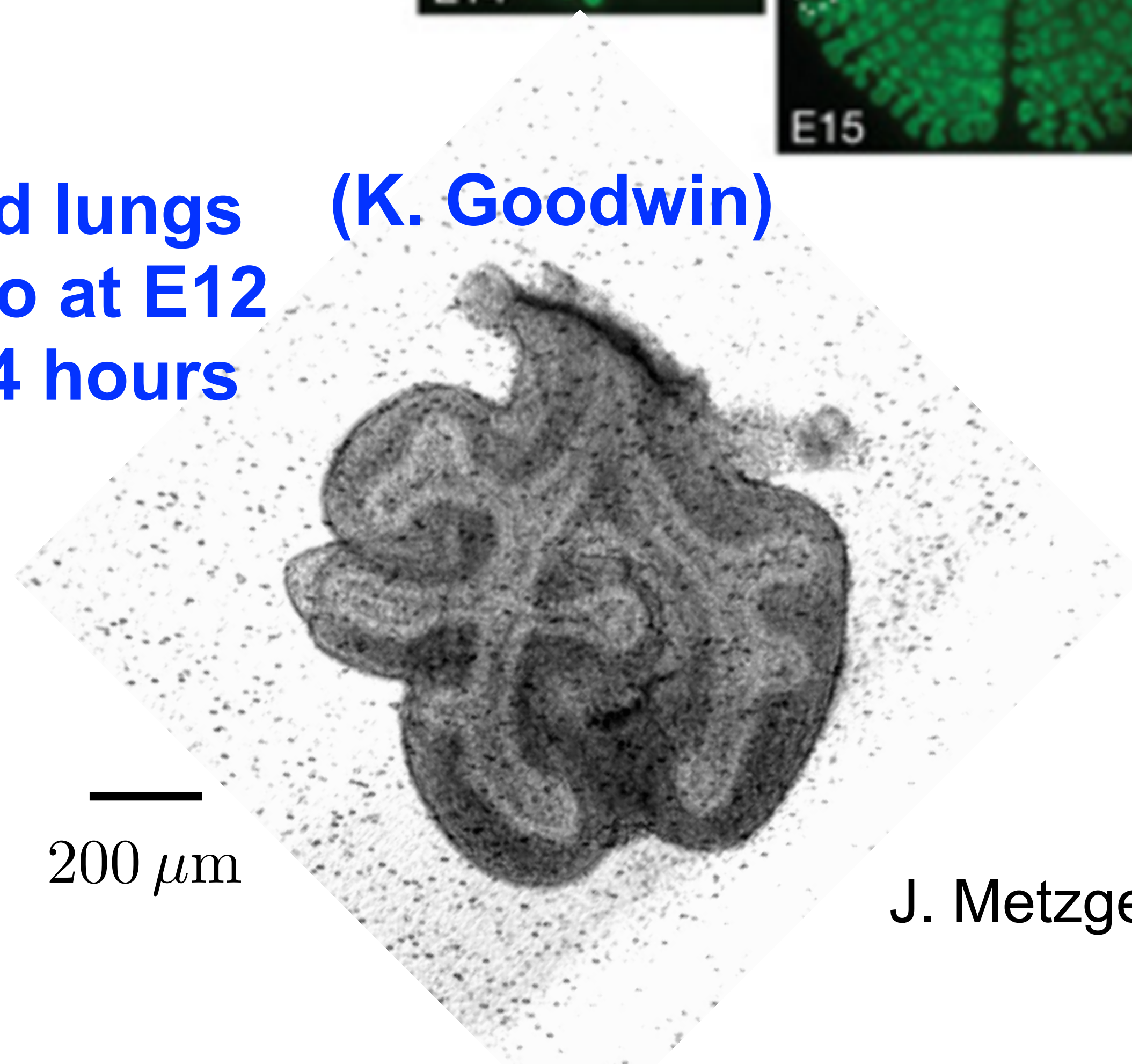
$200\,\mu$m

J. Metzger *et al.*, <u>Nature</u> **453**, 745 (2008)

# Mesenchyme directs branching of the epithelium

**Smooth muscles differentiate from the surrounding mesenchyme.**

**mesenchyme**

$$E \lesssim 1\,\text{kPa}$$

**Structure at E12.5 (K. Goodwin)**

**smooth muscles**

$$E \approx 5\text{-}20\,\text{kPa}$$

**epithelium**

$$E \approx 1\text{-}5\,\text{kPa}$$

$100\,\mu\text{m}$

sketch adapted from M. E. Kumar *et al.*, Science **346**, 1258810 (2014)
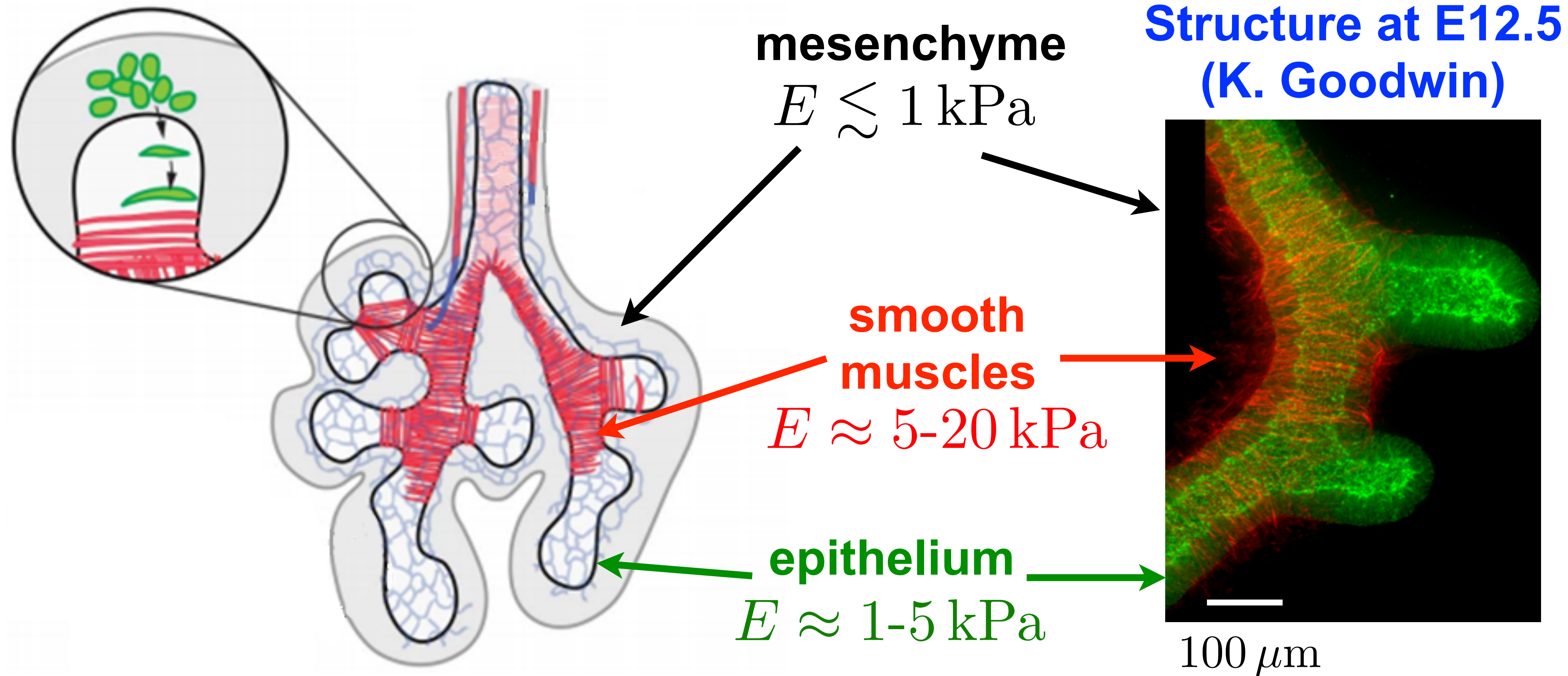
# Smooth muscle differentiation is required for airway bifurcation
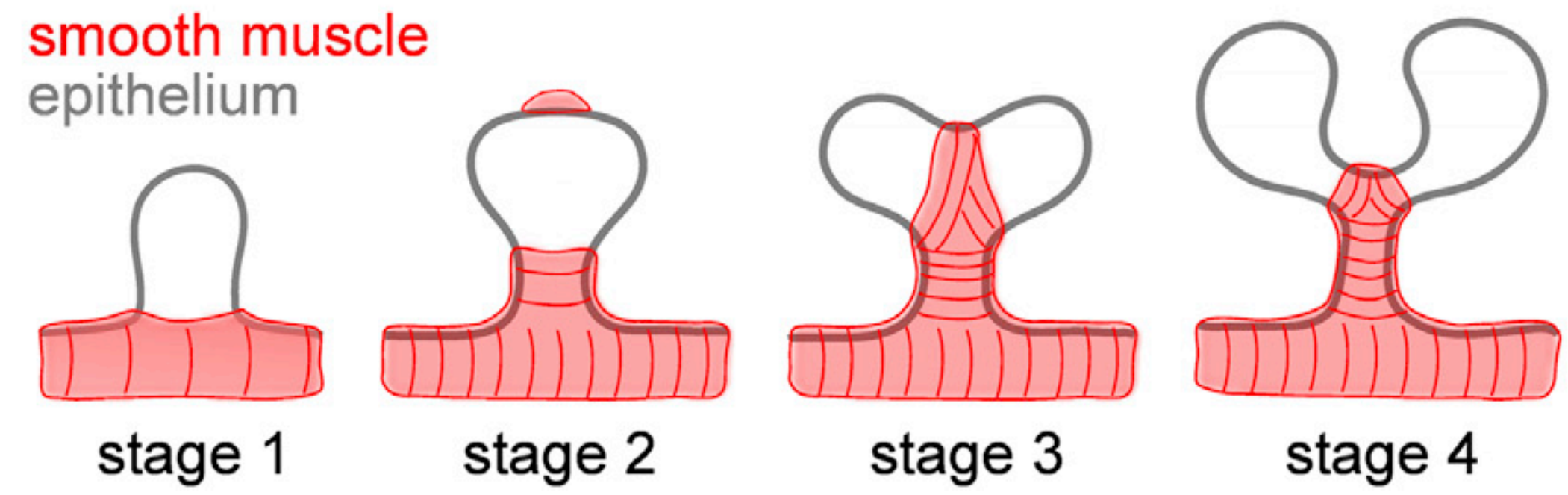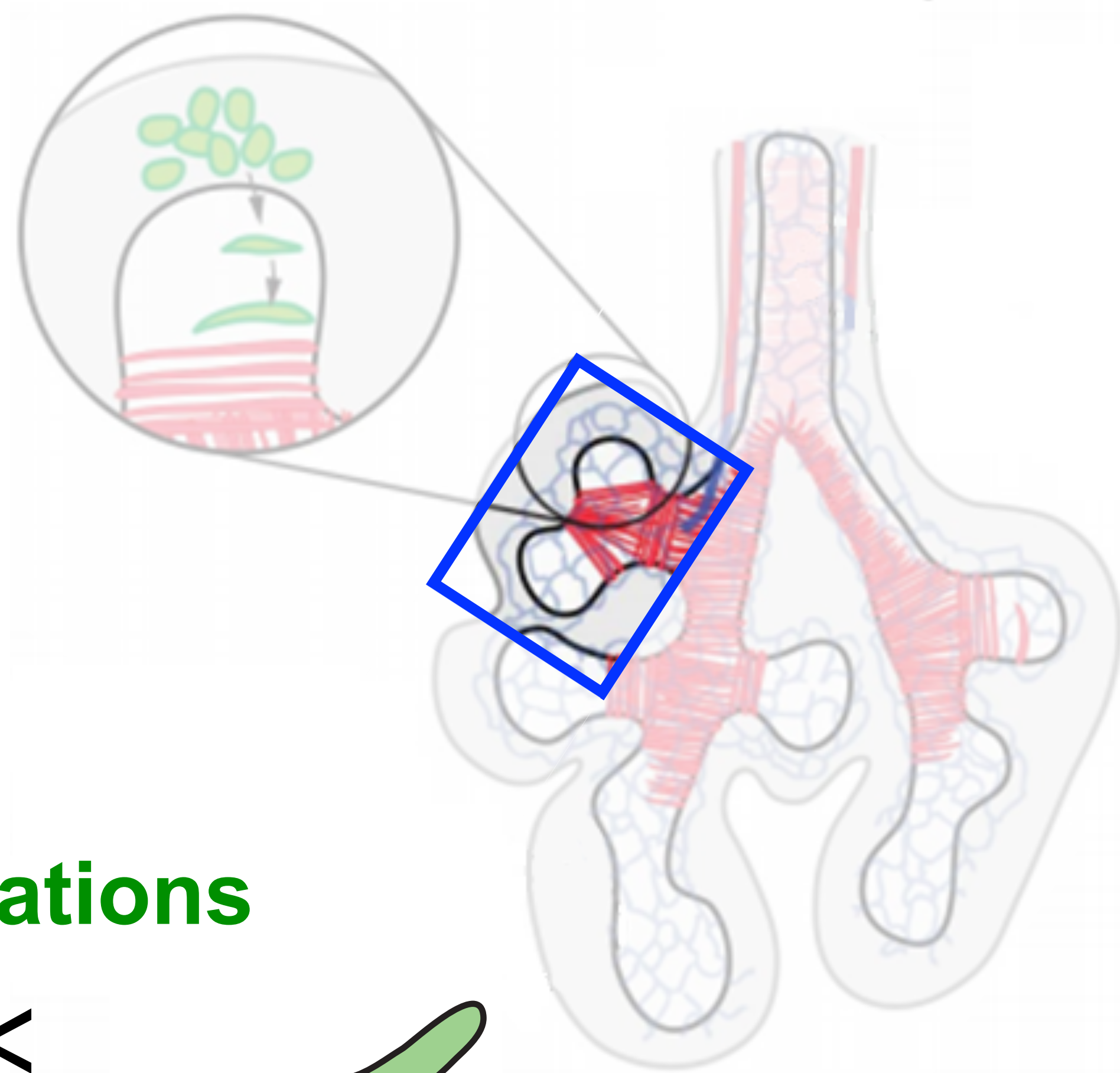


smooth muscle
epithelium

stage 1  stage 2  stage 3  stage 4

**bifurcations**

**normal conditions**

**blocked differentiation of smooth muscles**

$100\mu m$

H.Y. Kim, …, C.M. Nelson, Dev. Cell **34**, 719 (2015)

# Formation of new domain branch

**Surgically removed lungs
from mouse embryo at E11.5
and cultured for 24 hours**
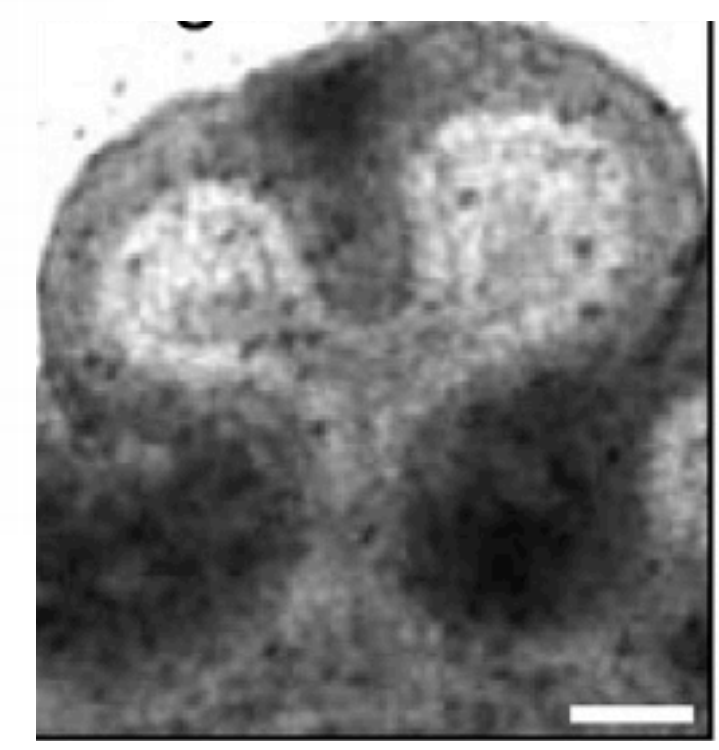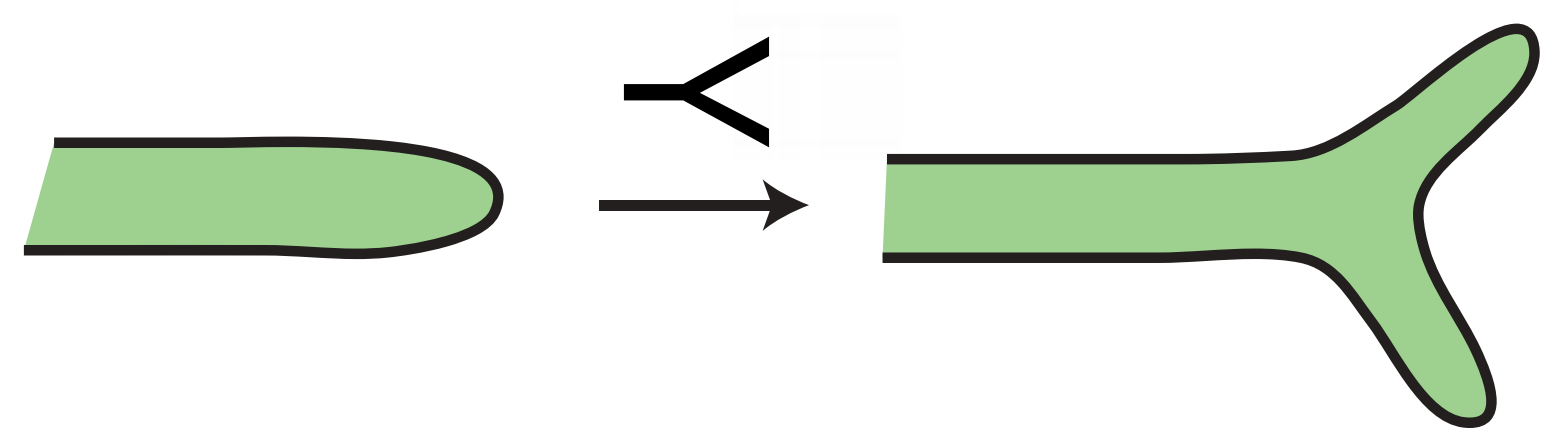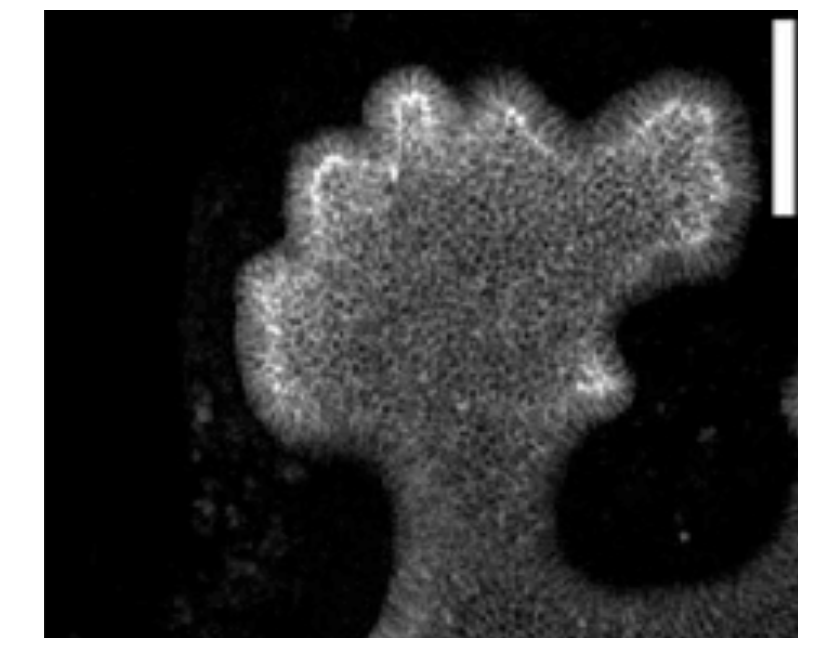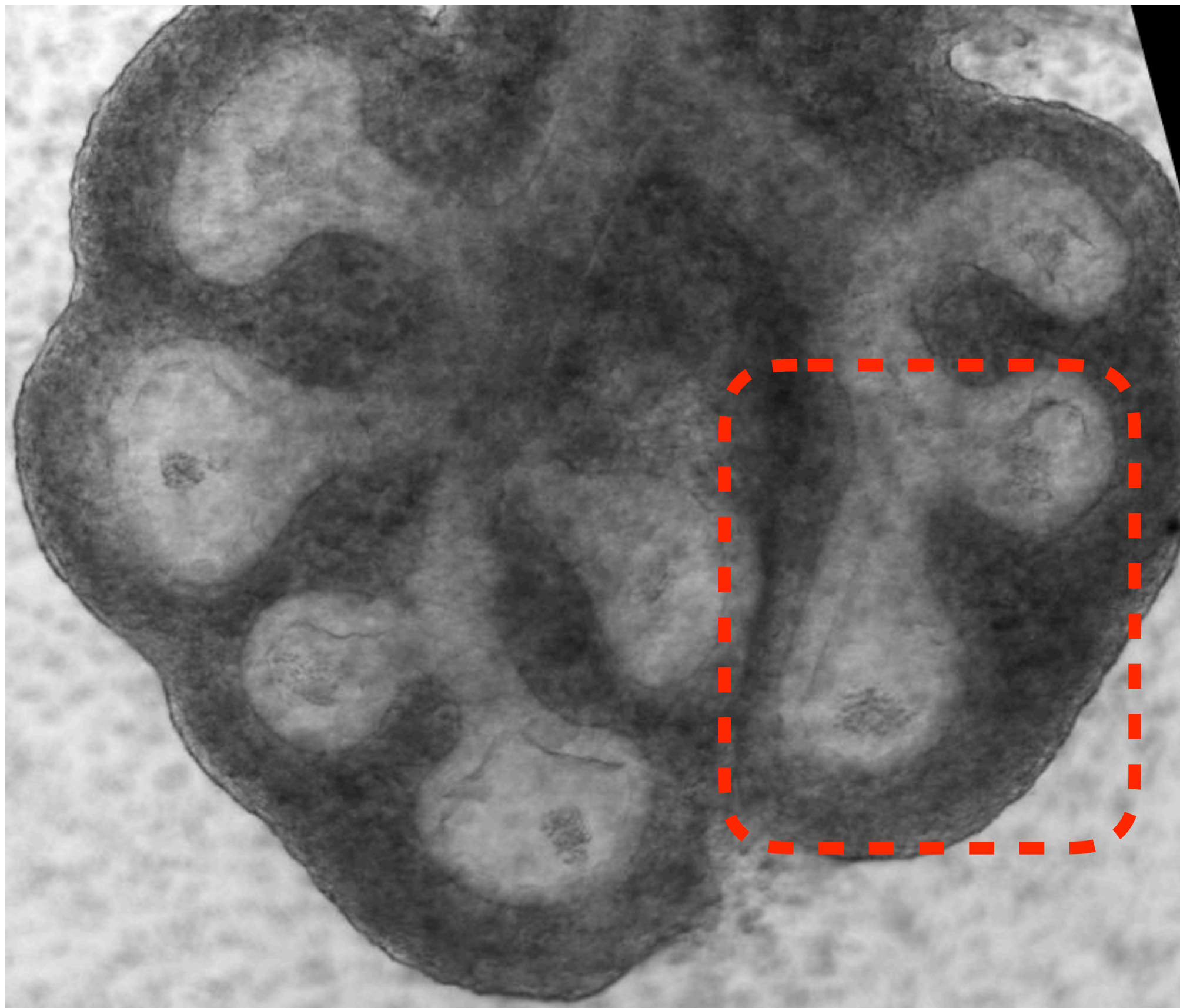


100 $\mu$m

**Surgically removed lungs from mouse embryo at E11.5 and cultured for 24 hours**



$100 \, \mu\text{m}$

# Formation of new domain branch

**Surgically removed lungs from mouse embryo at E11.5 and cultured for 24 hours**
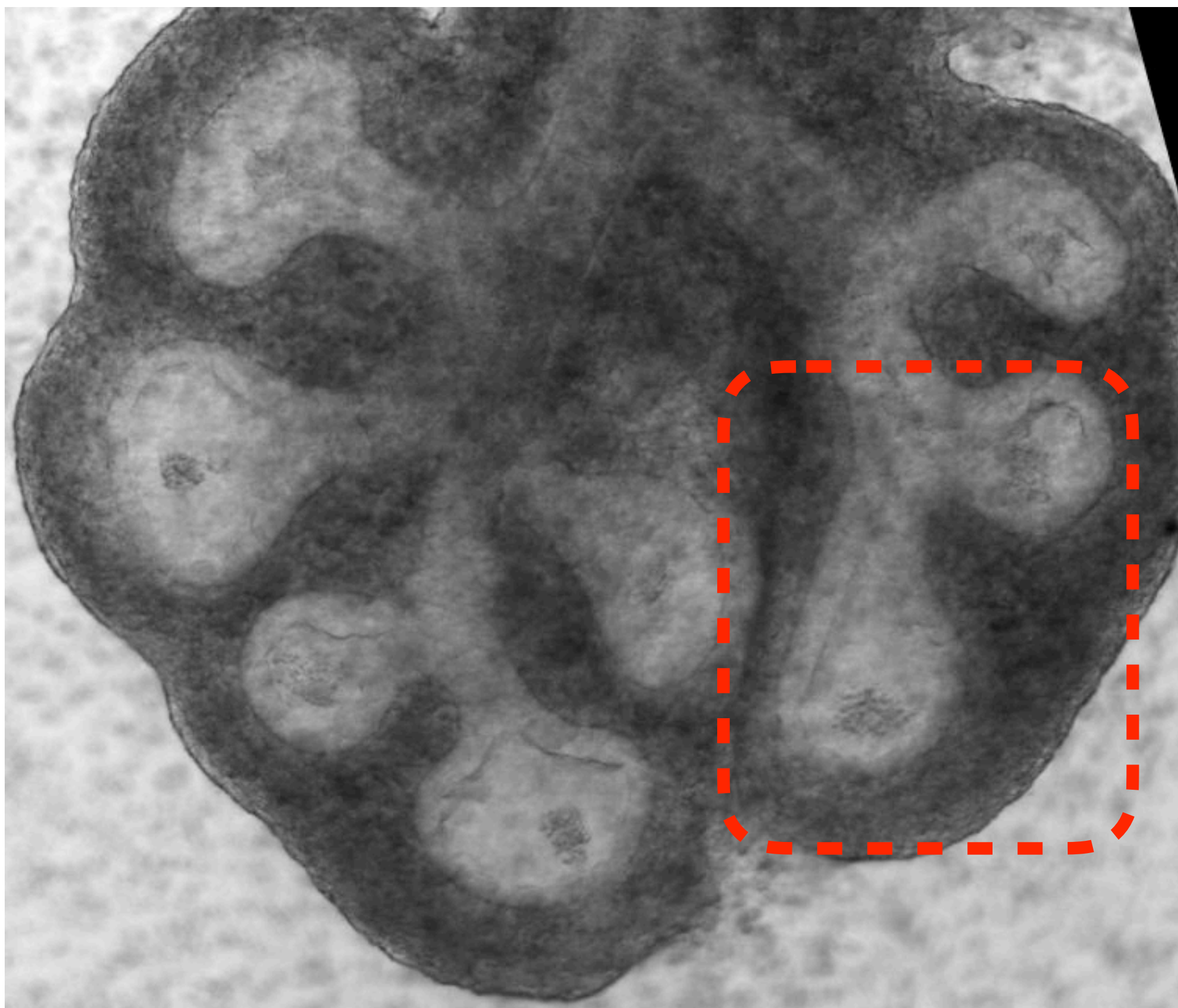
**What physical mechanisms drive the formation of new domain branches?**
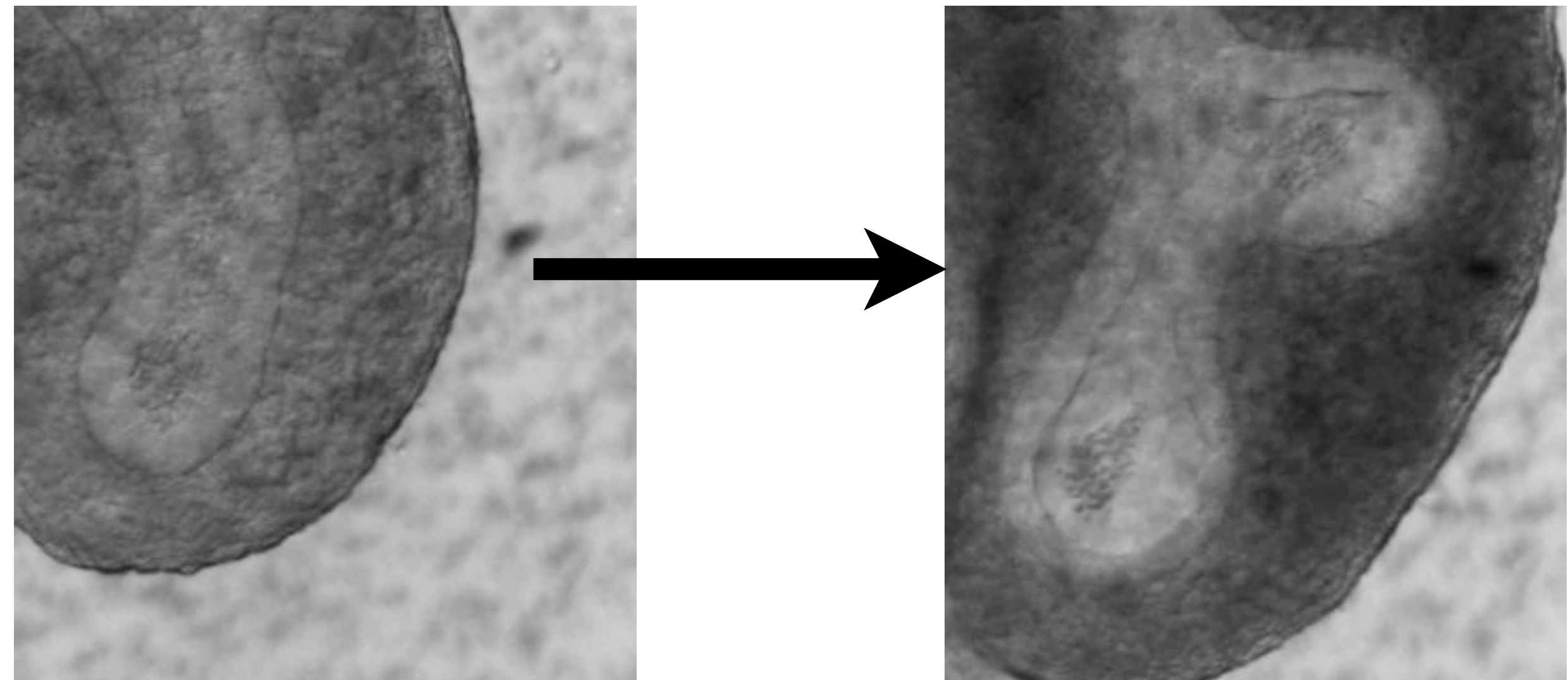


$100\,\mu\mathrm{m}$

# Smooth muscle differentiation is required for proper domain branching
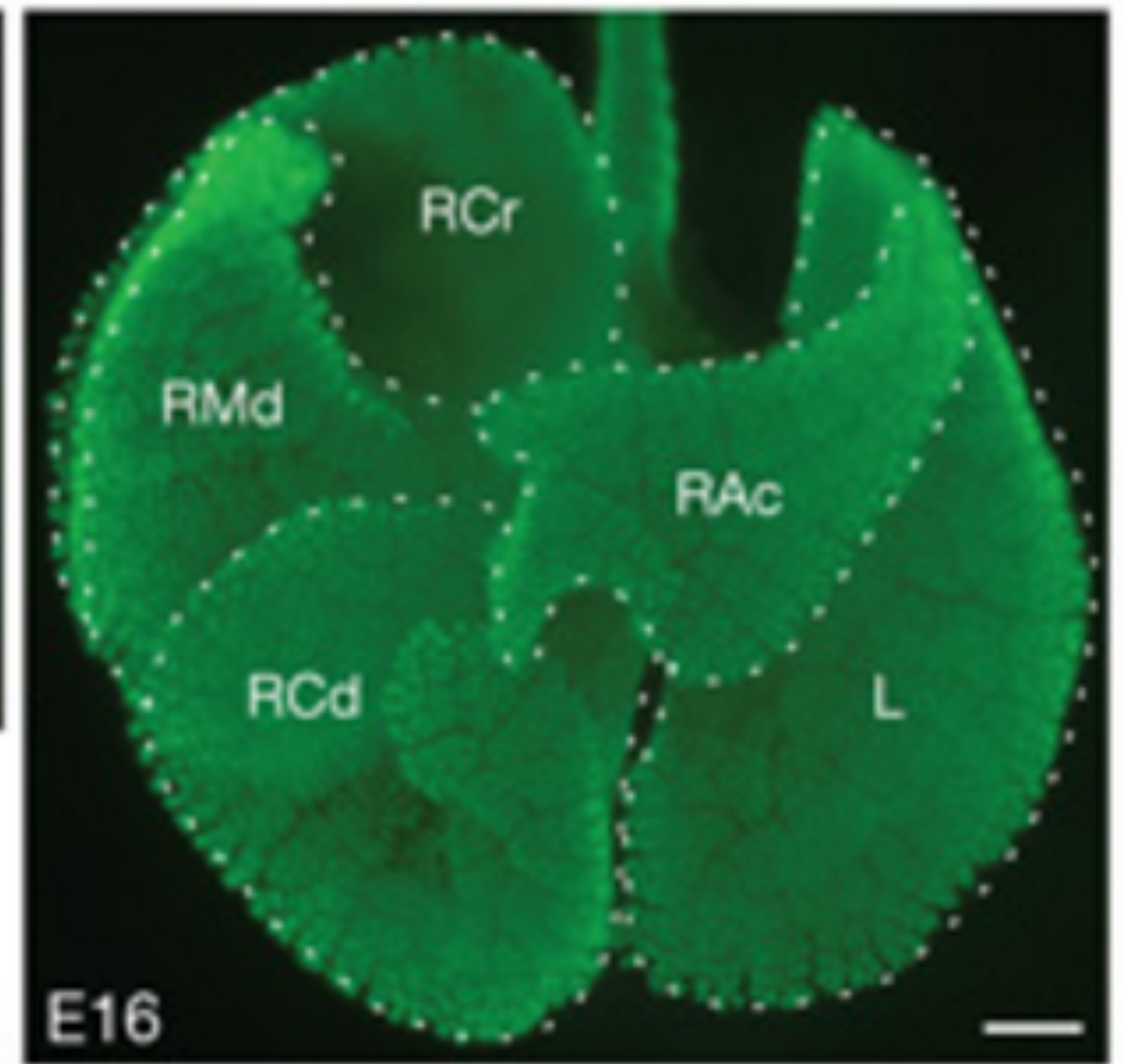


E11.5     E12     E12.5
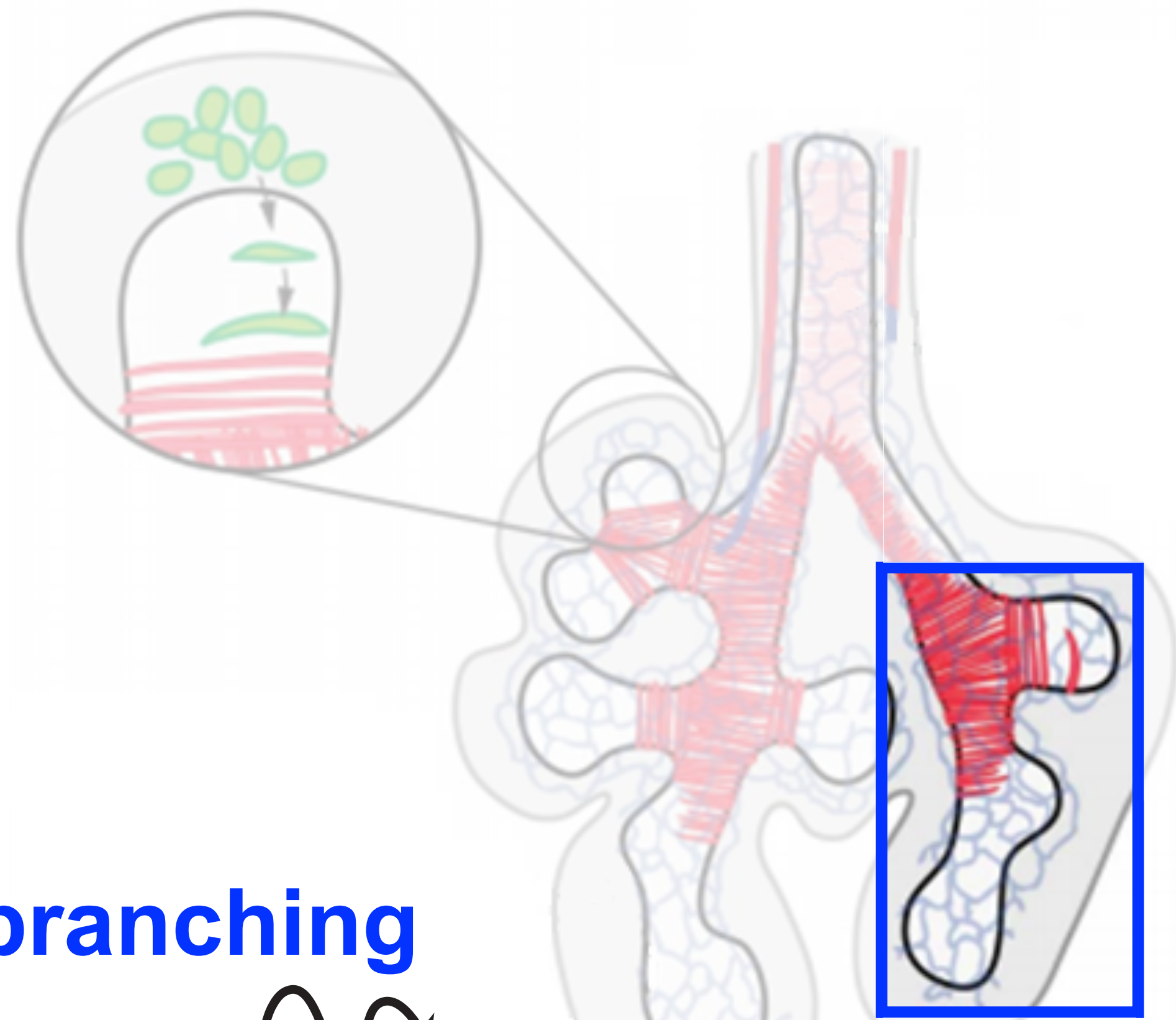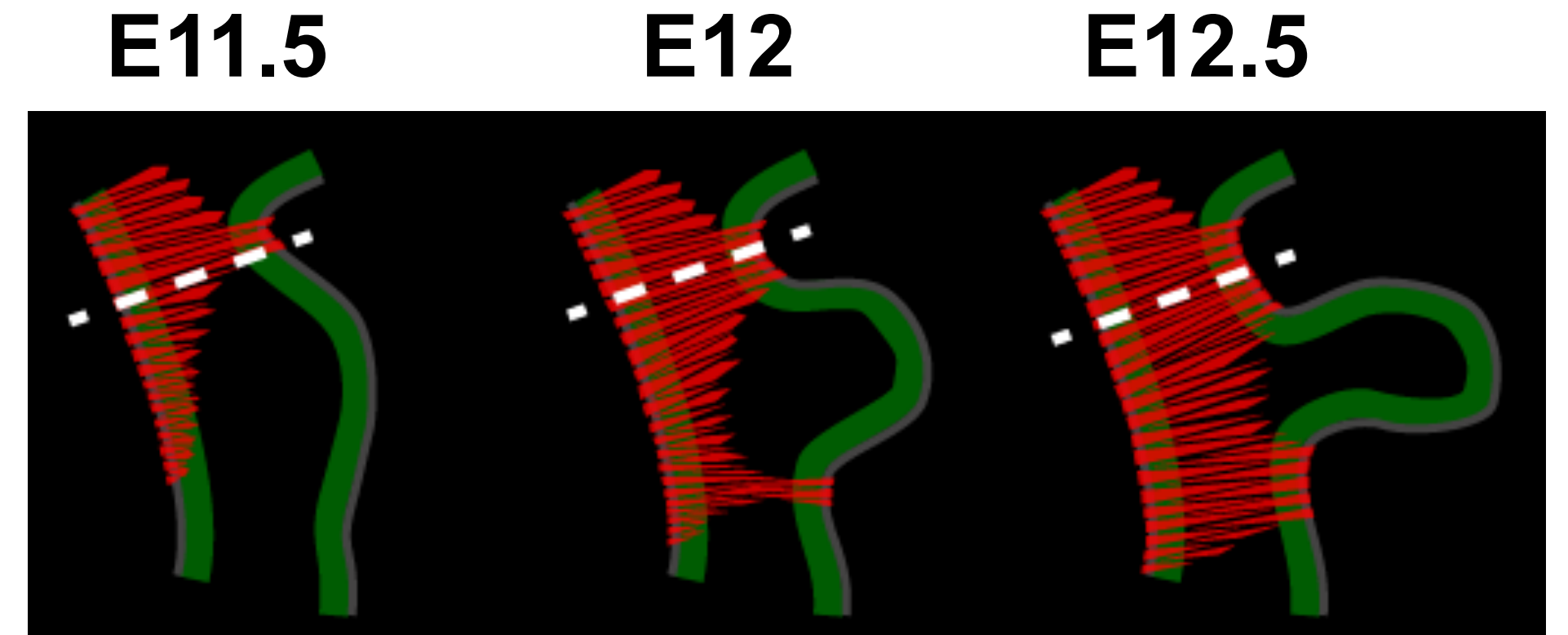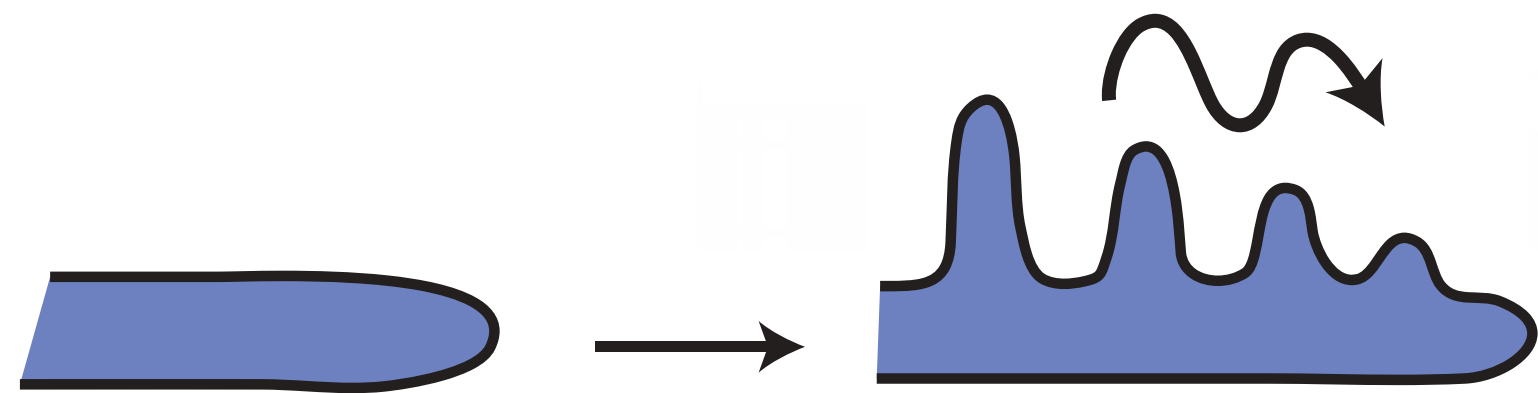
**normal conditions**

**blocked differentiation of smooth muscles (nifedipine, cyclopamine)**

**domain branching**

$100\mu m$
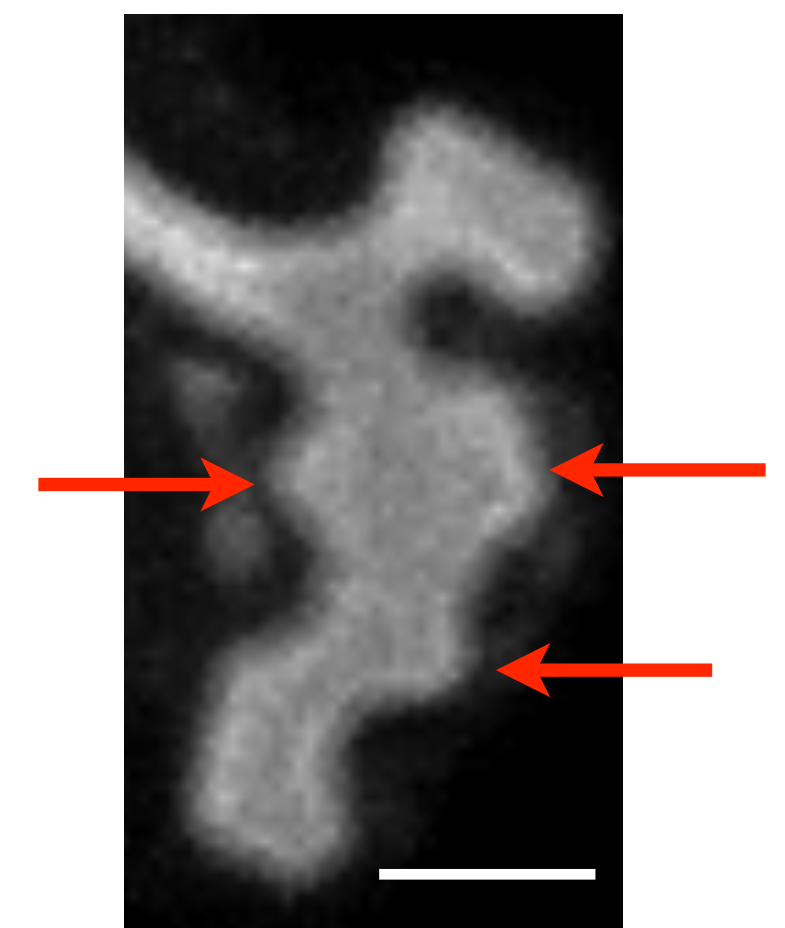
# Growth pattern of epithelium

**Lungs at E12**
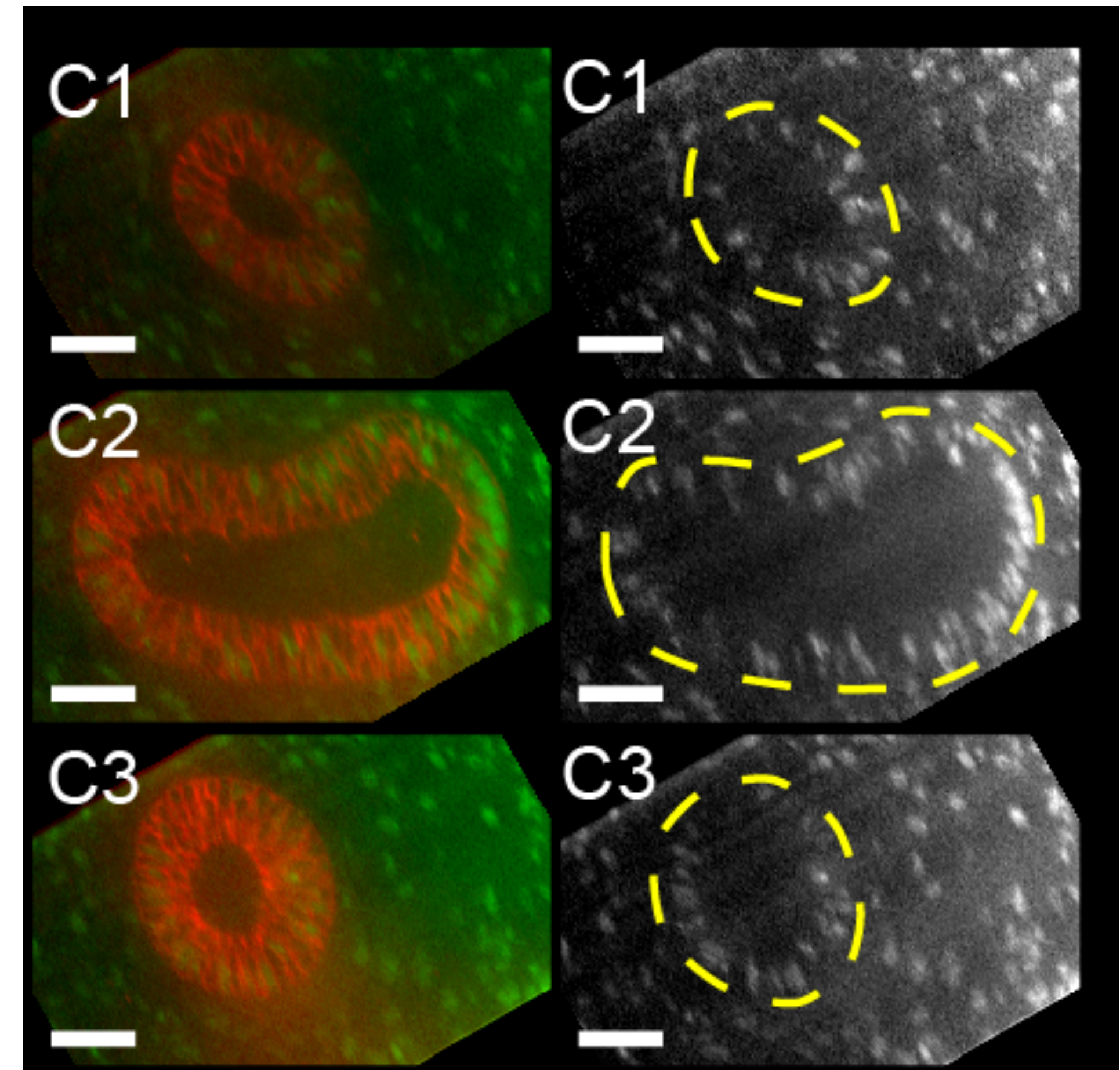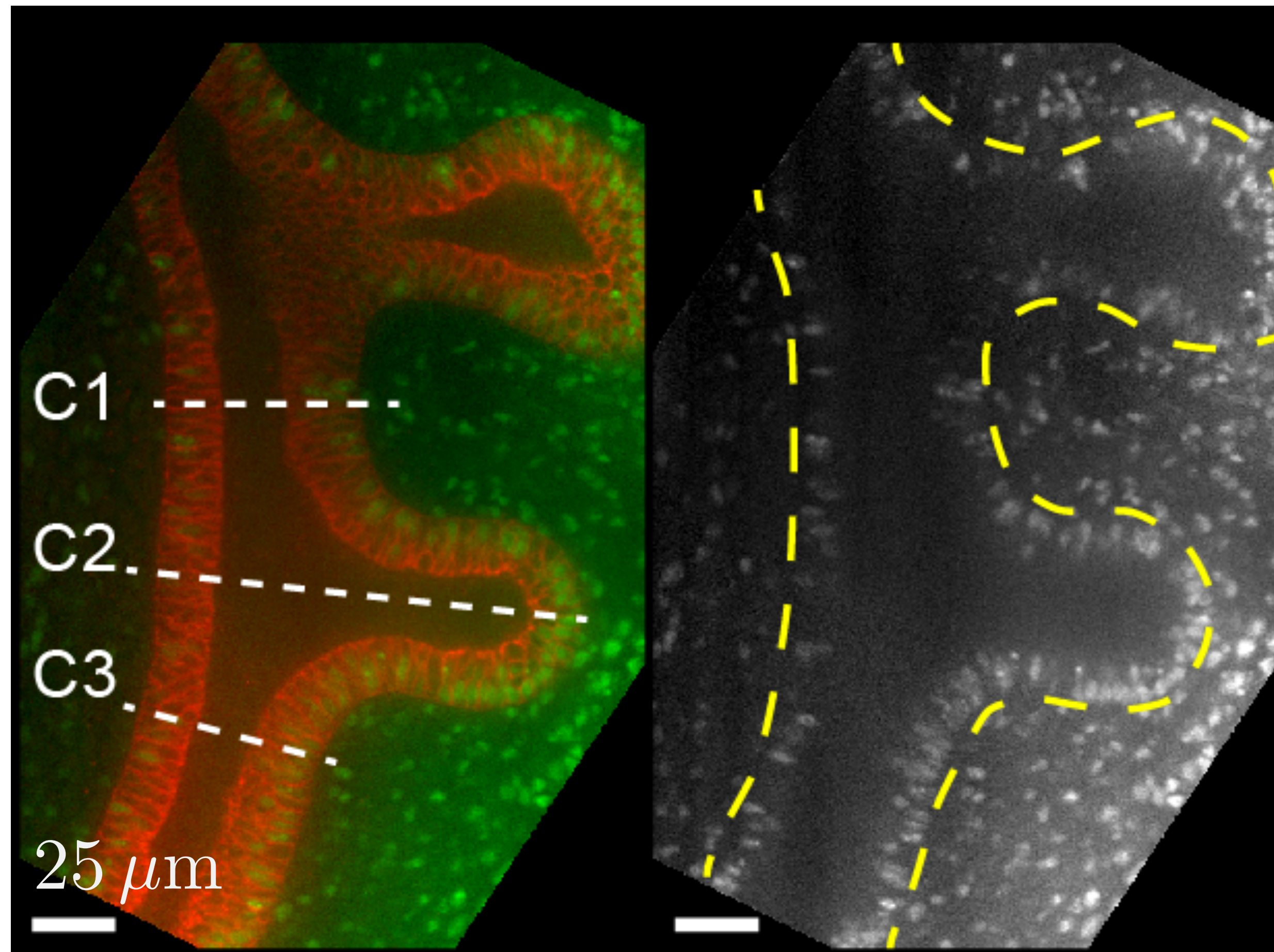
**cross-sections**



Ecad (epithelium)   EdU (proliferating cells)
EdU (proliferating cells)

25 μm

25 μm

# Growth pattern of epithelium



**Lungs at E12**

**cross-sections**

**onset of new branch formation**
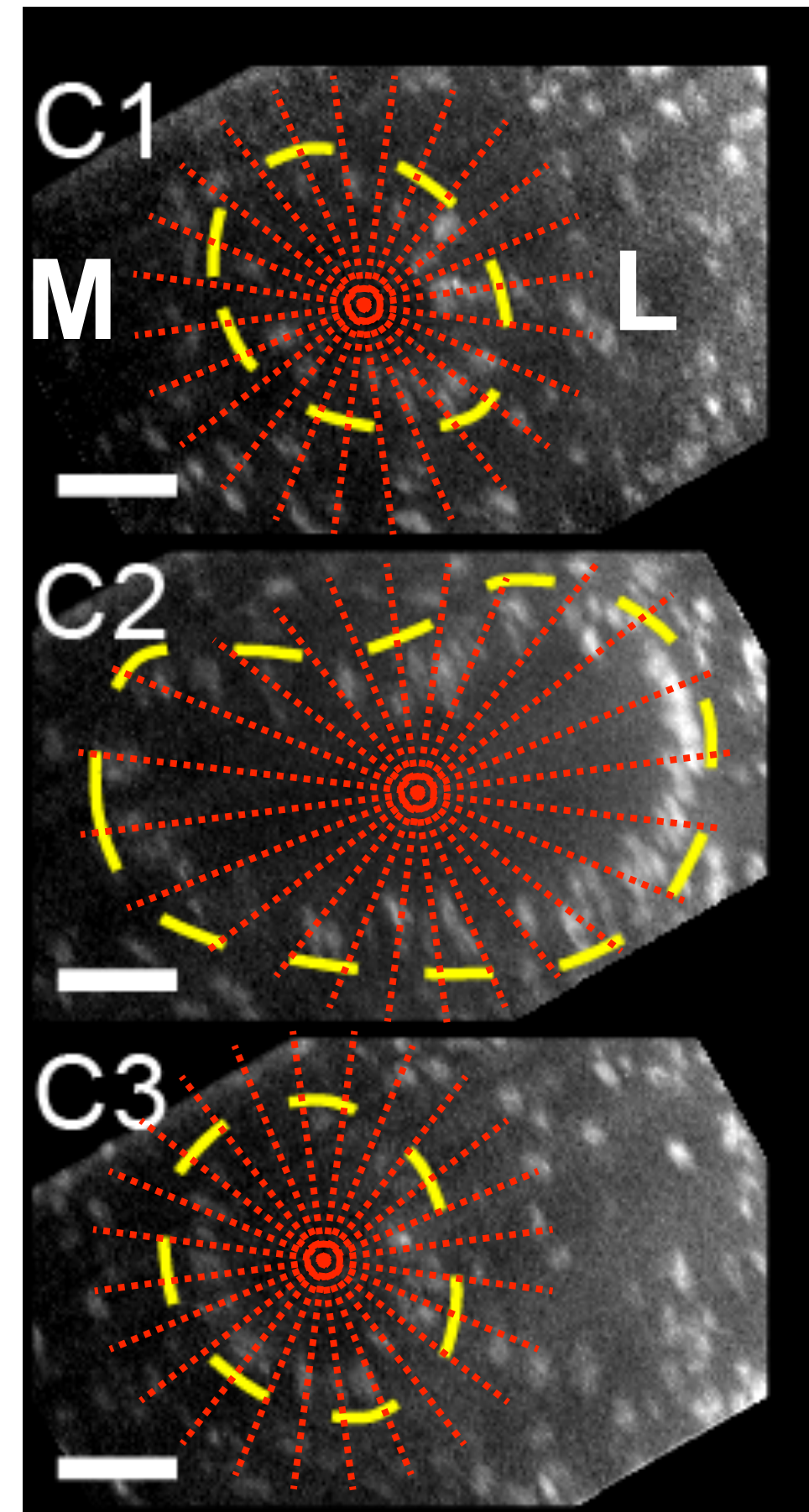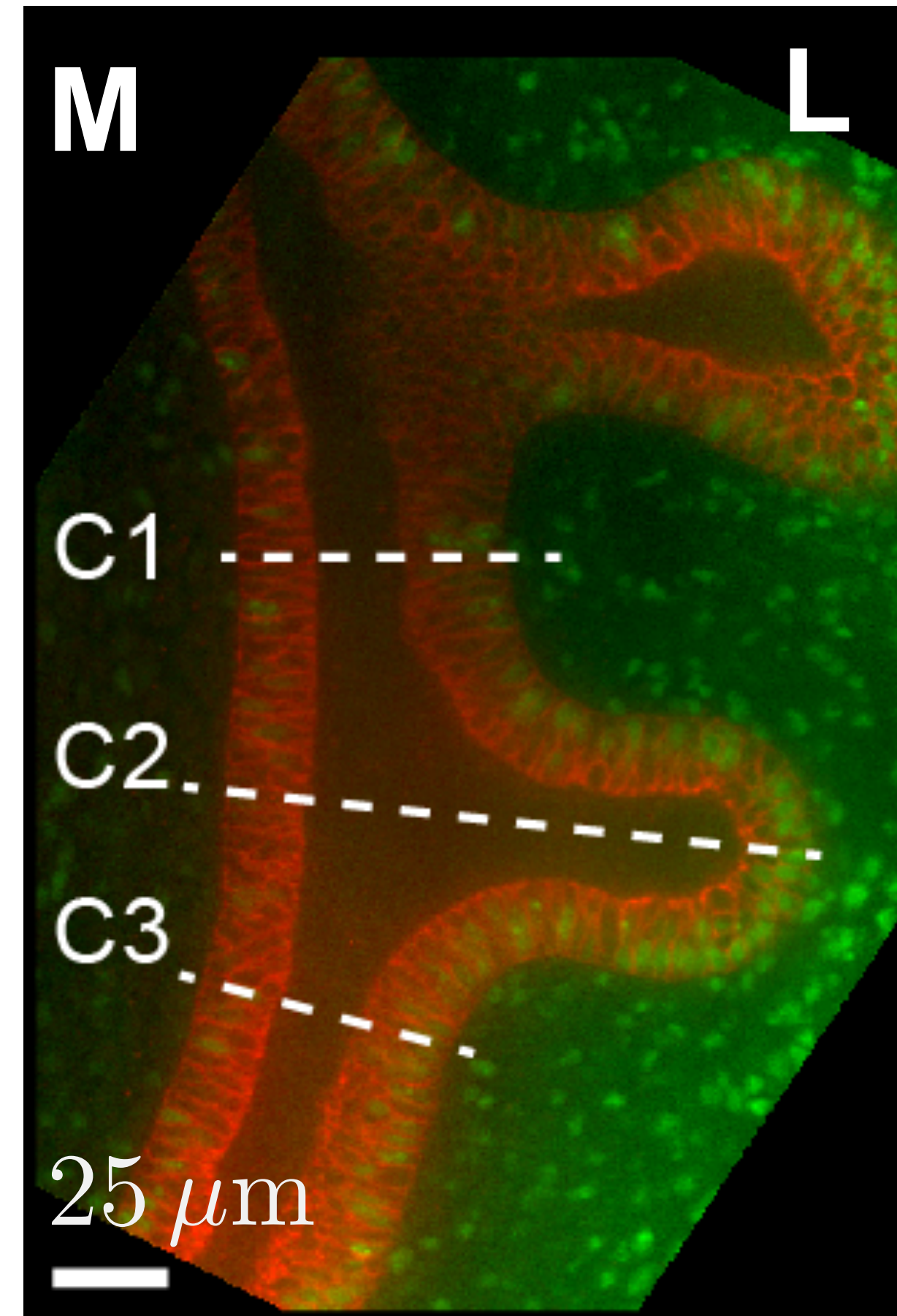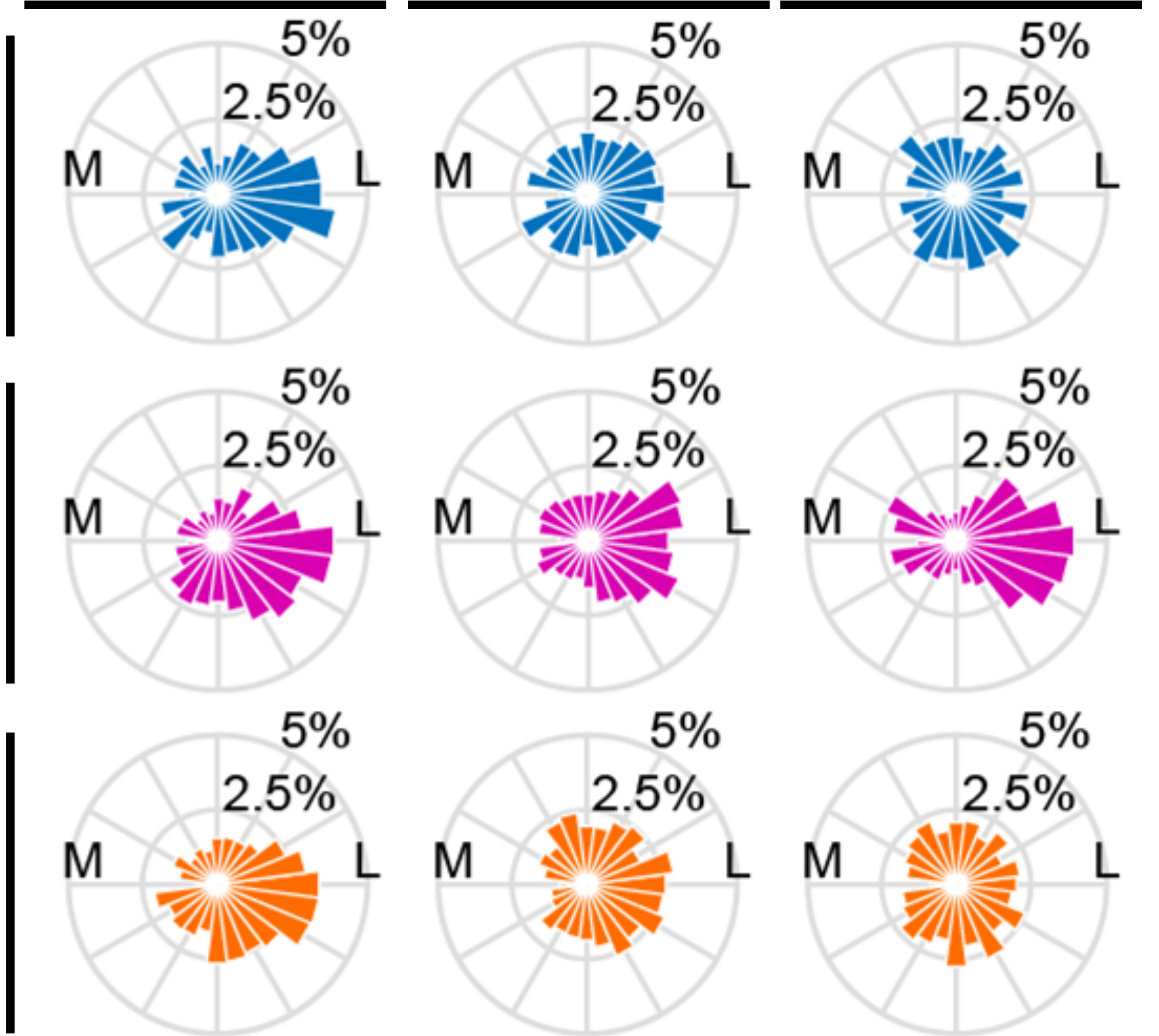
Ecad (epithelium)    EdU (proliferating cells)
EdU (proliferating cells)

# Formation of new domain branch

**What is the relative importance of differential growth and smooth muscle differentiation for the formation of new domain branch?**
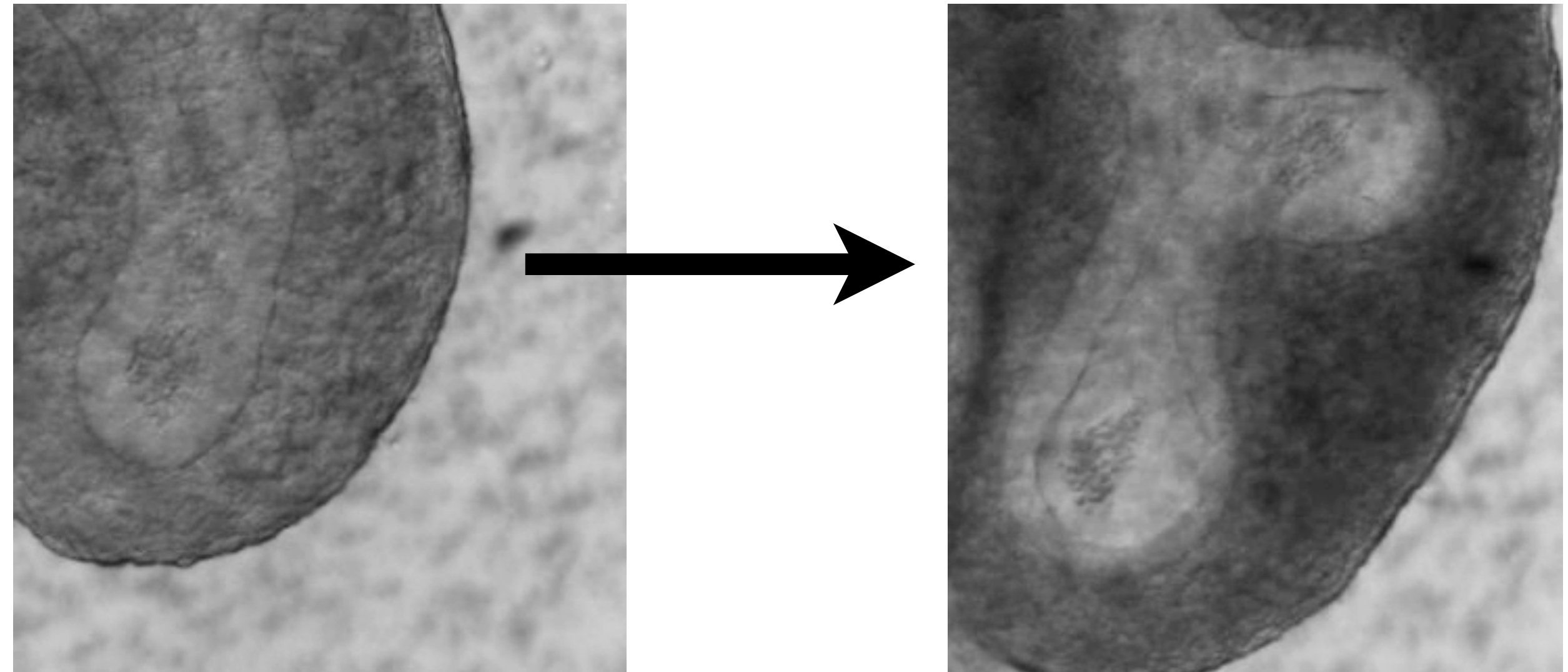


**Smooth muscle differentiation**

E11.5     E12     E12.5
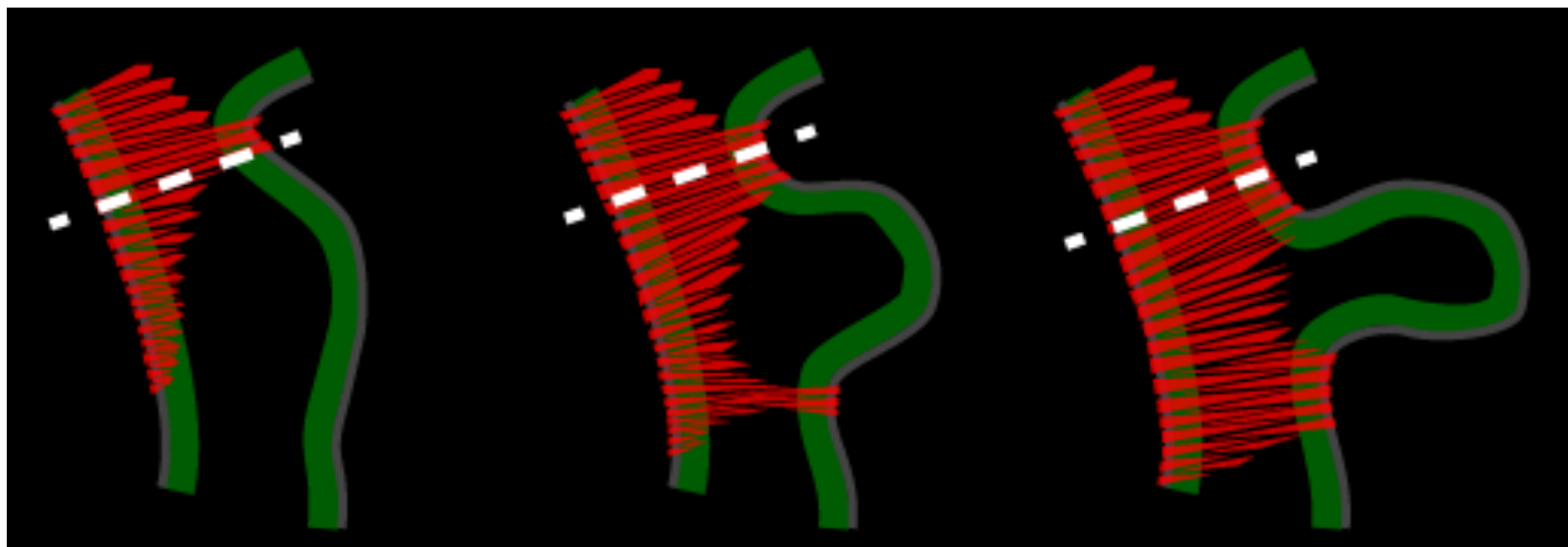


**Differential growth**

E11.5     E12     E12.5
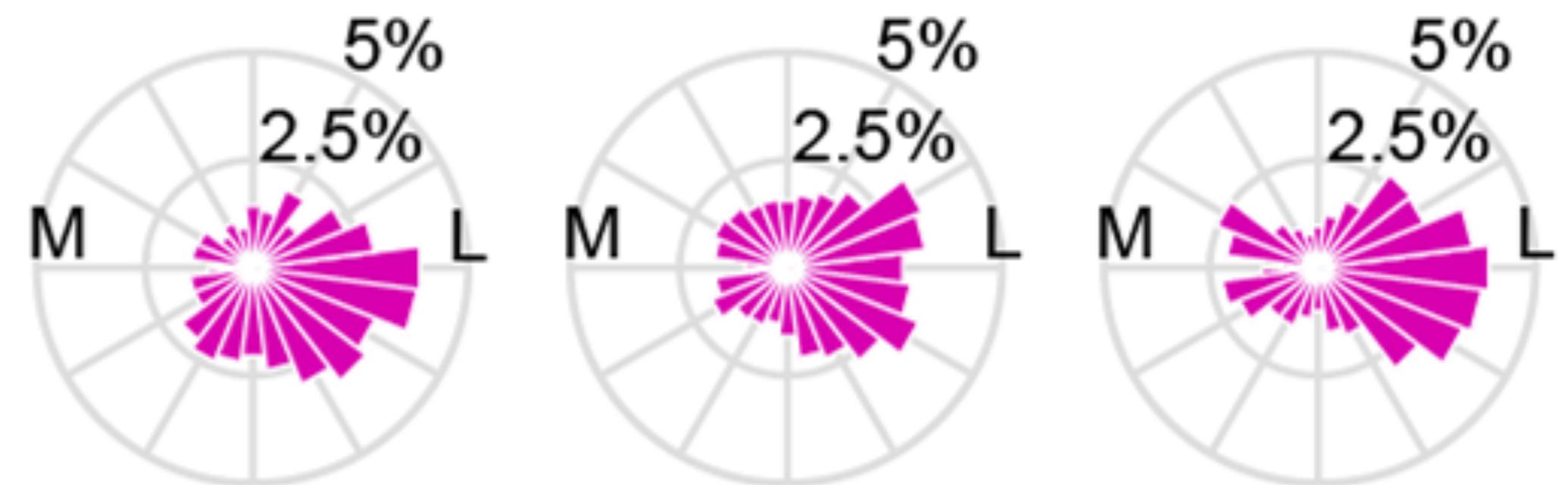
$$i, j, k \in x, y, z$$

**growth +**
**elastic deformation**

**deformed**
**state**

**reference**
**undeformed**
**state**
$$X_i$$

$$r_i = \phi_i(X_j)$$

$\mathcal{B}_0$

$\mathcal{B}_t$
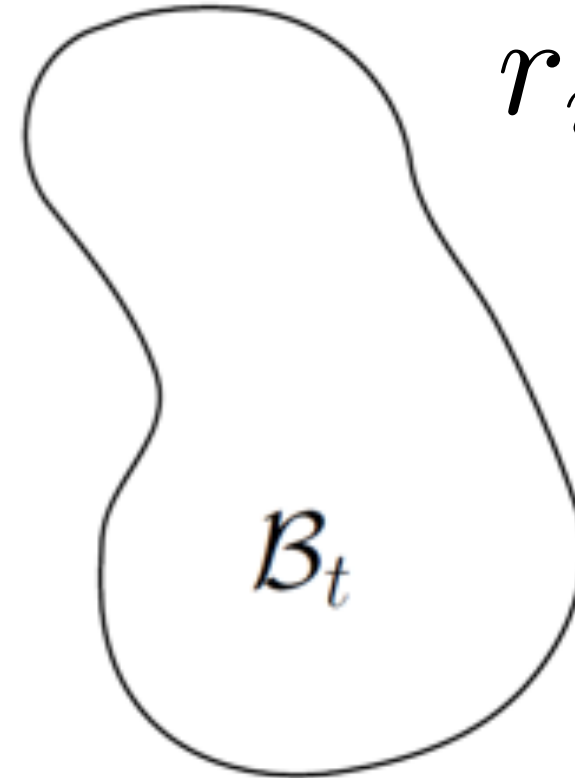
# Modeling growing tissues

$$i, j, k \in x, y, z$$

**reference undeformed state**
$X_i$
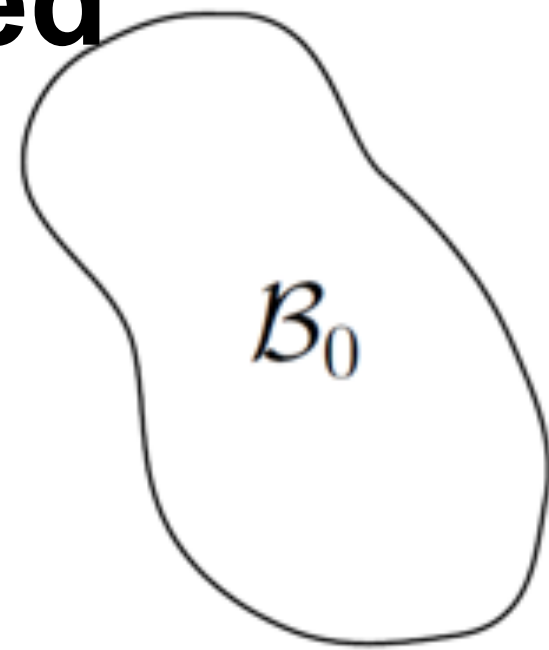
**growth + elastic deformation**

**deformed state**
$$r_i = \phi_i(X_j)$$



$\mathcal{B}_0$

$\mathcal{B}_t$

$\mathcal{B}_g$
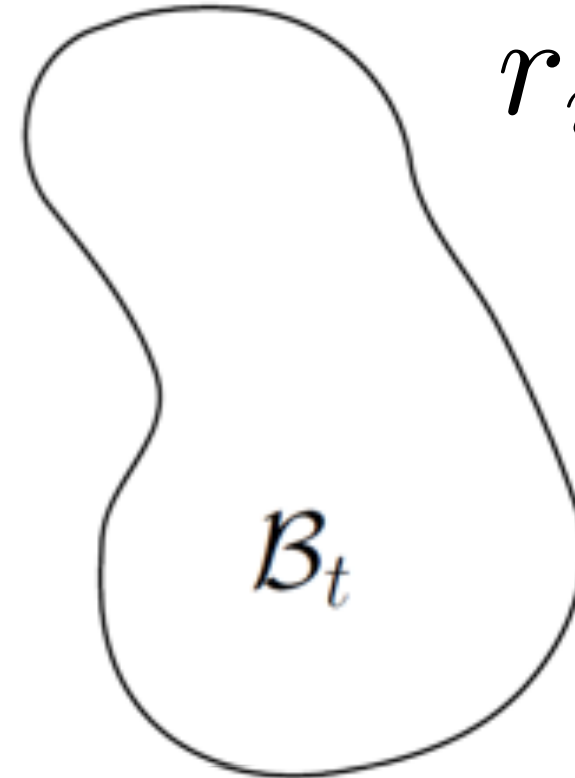
**growth**
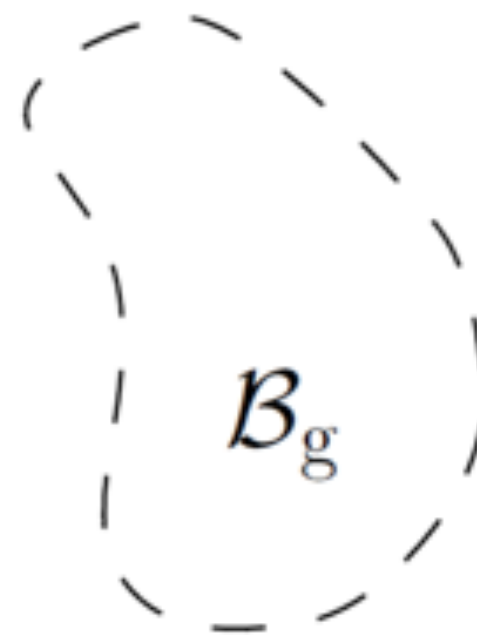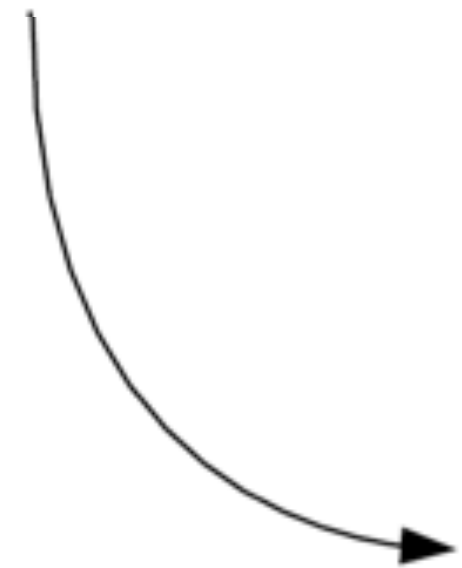
**elastic deformation**

# Modeling growing tissues

$$i, j, k \in x, y, z$$

**reference undeformed state**
$$X_i$$

**growth + elastic deformation**

$$F_{ij} = \sum_k F_{ik}^e F_{kj}^g$$

**deformed state**
$$r_i = \phi_i(X_j)$$

$\mathcal{B}_0$

$\mathcal{B}_t$

$F_{ij}^g$

**growth**

$\mathcal{B}_g$
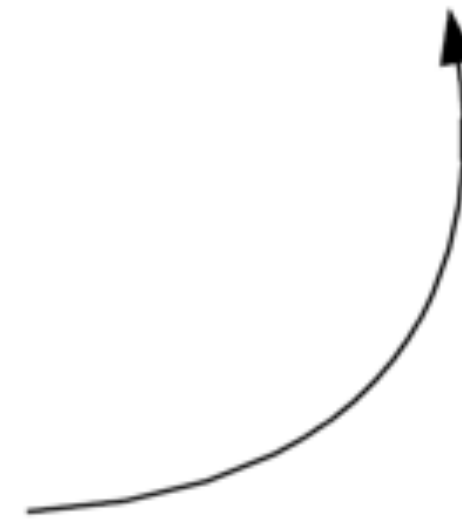
$F_{ij}^e$

**elastic deformation**
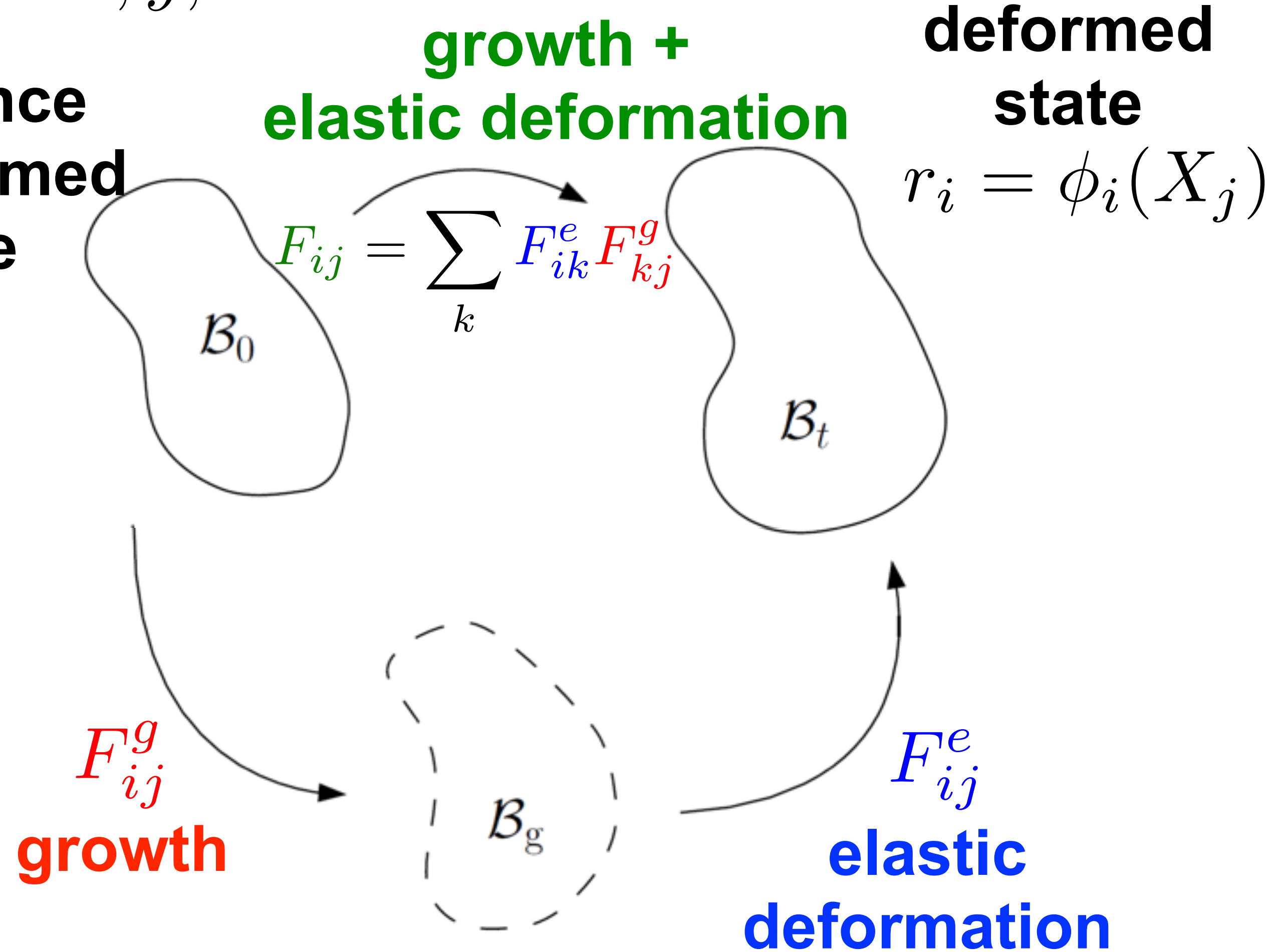
**deformation gradient tensor**

$$F_{ij} = \frac{\partial \phi_i}{\partial X_j}$$

**only elastic part of deformation gradient is associated with stresses in tissues**

$$F_{ij}^e = \sum_k F_{ik} \left(F_g^{-1}\right)_{kj}$$

$i, j, k \in x, y, z$

**reference undeformed state**
$X_i$

**growth + elastic deformation**

$F_{ij} = \sum_k F_{ik}^e F_{kj}^g$
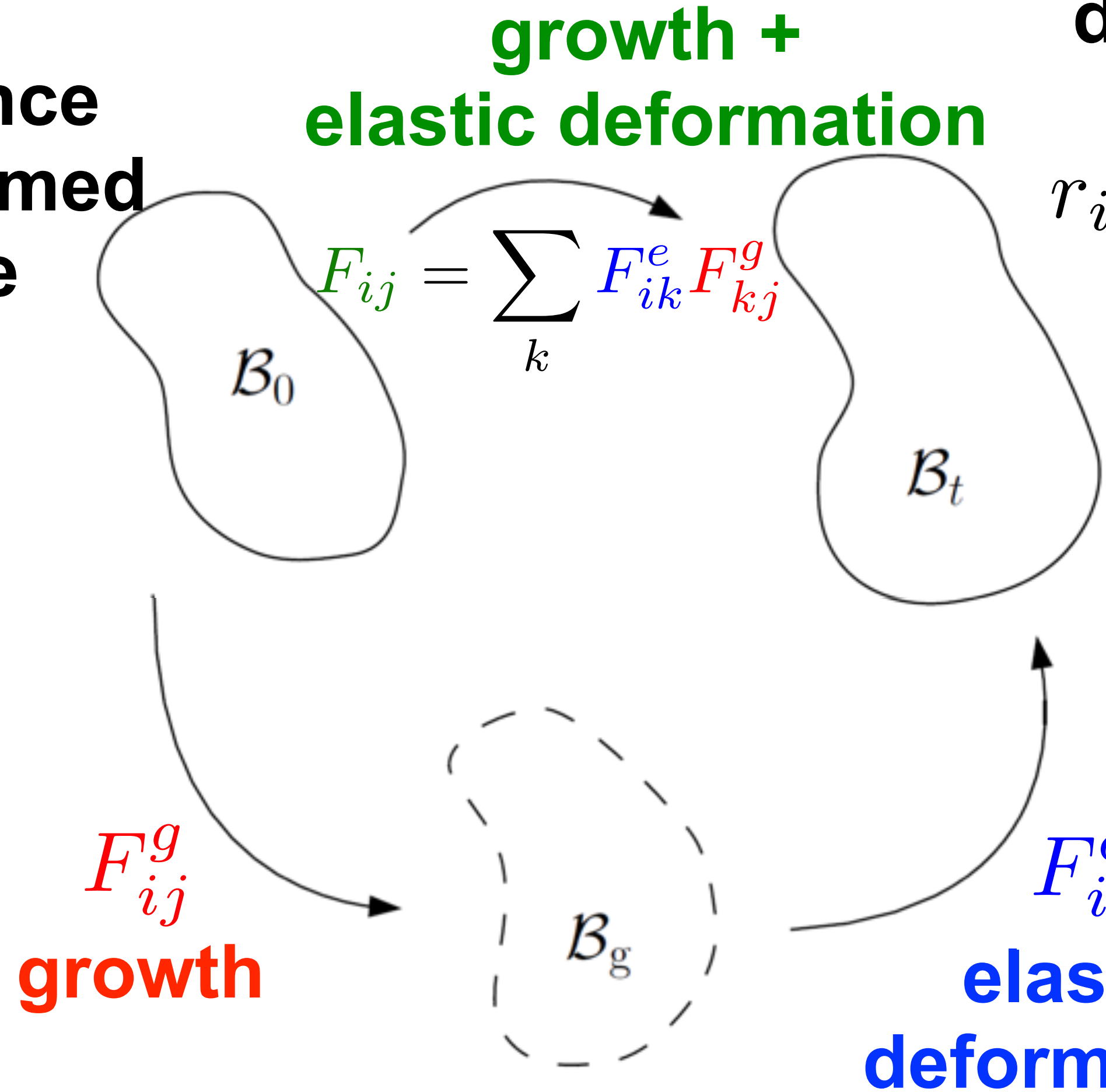
**deformed state**
$r_i = \phi_i(X_j)$

**elastic part of deformation gradient**

$F_{ij}^e = \sum_k F_{ik} \left( F_g^{-1} \right)_{kj}$

$\mathcal{B}_0$

$\mathcal{B}_t$

**Elastic energy (neo Hookean model)**

$$U = \int dX_i \, J_g \left( \frac{\mu}{2} (I_c - 3) - \mu \ln J + \frac{\lambda}{2} [\ln J]^2 \right)$$

$J_g = \det \left( F_{ij}^g \right)$

$J = \det \left( F_{ij}^e \right)$

$I_c = \sum_{i,j} \left( F_{ij}^e F_{ij}^e \right)$

$F_{ij}^g$
**growth**

$\mathcal{B}_g$

$F_{ij}^e$
**elastic deformation**

**Lame constants**

$$\mu = \frac{E}{2(1 + \nu)} \qquad \lambda = \frac{E\nu}{(1 + \nu)(1 - 2\nu)}$$

**Young's modulus** $E$ **Poisson's ratio** $\nu$

# Modeling growing tissues

**top view**

$360\,\mu\text{m}$

$60\,\mu\text{m}$

$\theta$

$r$

**mesenchyme**

$t_{\text{mes}} = 120\,\mu\text{m}$

**smooth muscles**

$t_{\text{sm}} = 10\,\mu\text{m}$

**side view**

$z$

$540\,\mu\text{m}$

Young's modulus $E/E_{mes}$

10

5

2

1

**epithelium**

$t_e = 20\mu\text{m}$

## growth tensor

$rd\theta$

$dz$

$dr$

$g_{\theta\theta}rd\theta$

$g_{zz}dz$

$g_{rr}dr$
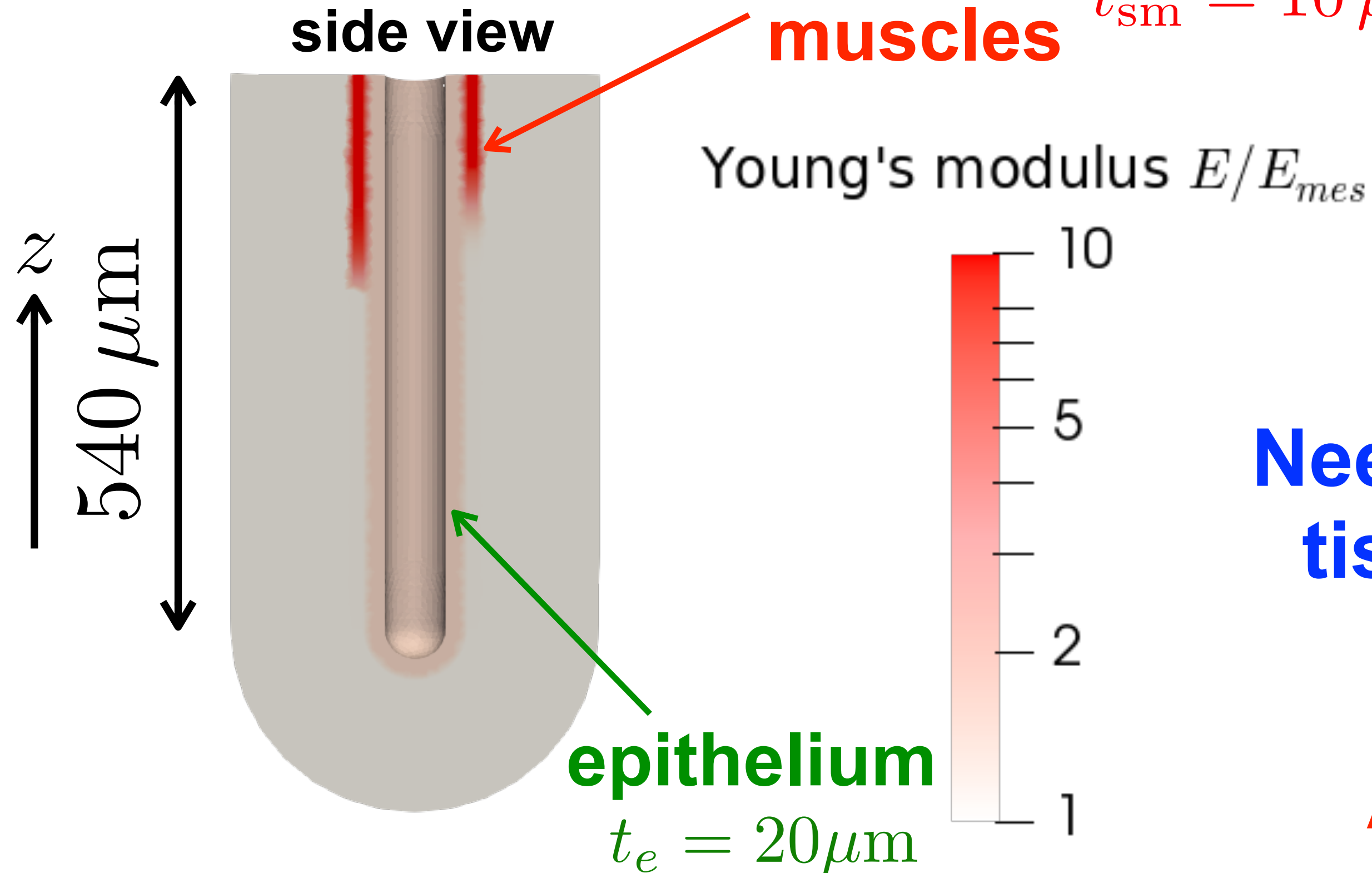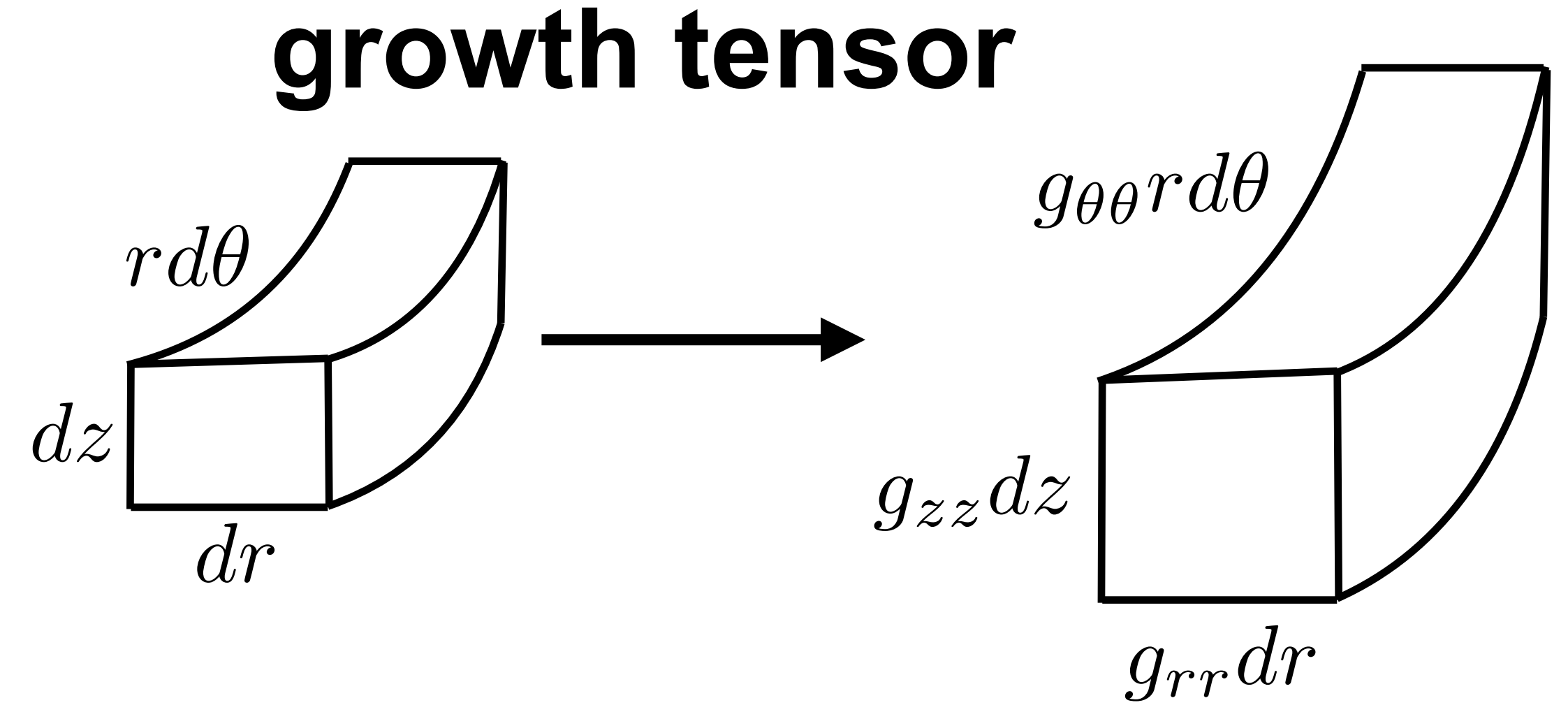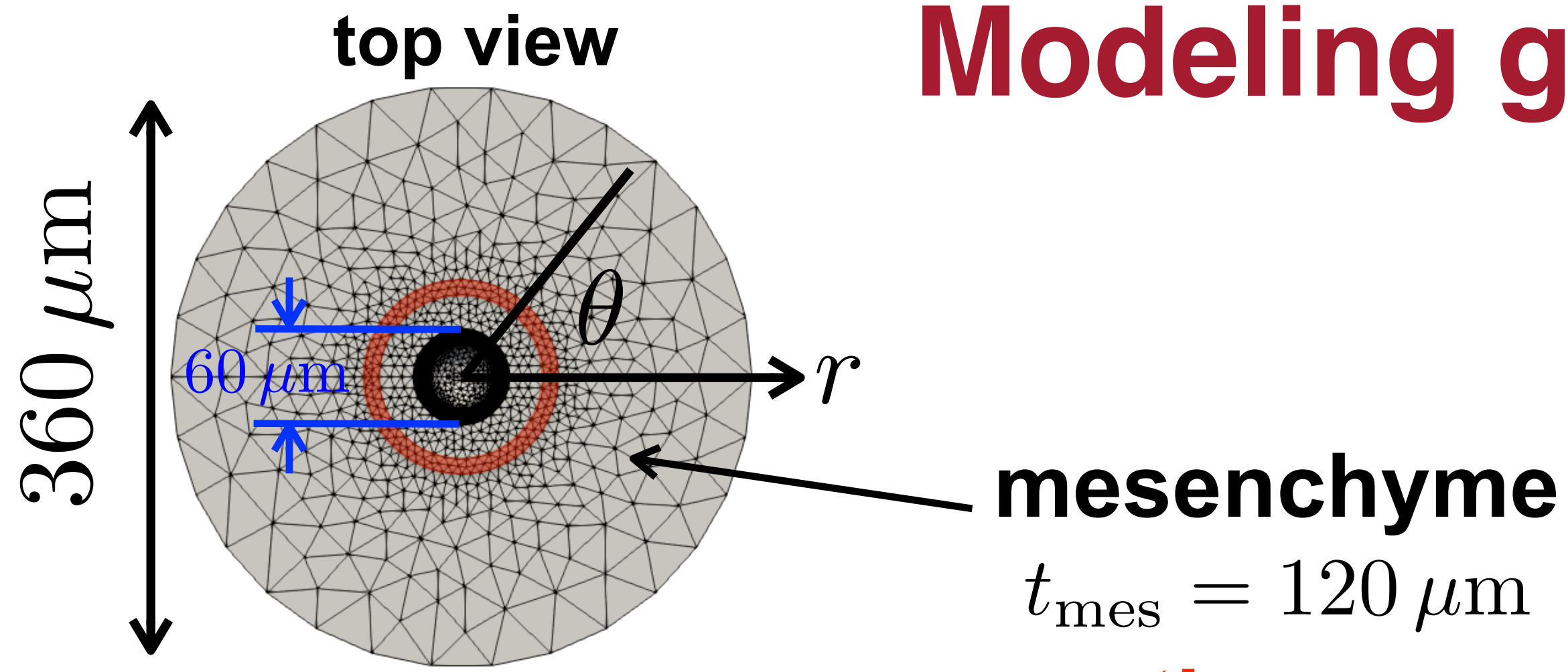
$$F_{ij}^{g} = \begin{pmatrix} g_{rr}, & 0, & 0 \\ 0, & g_{\theta\theta}, & 0 \\ 0, & 0, & g_{zz} \end{pmatrix}$$

**Need to specify growth tensors for all tissues and then minimize the total elastic deformation energy.**

**Assume no mesenchyme growth.**

# Blocked smooth muscle differentiation leads to wrinkling instability of growing epithelium

**assume epithelium growth only along the z axis**

$$g_{rr} = g_{\theta\theta} \equiv 1 \quad g_{zz} = 1 + \frac{(g-1)}{3}(2 + cos(\theta))$$

Growth: $g_0 = 4.50$

Young's modulus $E/E_{mes}$

# Blocked smooth muscle differentiation leads to wrinkling instability of growing epithelium

**assume epithelium growth only along the z axis**

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# Blocked smooth muscle differentiation leads to wrinkling instability of growing epithelium
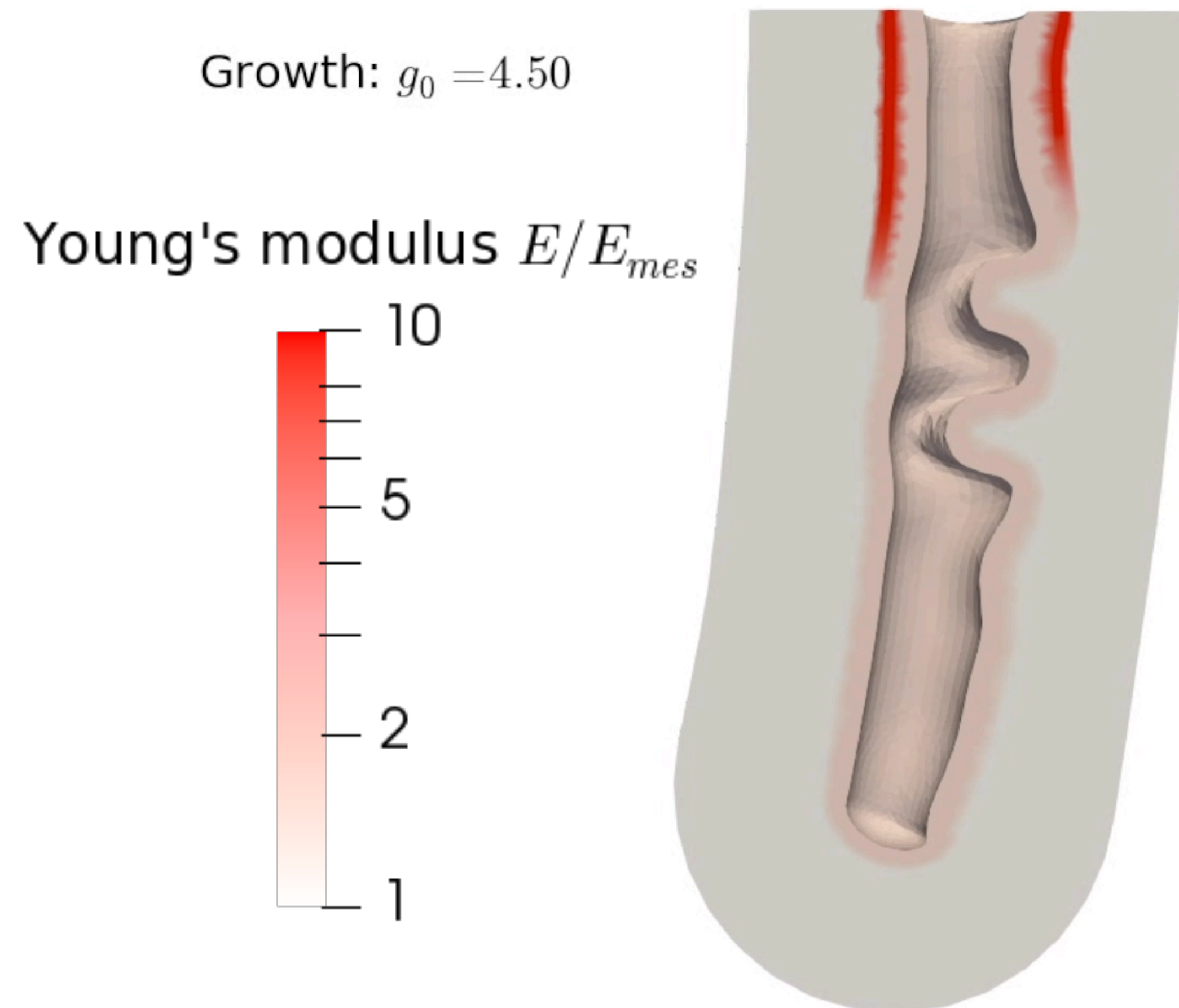
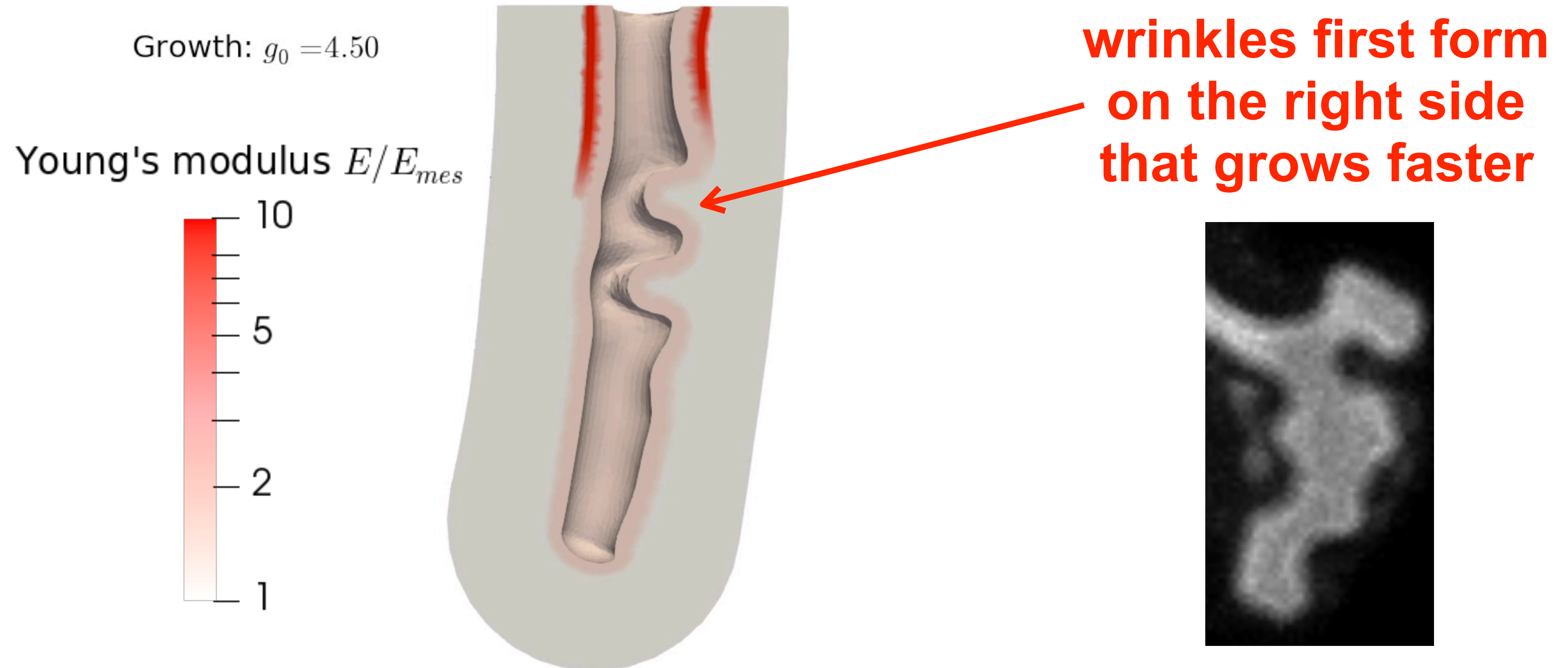**assume epithelium growth only along the z axis**

$$g_{rr} = g_{\theta\theta} \equiv 1 \quad g_{zz} = 1 + \frac{(g-1)}{3}(2 + cos(\theta))$$



Growth: $g_0 = 4.50$

Young's modulus $E/E_{mes}$

**wrinkles first form on the right side that grows faster**
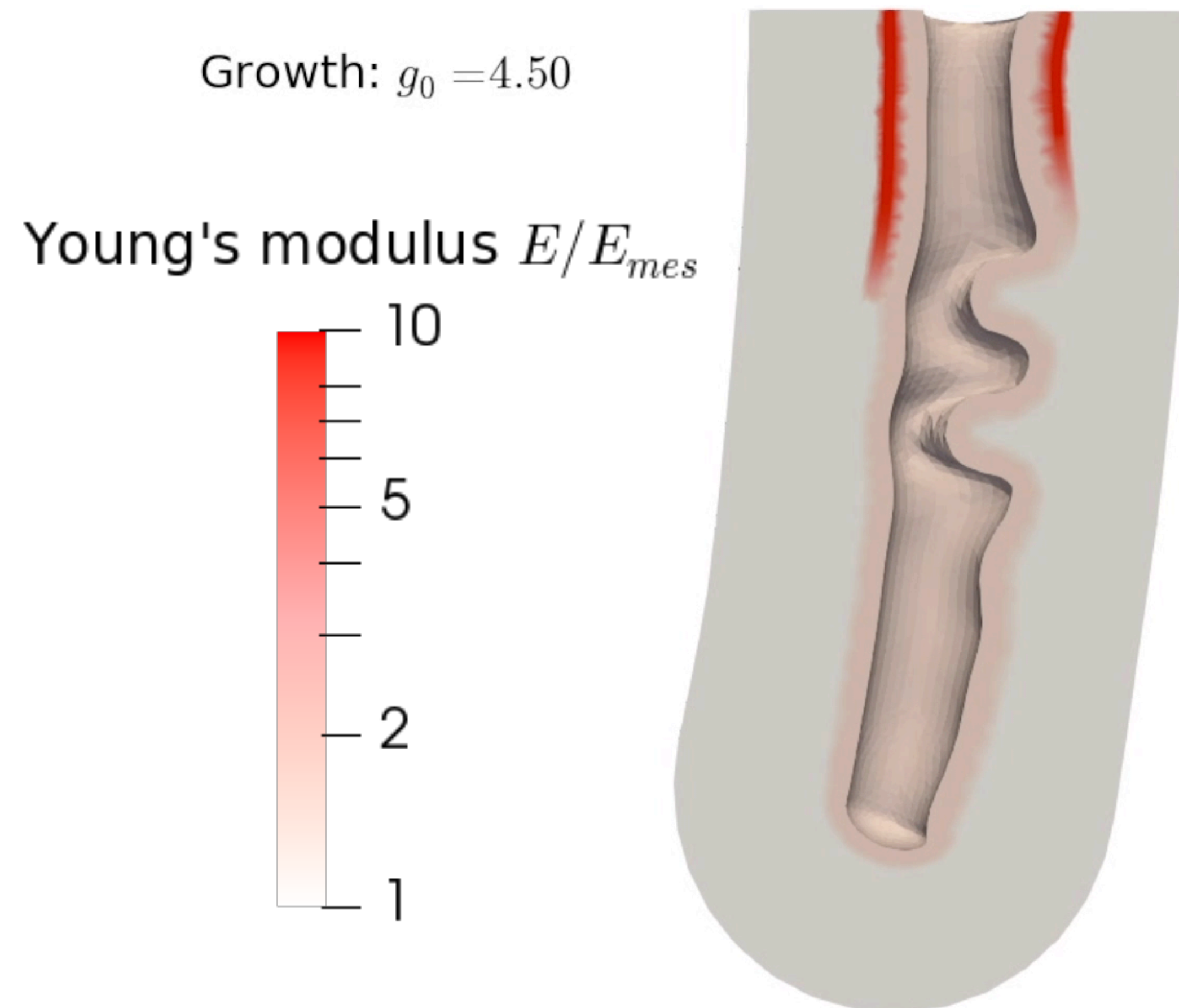
# Differentiation of smooth muscles



**initial state**

**final state**

linear
interpolation
in time

**mesenchyme**
$t_{\mathrm{mes}} = 120\,\mu\mathrm{m}$

**epithelium**
$t_e = 20\mu\mathrm{m}$

**smooth
muscles**
$t_{\mathrm{sm}} = 10\,\mu\mathrm{m}$
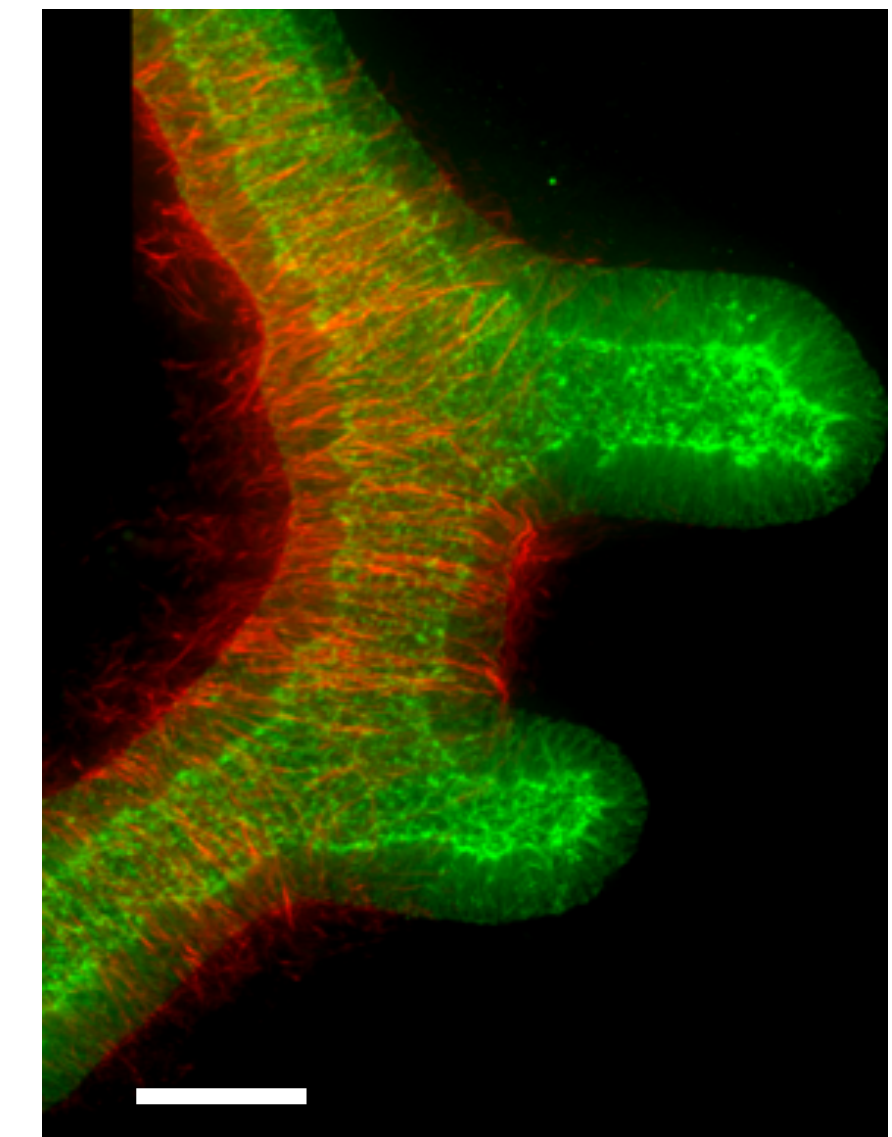
$100\,\mu\mathrm{m}$

Young's modulus $E/E_{mes}$
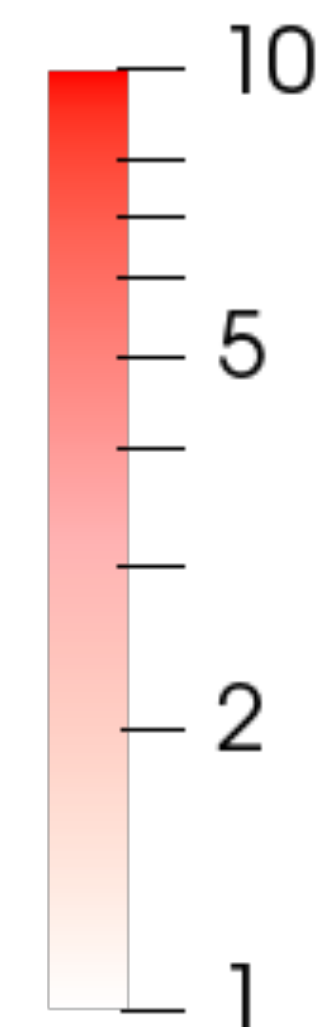
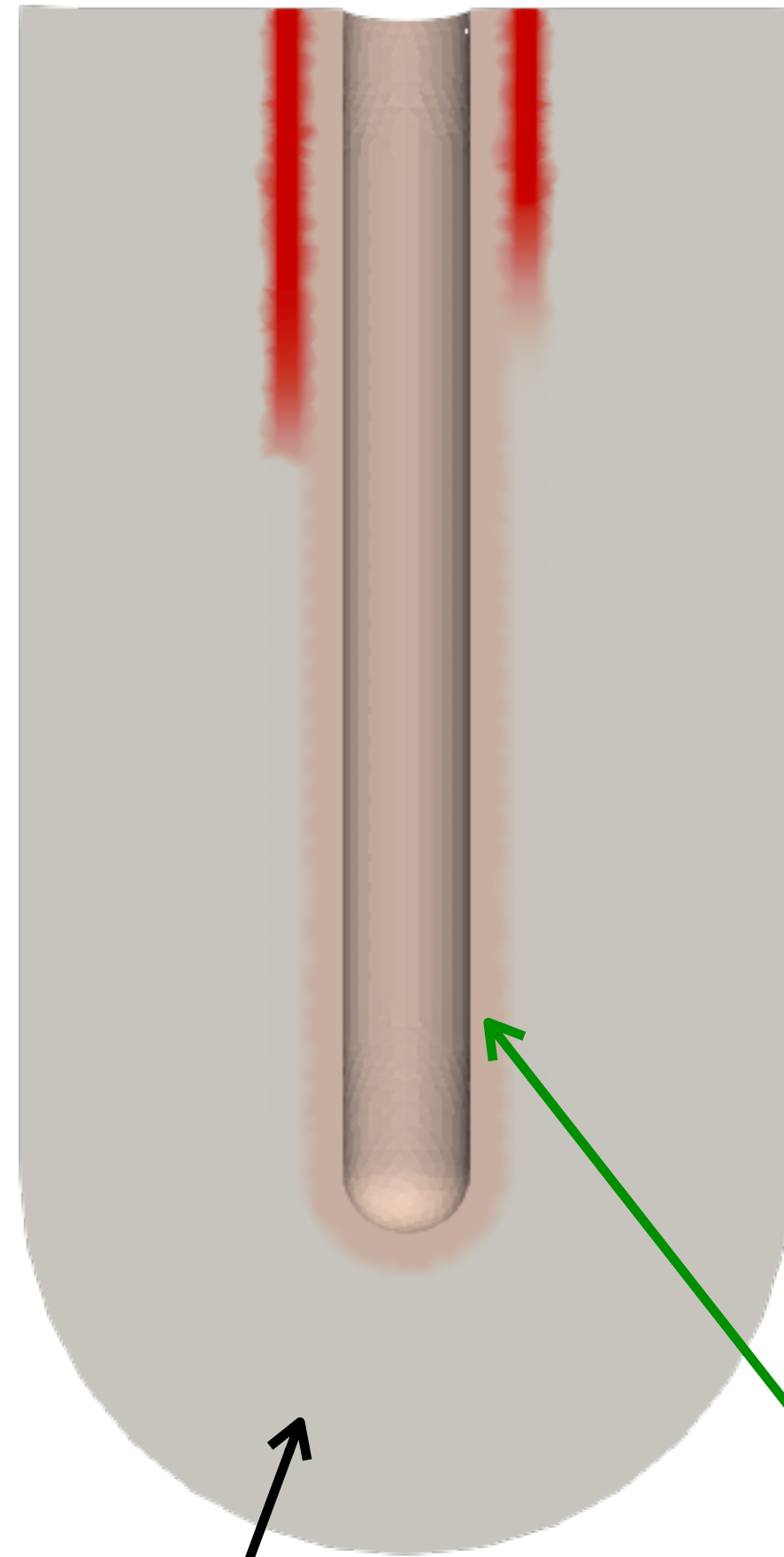# Differentiation of smooth muscles can guide the buckling of growing epithelium to form a new branch

**assume epithelium growth only along the z axis**

$$g_{rr} = g_{\theta\theta} \equiv 1 \qquad g_{zz} = 1 + \frac{(g-1)}{3}(2 + cos(\theta))$$



Growth: $g_0 = 4.50$

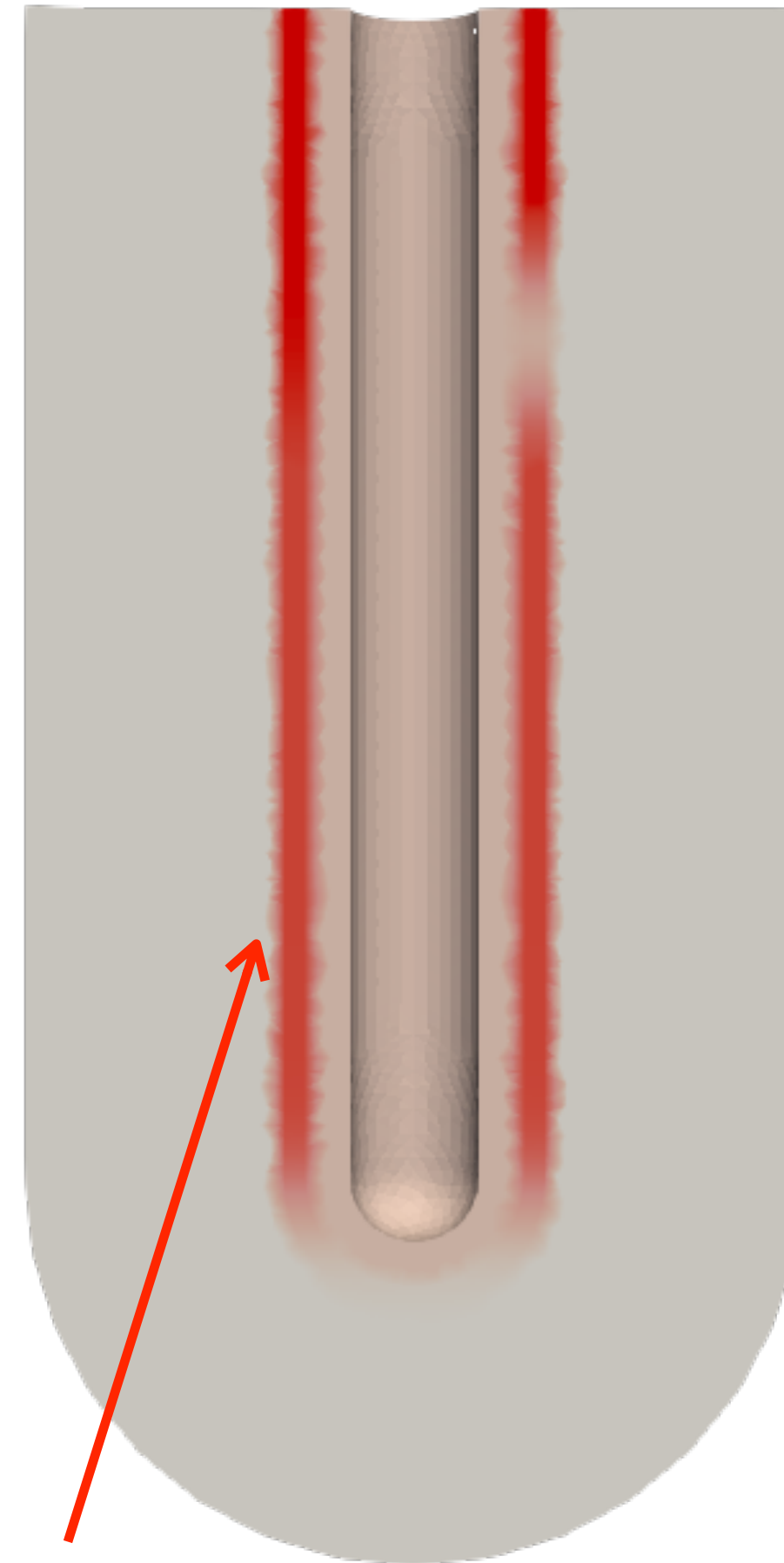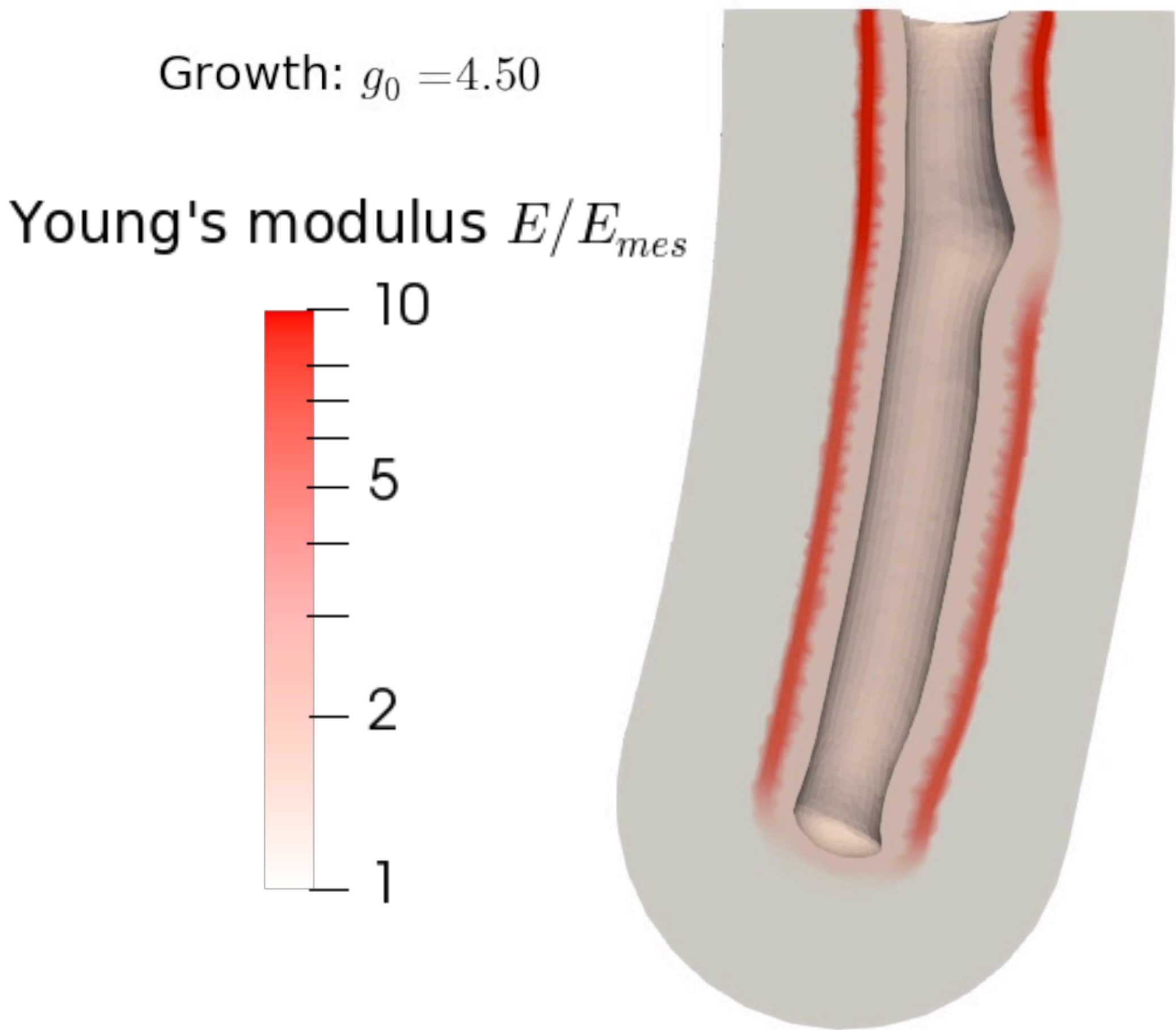Young's modulus $E/E_{mes}$

# Differentiation of smooth muscles can guide the buckling of growing epithelium to form a new branch
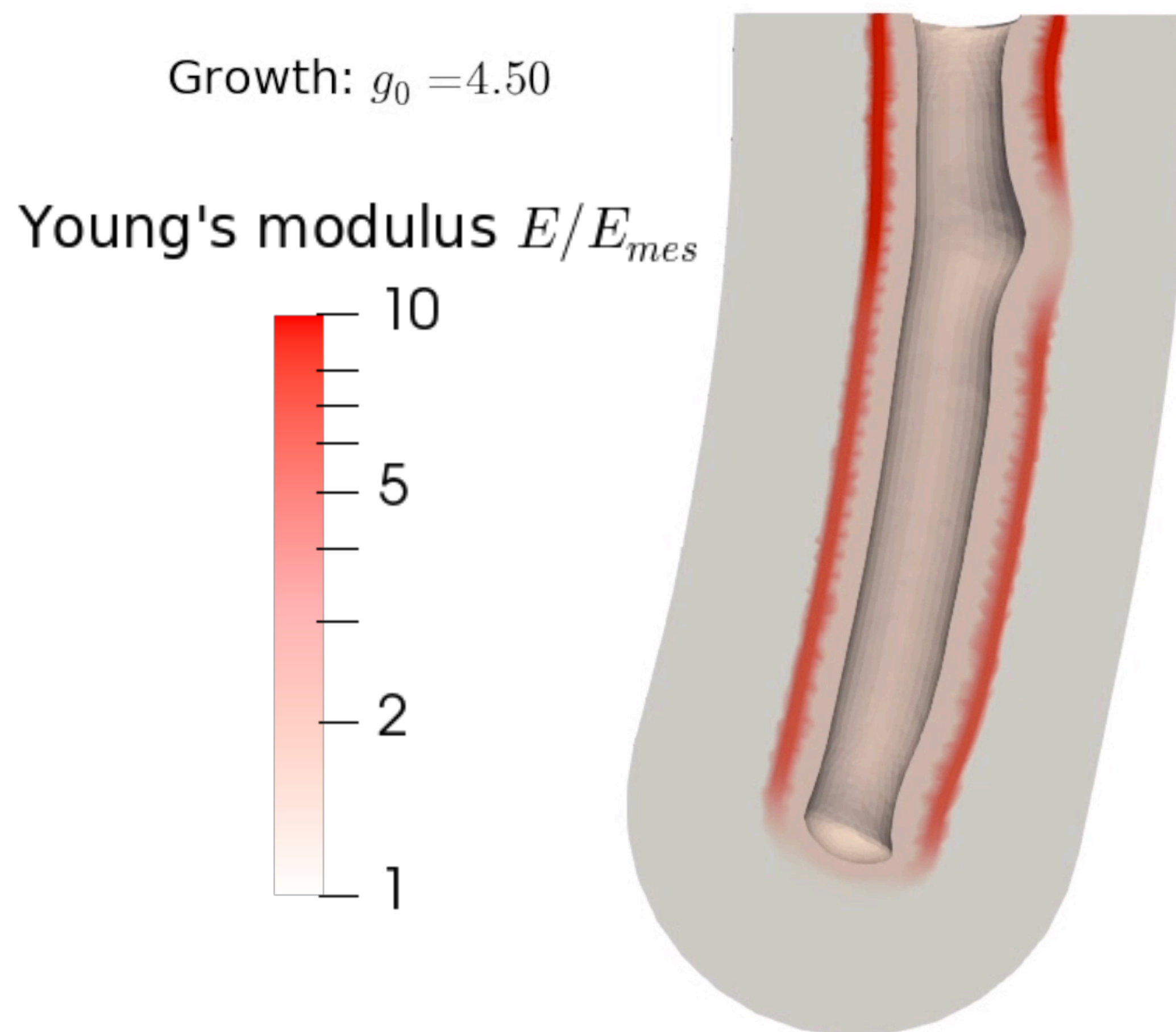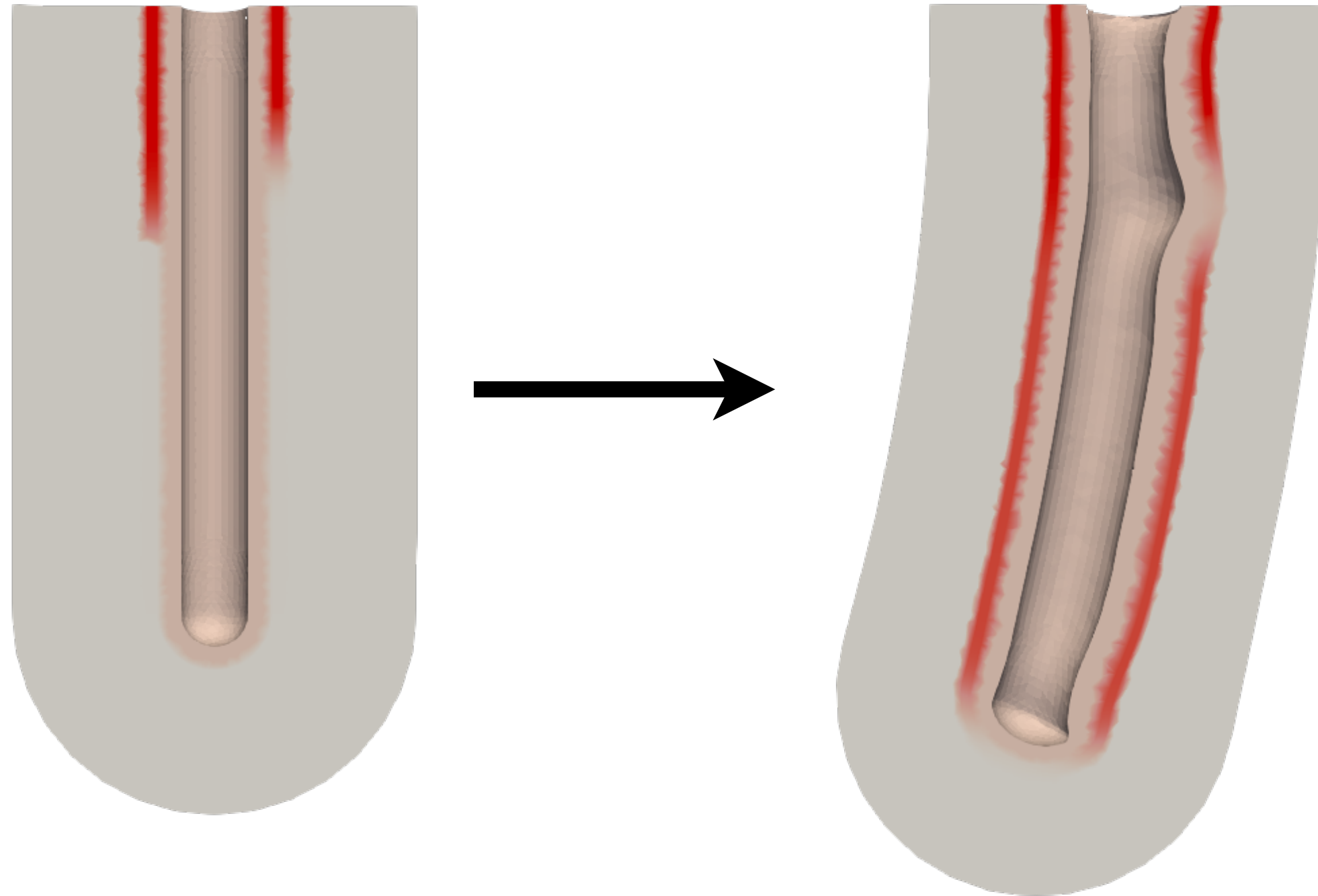
**assume epithelium growth only along the z axis**

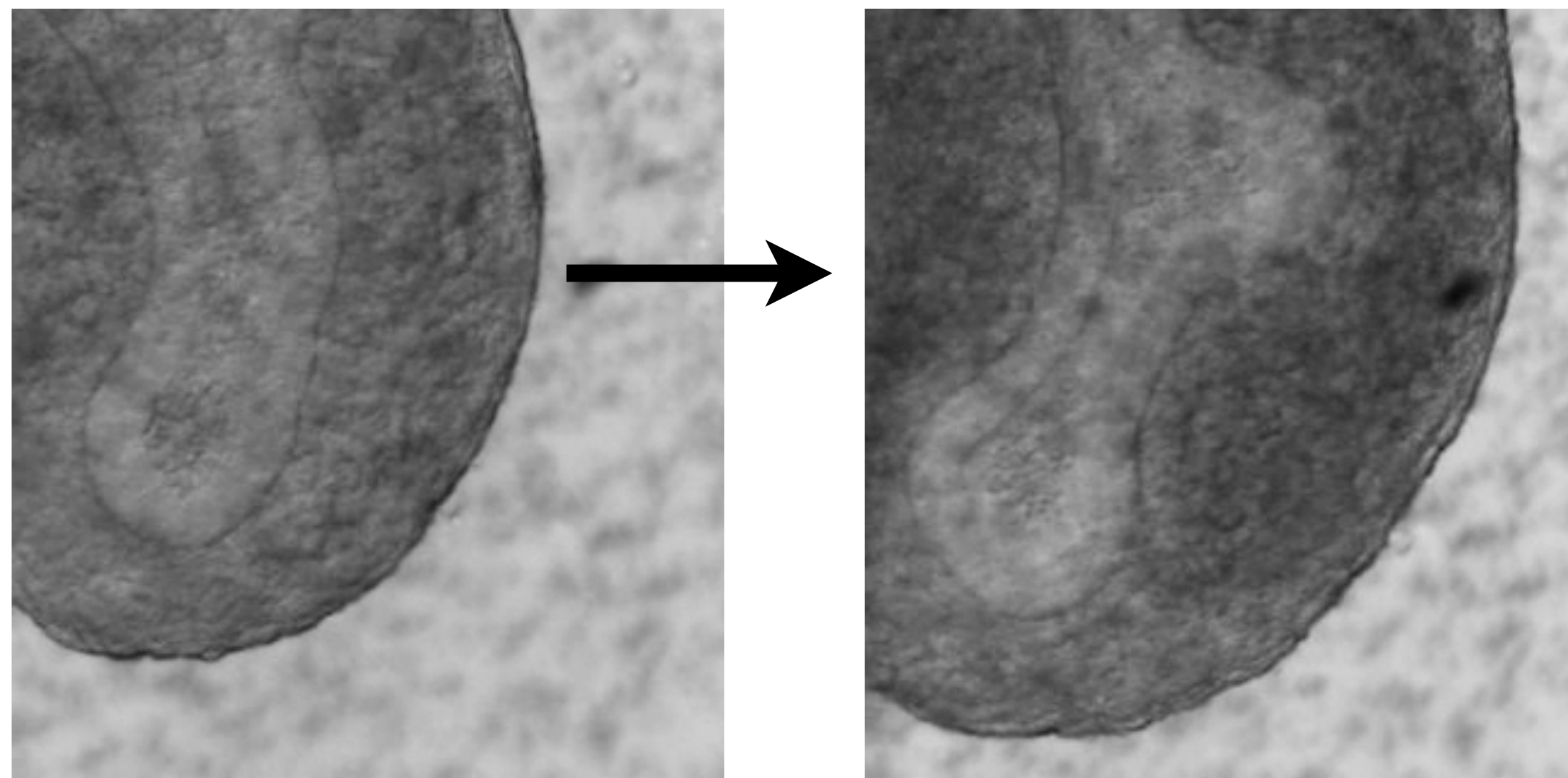$$g_{rr} = g_{\theta\theta} \equiv 1 \qquad g_{zz} = 1 + \frac{(g-1)}{3}(2 + cos(\theta))$$

Growth: $g_0 = 4.50$

Young's modulus $E/E_{mes}$

# Future directions



**Analyze mesenchyme growth patterns**

**Treat mesenchyme as viscoelastic solid to enable elongation of the new branch.**

**Test the hypothesis that smooth muscles are actively contracting the epithelium.**

**Long term goals:** Identify relevant morphogens that are responsible for patterned growth and for the differentiation of mesenchyme to smooth muscles. Implement the relevant reaction diffusion equations.

**the onset of wrinkling in bacterial biofilms is due to mechanical instability**
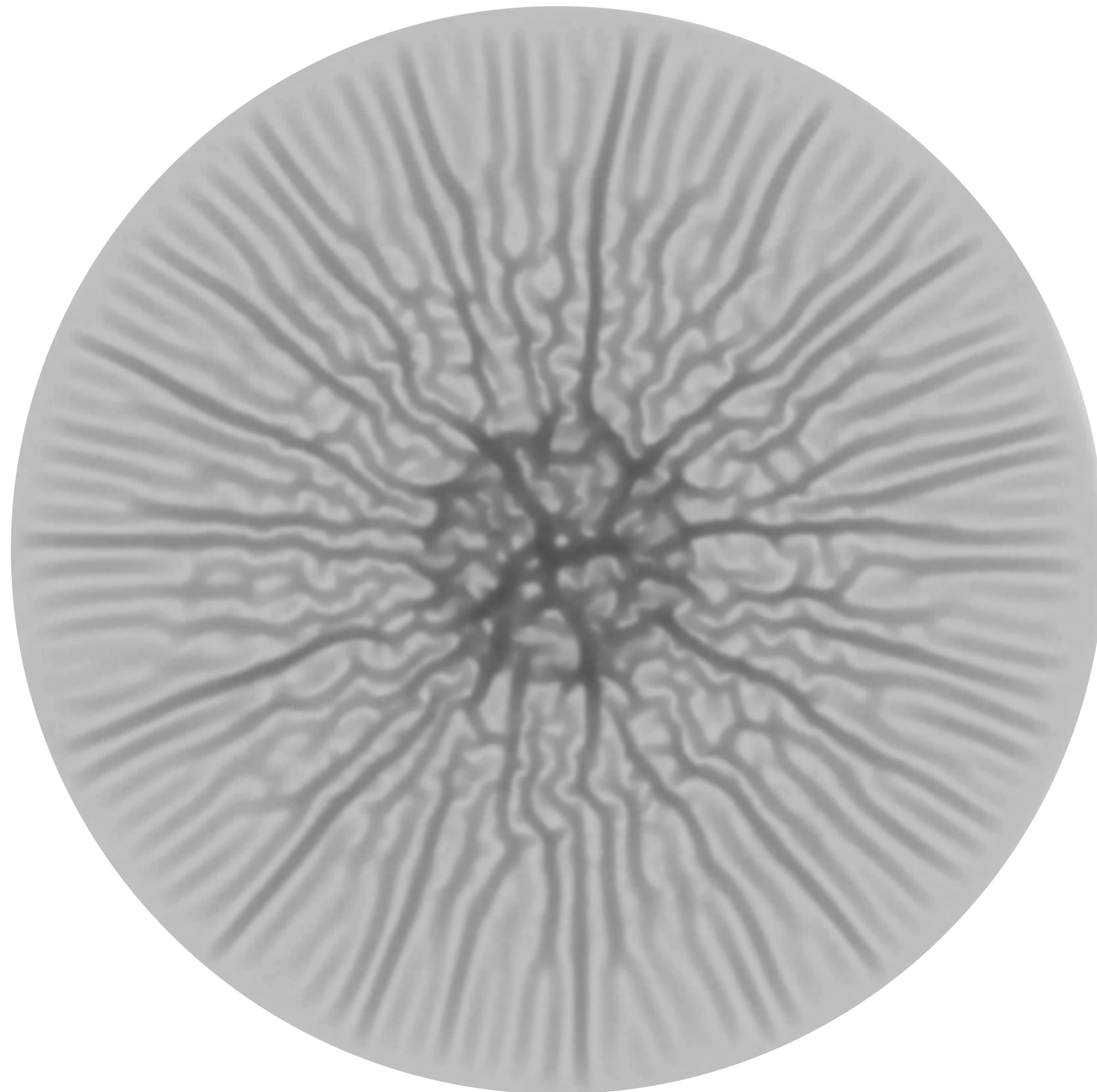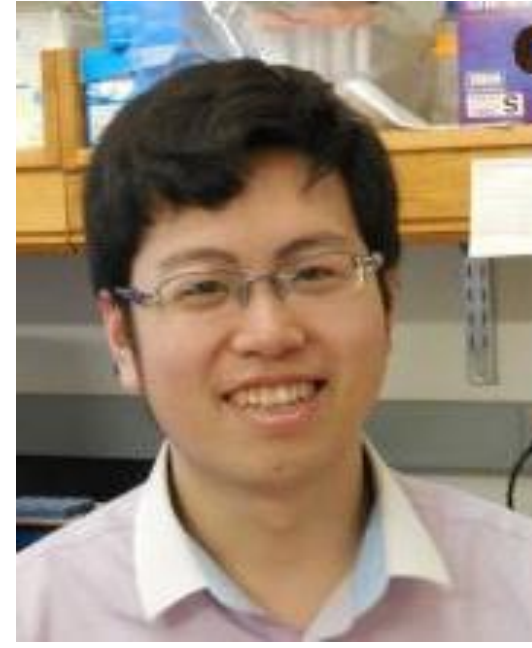
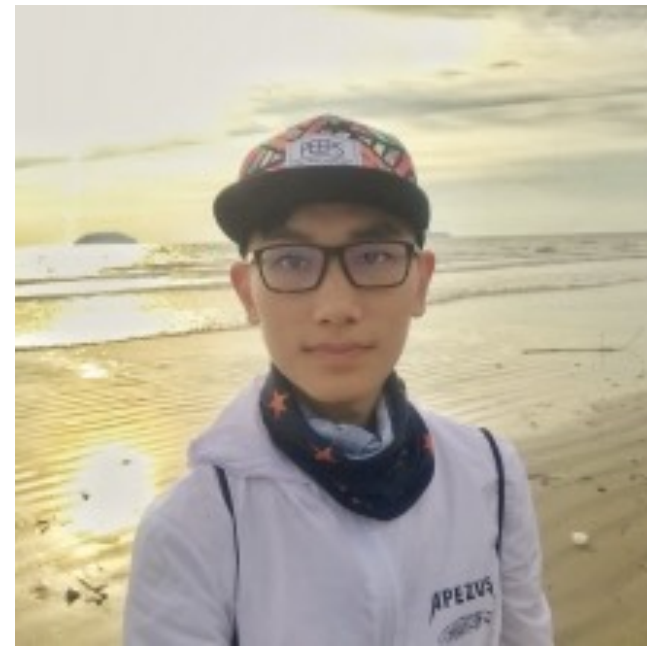**differentiated stiff smooth muscles guide the formation of new domain branches**
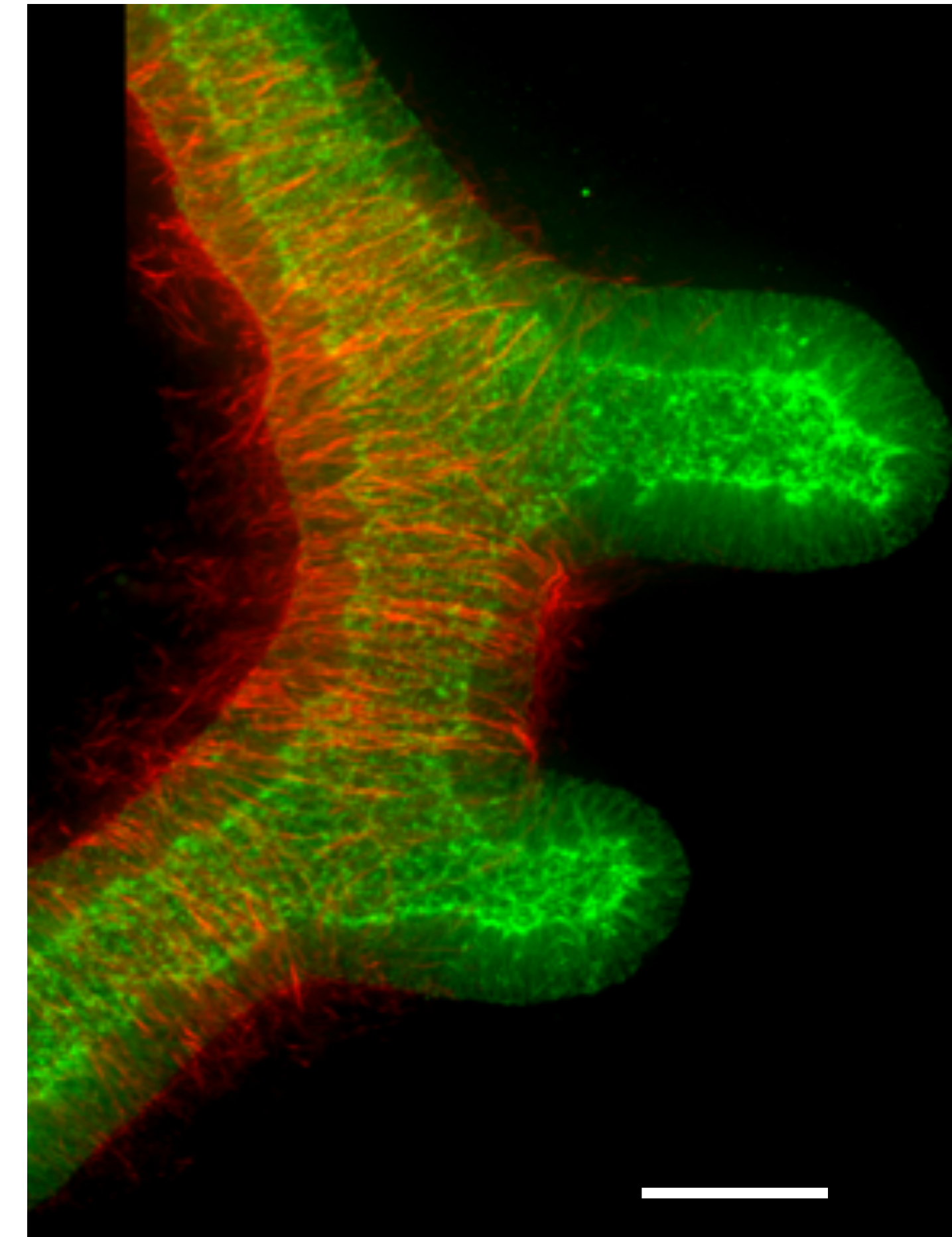
# Acknowledgements

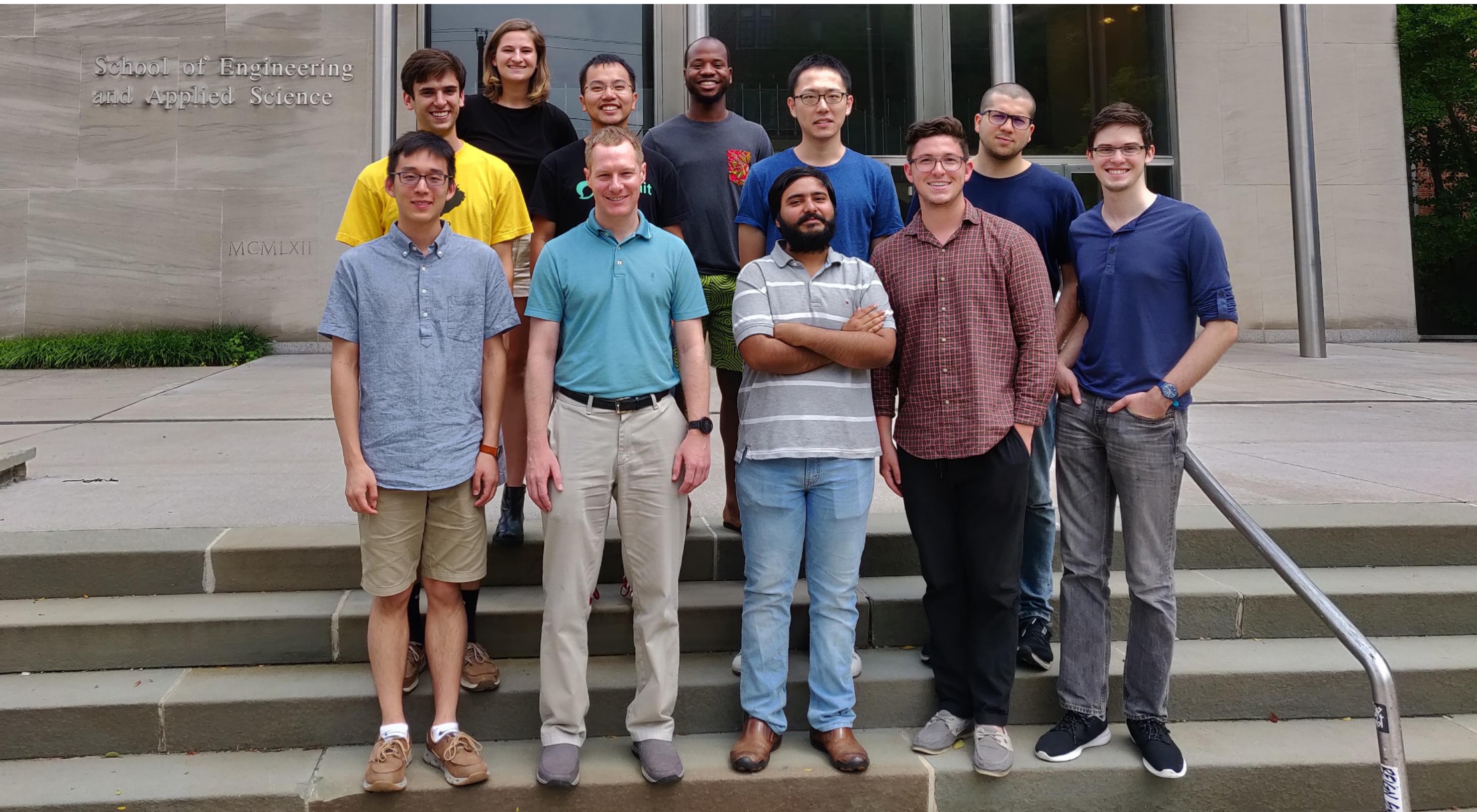N. Wingreen,
B. Bassler, H. Stone

J. Yan

C. Fei

S. Mao

C. Nelson

K. Goodwin

T. Guyomar

# Wrinkling patterns in bacterial biofilms
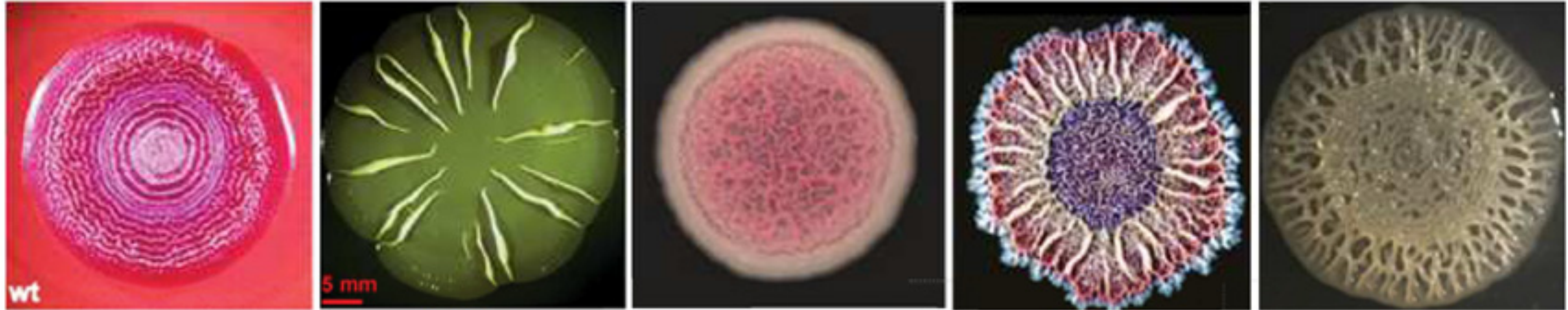


*E.coli* W3110    *E.coli* AR3110    *P. aeruginosa*    *B. subtilis*    *E.coli* UTI89

C. Zhang *et al.*, Appl. Phys. Lett. **109**, 143701 (2016)