Multi-Scale Modeling and Simulation of the Growth of Bacterial Colony with Cell-Cell Mechanical Interactions

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Founding

NSF, NIH, Simons Foundation, and SDSC

OUTLINE

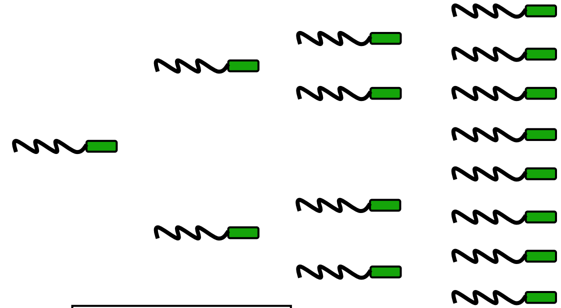
1. Introduction
2. A Two-Scale Model
3. Computational Analysis
4. Conclusions

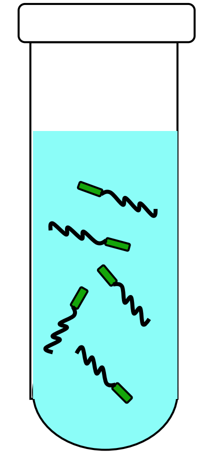
1. Introduction

Quick Facts about Bacteria

- Single-celled life – simplest form of life. Bacterial cells grow, divide, and die.
- Diversity:10,000,000 ~ 1,000,000,000 types. Different morphologies: rod-shaped, spheres, and spirals; 0.5 ~ 5 μm in length or diameter.
- 40 million bacterial cells in a gram of soil and 1 million in a milliliter of fresh water.
- Human body: bacterial cells are 10 times as many as human cells. Most bacteria are in skins and gut.
- Only 1% of bacteria are found to be harmful.

Planktonic Bacteria

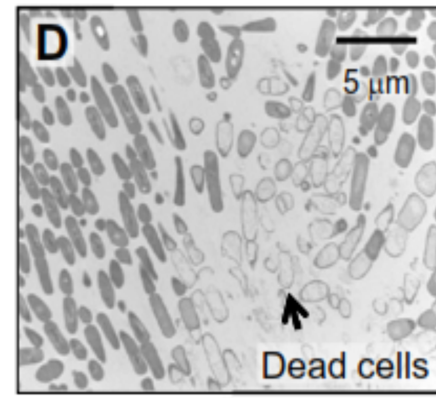




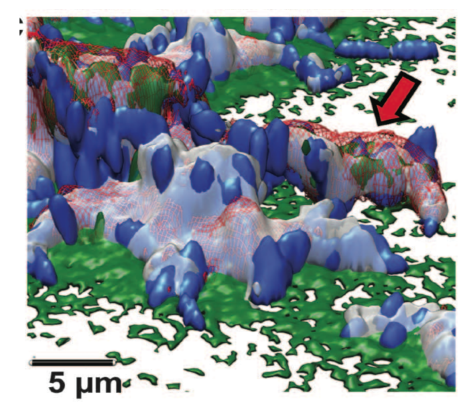
- Free swimming
- Seek out food sources
- Exponentially growing at a steady rate

$$\frac{dN}{dt} = \lambda N$$

Highly Dense Bacteria (Biofilms)

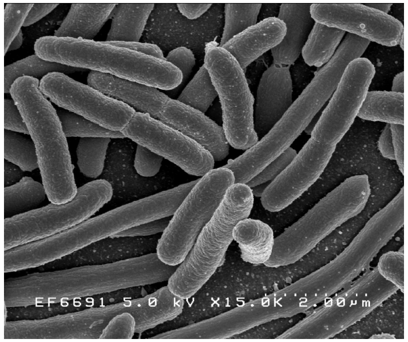


- Push each other instead of swimming or swarming.
- Compete for resources such as nutrient and space.
- Grow slowly, and die.
- Communicate with quorum sensing for differentiation and coordinated virulence.
- Produce extra-cellular, viscoelastic, multi-component, polymer matrix to stick together.

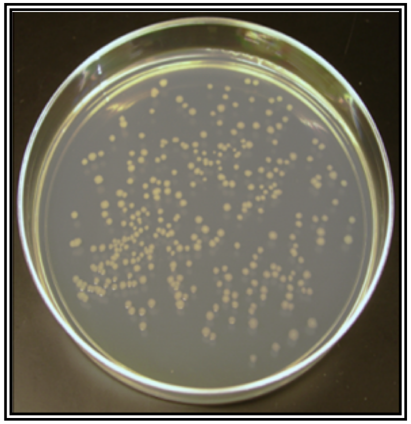


Start from a simpler problem

E. coli Growing on Agar



- No swimming
- Expansion only through force of growth
- Minimal quorum sensing
- No extracellular polymer matrix
- Metabolism in crowded environment
- Physical forces at the microscopic scale



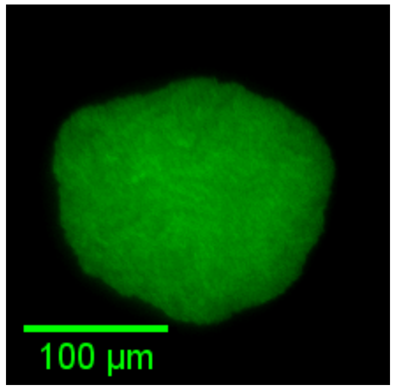
Goals

- Explain experimental findings such as growth laws.
- Identify key parameters that control the growth dynamics.
- Understand the genetic origins.

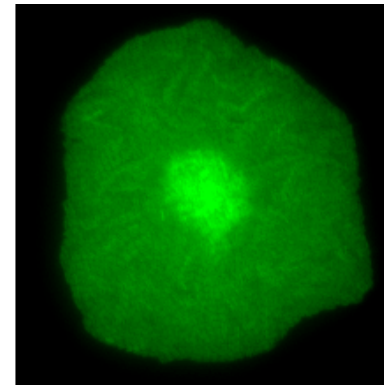
Experimental Observations (Warren/Hwa, UCSD)

From a single cell …

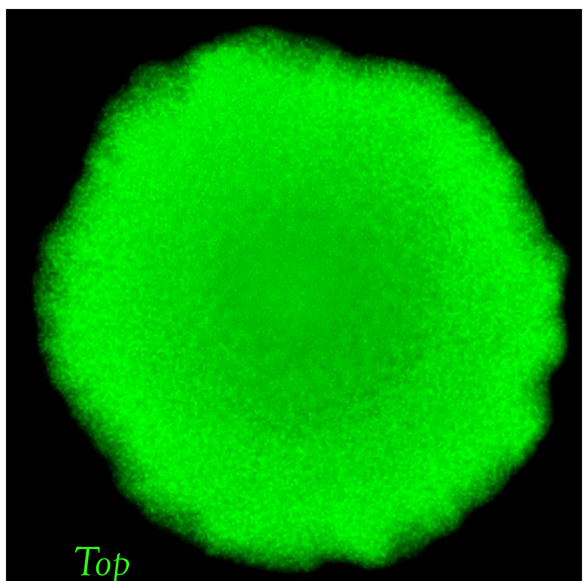
Exponentially growing, close-packed monolayer

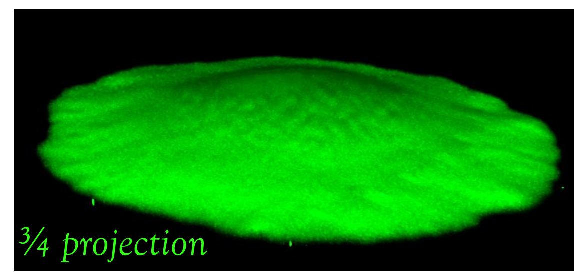


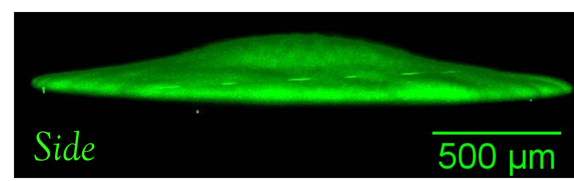
Buckling near the center to form a bilayer, trilayer …



And eventually, a full 3D colony

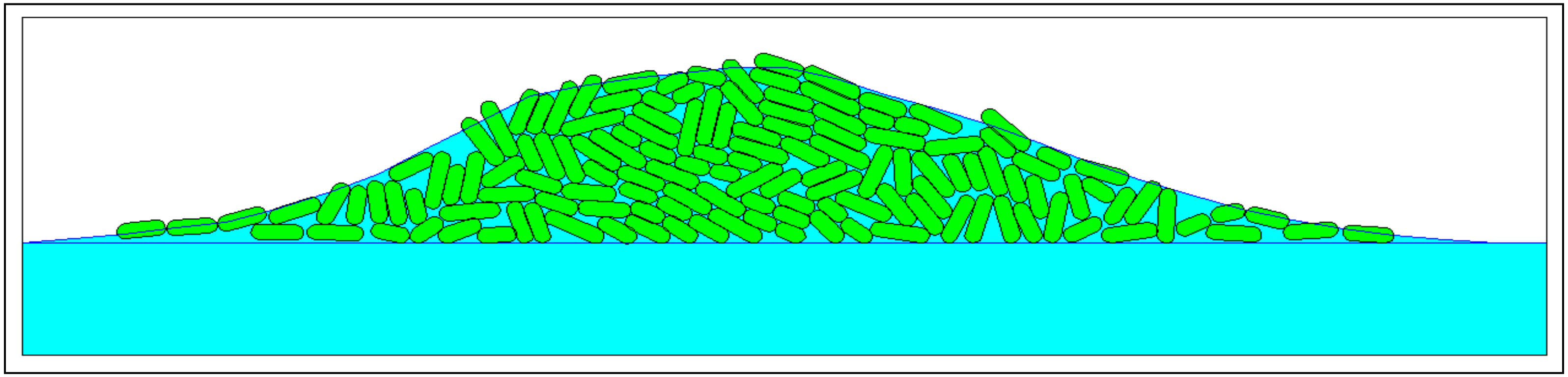






- 2 photon microscopy
- Rich media ~24hr

2. A Two-Scale Model

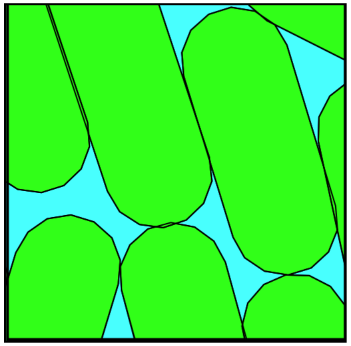


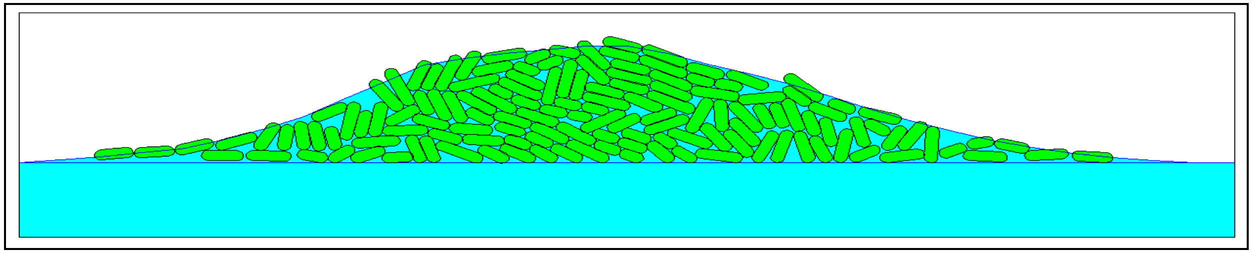
Model Assumptions

- Only one type of cells
- Only one type of nutrient
- No waste
- No dead cells
- Cells do not dip into agar
- No extracellular polymer matrix

Main Variables

- **Individual cells described as elastic sphero-cylinders**
- **Nutrient concentration**
- **Colony boundary**





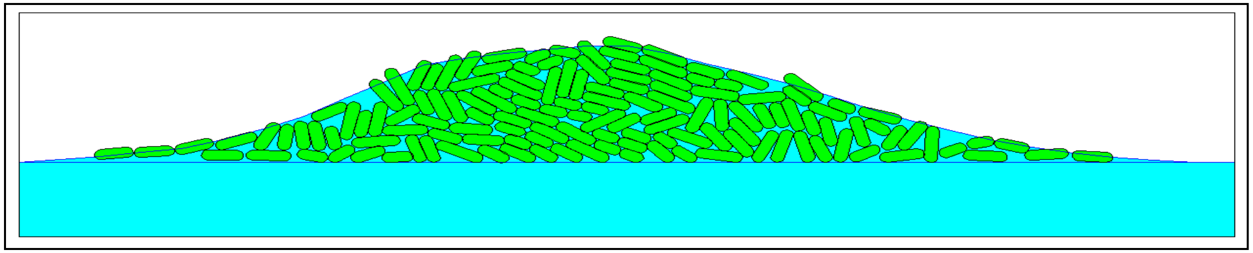
Computational Model

- Cover a computational box with a finite-difference grid
- Initialization:
  - Randomly distribute a few cells;
  - Create a rough agar surface;
  - Set constant nutrient concentration in agar region.
- Time iteration

Main Loop

Step 1. Generate the cell density and colony boundary.
Step 2. Update the nutrient concentration and cell growth rate.
Step 3. Simulate the cell growth, division, and movement.

- Nutrient update with every 100 ~ 1000 micro steps of calculations of cell activities.
- Cell motion by Newton’s law with mechanical interaction forces.



Step 1. Generate the cell density and colony boundary

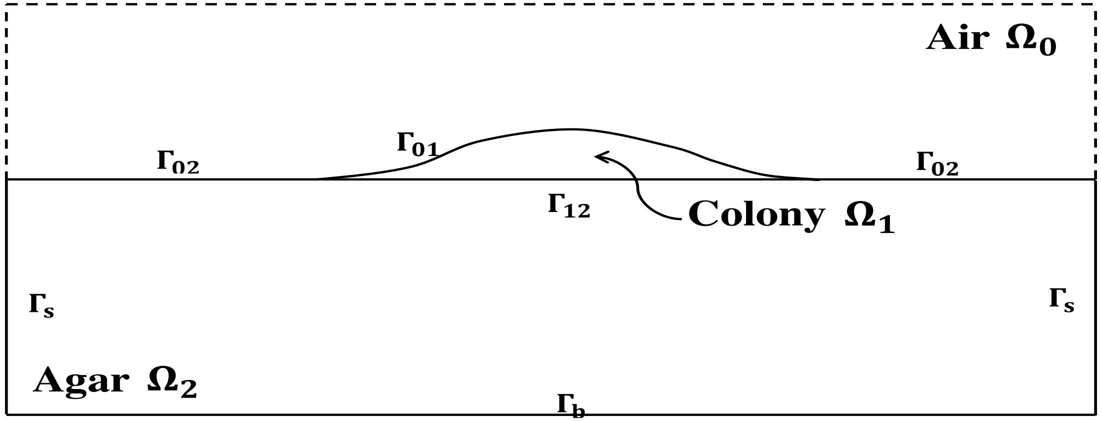
Volume fraction

$$\phi = \frac{\text{sum of volumes of those cells that are inside the grid box}}{\text{volume of grid box}}$$

Smooth locally by convoluting with $$G(\mathbf{x}) = \frac{1}{(\sqrt{2\pi\sigma^2})^3} e^{-\frac{|\mathbf{x}|^2}{2\sigma^2}}$$

Cell density: $$\rho(\mathbf{x}, t) = \phi(\mathbf{x}, t)\rho_{\text{cell}}$$

Colony boundary: threshold the volume fraction.



Step 2. Update the nutrient and growth rate

Nutrient update

$$\frac{\partial C_1}{\partial t} = D_1 \Delta C_1 - \frac{\lambda_{\max}\rho}{Y}\frac{C_1}{C_1 + K_S} \quad \text{in } \Omega_1(t)$$

$$\frac{\partial C_2}{\partial t} = D_2 \Delta C_2 \quad \text{in } \Omega_2$$

- Forward Euler for time
- Finite difference for Laplacian with ghost points near boundary
- Iteration by sweeping over grids
- Multi-resolution meshes

$$C_1 = C_2 \quad \text{on } \Gamma_{12}(t)$$

$$D_1 \frac{\partial C_1}{\partial z} = D_2 \frac{\partial C_2}{\partial z} \quad \text{on } \Gamma_{12}(t)$$

$$\frac{\partial C_1}{\partial n} = 0 \quad \text{on } \Gamma_{01}(t)$$

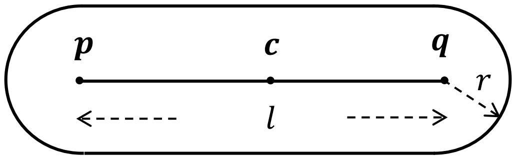
$$\frac{\partial C_2}{\partial z} = 0 \quad \text{on } \Gamma_{02}(t) \cup \Gamma_b$$

$$C_2 = C_s \quad \text{on } \Gamma_s$$

Growth rate update by the Monod equation

$$\lambda(\mathbf{x}, t) = \lambda_{\max} \frac{C_1(\mathbf{x}, t)}{C_1(\mathbf{x}, t) + K_S}$$

Step 3. Simulate the Cell Growth, Division, and Movement



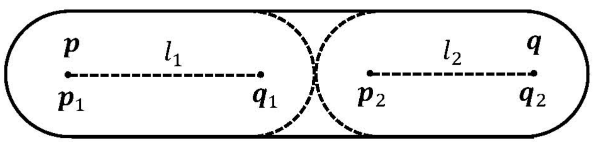
$$V_{\text{cell}} = \pi r^2 l + \frac{4}{3}\pi r^3 \qquad M_{\text{cell}} = \rho_{\text{cell}} V_{\text{cell}}$$

Cell growth

$$\frac{dl(t)}{dt} = \lambda(\mathbf{c}, t) l(t)$$

$$l(t + \Delta t) = l(t) + \lambda(\mathbf{c}, t)\Delta t$$

Cell division If $l \geq l_{\text{div}}$ then the cell splits into two cells.



$$l_1 = \frac{1}{2} l - r + l_{\text{ran}} \eta$$

$$l_2 = \frac{1}{2} l - r - l_{\text{ran}} \eta$$

Rotational fluctuations of each of the two daughter cells

Velocity of daughter cells: same as that of mother cell

Angular velocities: $\omega_1 = (0, 0, 0)$ and $\omega_2 = \omega_{\text{ran}}(0, 0, \xi)$

Cell movement: velocity-Verlet algorithm

First half-step velocity and angular velocity

$$\mathbf{v}_{\text{half}} = \mathbf{v}_{\text{old}} + \frac{\Delta t}{2} \frac{\mathbf{F}_{\text{half}}}{M_{\text{old}}}$$

$$\mathbf{T}_{\text{half,n}} = (\mathbf{T}_{\text{half}} \cdot \mathbf{n}_{\text{old}})\mathbf{n}_{\text{old}}$$

$$\mathbf{T}_{\text{half,t}} = \mathbf{T}_{\text{half}} - \mathbf{T}_{\text{half,n}}$$

$$\boldsymbol{\omega}_{\text{half}} = \boldsymbol{\omega}_{\text{old}} + \frac{\Delta t}{2} \left(\frac{\mathbf{T}_{\text{half,n}}}{I_{\text{old,n}}} + \frac{\mathbf{T}_{\text{half,t}}}{I_{\text{old,t}}} \right)$$

Cell positions update

$$\mathbf{p}_{\text{new}} = \mathbf{p}_{\text{old}} + \Delta t \left(\mathbf{v}_{\text{half}} + \boldsymbol{\omega}_{\text{half}} \times \frac{\mathbf{p}_{\text{old}} - \mathbf{q}_{\text{old}}}{2} \right)$$

$$\mathbf{q}_{\text{new}} = \mathbf{q}_{\text{old}} + \Delta t \left(\mathbf{v}_{\text{half}} + \boldsymbol{\omega}_{\text{half}} \times \frac{\mathbf{q}_{\text{old}} - \mathbf{p}_{\text{old}}}{2} \right)$$

Second half-step velocity and angular velocity

$$\mathbf{v}_{\text{new}} = \mathbf{v}_{\text{half}} + \frac{\Delta t}{2} \frac{\mathbf{F}_{\text{new}}}{M_{\text{new}}}$$

$$\mathbf{T}_{\text{new,n}} = (\mathbf{T}_{\text{new}} \cdot \mathbf{n}_{\text{new}})\mathbf{n}_{\text{new}}$$

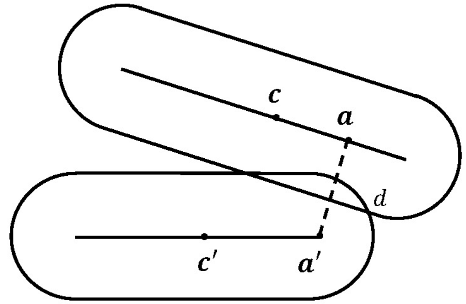
$$\mathbf{T}_{\text{new,t}} = \mathbf{T}_{\text{new}} - \mathbf{T}_{\text{new,n}}$$

$$\boldsymbol{\omega}_{\text{new}} = \boldsymbol{\omega}_{\text{half}} + \frac{\Delta t}{2} \left(\frac{\mathbf{T}_{\text{new,n}}}{I_{\text{new,n}}} + \frac{\mathbf{T}_{\text{new,t}}}{I_{\text{new,t}}} \right)$$

Force Calculations

$$\mathbf{F} = \mathbf{F}_{\mathrm{cc}} + \mathbf{F}_{\mathrm{ca}} + \mathbf{F}_{\mathrm{surf}} + \mathbf{F}_{\mathrm{visc}}$$

Cell-cell interaction



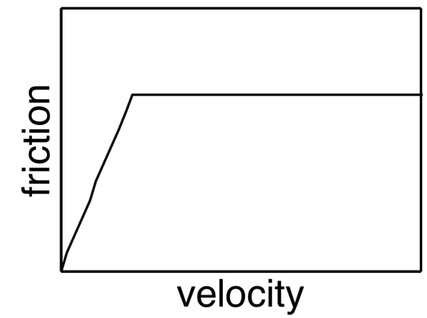
$$\delta_{\mathrm{cc}} = 2r - d$$

$$\mathbf{v}_{\mathrm{diff}} = \mathbf{V} - \mathbf{V}'$$

$$\mathbf{F}_{\mathrm{cc}} = \mathbf{F}_{\mathrm{cc,elas}} + \mathbf{F}_{\mathrm{cc,diss,n}} + \mathbf{F}_{\mathrm{c}}$$

$$\mathbf{T}_{\mathrm{cc}} = (\mathbf{r}_{\mathrm{cc}} - \mathbf{c}) \times \mathbf{F}_{\mathrm{cc}}$$

$$\mathbf{F}_{\mathrm{cc,elas}} = k_{\mathrm{cc}} \sqrt{r}\, \delta_{\mathrm{cc}}^{3/2} \mathbf{n}_d$$ (Hertzian contact force)

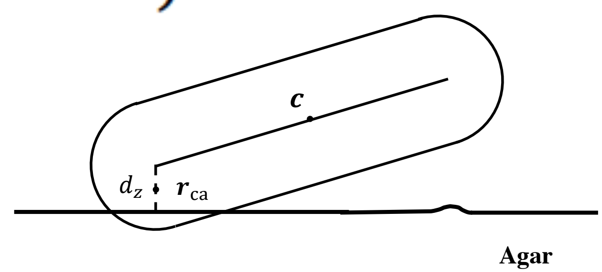


$$\mathbf{F}_{\mathrm{cc,diss,n}} = -\gamma_{\mathrm{cc,n}} M_{\mathrm{eff}} \delta_{\mathrm{cc}} (\mathbf{v}_{\mathrm{diff}} \cdot \mathbf{n}_d) \mathbf{n}_d$$

Tangential force

$$\mathbf{F}_{\mathrm{cc,diss,t}} = -\min\left\{ \gamma_{\mathrm{cc,t}} M_{\mathrm{eff}} \delta_{\mathrm{cc}}^{1/2}, \frac{\mu_{\mathrm{cc}} k_{\mathrm{cc}} \sqrt{r}\, \delta_{\mathrm{cc}}^{3/2}}{|\mathbf{v}_{\mathrm{diff}} - (\mathbf{v}_{\mathrm{diff}} \cdot \mathbf{n}_d)\mathbf{n}_d|} \right\} (\mathbf{v}_{\mathrm{diff}} - (\mathbf{v}_{\mathrm{diff}} \cdot \mathbf{n}_d)\mathbf{n}_d)$$

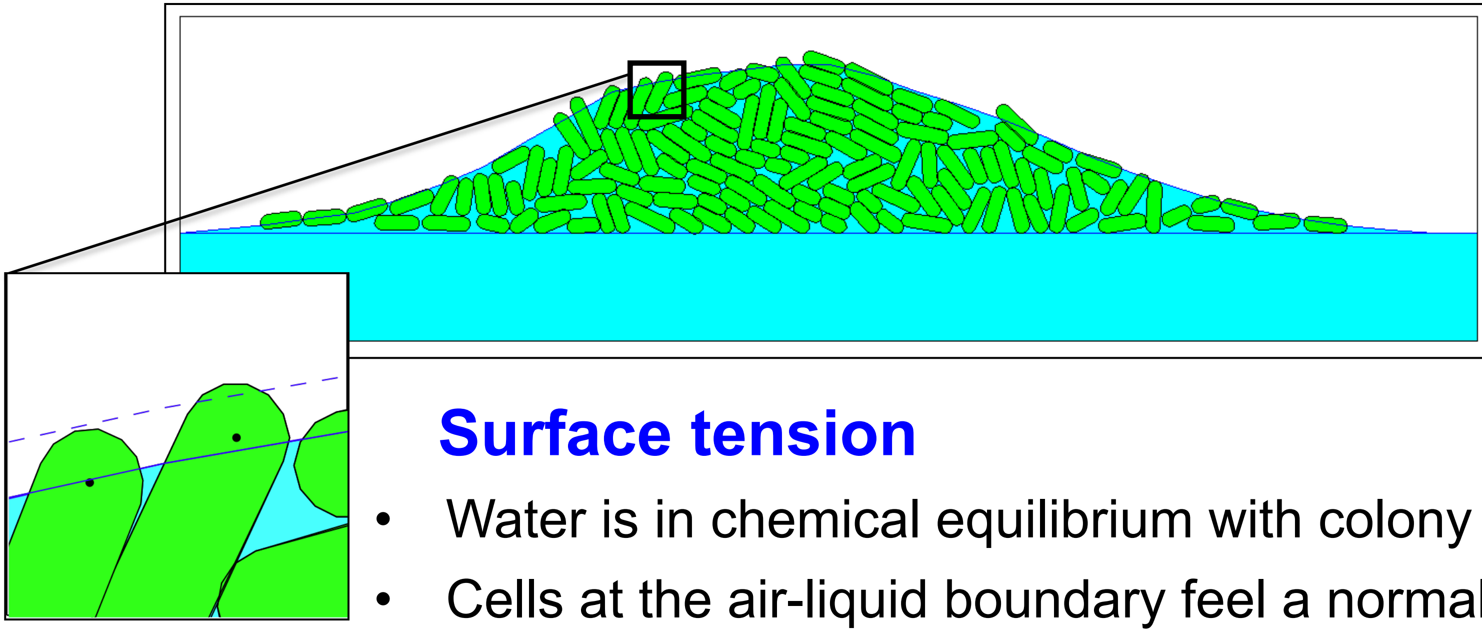
Cell-agar interaction



$$\mathbf{F}_{\mathrm{ca}} = \mathbf{F}_{\mathrm{ca,elas}} + \mathbf{F}_{\mathrm{ca,diss,n}} + \mathbf{F}_{\mathrm{ca,diss,t}}$$

$$\mathbf{T}_{\mathrm{ca}} = (\mathbf{r}_{\mathrm{ca}} - \mathbf{c}) \times \mathbf{F}_{\mathrm{ca}}$$

$$\mathbf{v}_{\mathrm{ca}} = \mathbf{v} + \boldsymbol{\omega} \times (\mathbf{r}_{\mathrm{ca}} - \mathbf{c})$$



Surface tension

- Water is in chemical equilibrium with colony at all times
- Cells at the air-liquid boundary feel a normal force due to <u>cell</u> curvature, not the macroscopic colony curvature
- Agar is also "water" which accounts for contact line

$$\mathbf{F}_{\mathrm{surf}} = \mathbf{F}_{\mathrm{surf,p}} + \mathbf{F}_{\mathrm{surf,q}}$$

$$\mathbf{T}_{\mathrm{surf}} = (\mathbf{p} - \mathbf{c}) \times \mathbf{F}_{\mathrm{surf,p}} + (\mathbf{q} - \mathbf{c}) \times \mathbf{F}_{\mathrm{surf,q}}$$

$$\mathbf{F}_{\mathrm{surf,i}} = 2\pi\gamma_{\mathrm{surf}} \min\left\{\frac{\max\{0, (h_{\mathrm{i}} - z_{\mathrm{i}})\mathbf{n}_s \cdot \mathbf{n}_z + \delta_h\}}{r/5.0}, 1\right\}\mathbf{n}_{\mathrm{s}} \quad (\mathbf{i} = \mathbf{p} \text{ or } \mathbf{q})$$

Viscous force

$\mathbf{F}_{\mathrm{visc}} = -6\pi\mu_{\mathrm{liq}} r \mathbf{v}$ and $\mathbf{T}_{\mathrm{visc}} = -8\pi\mu_{\mathrm{liq}} r^3 \boldsymbol{\omega}$

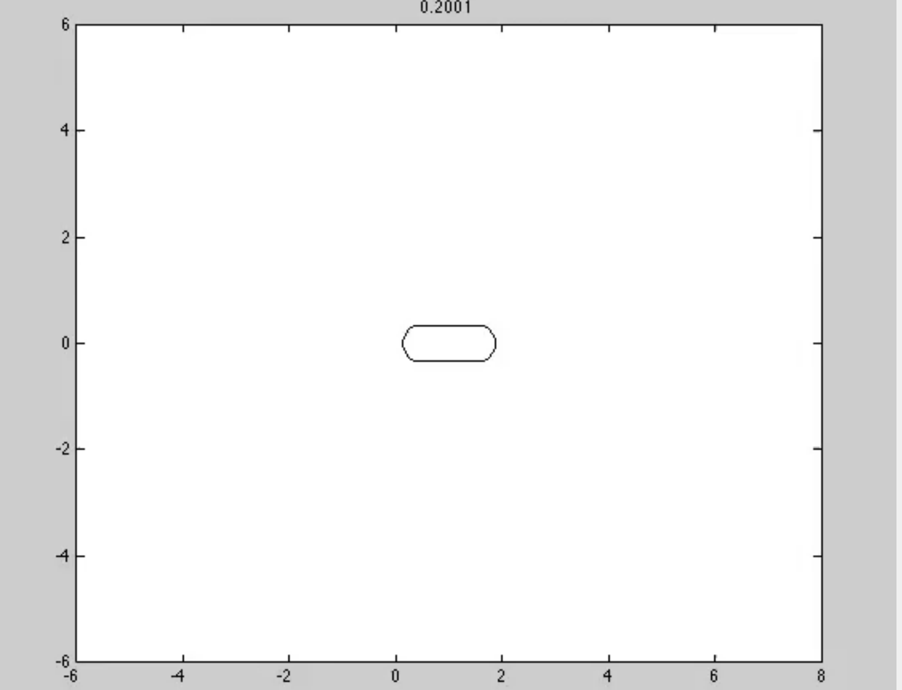
Key Parameters

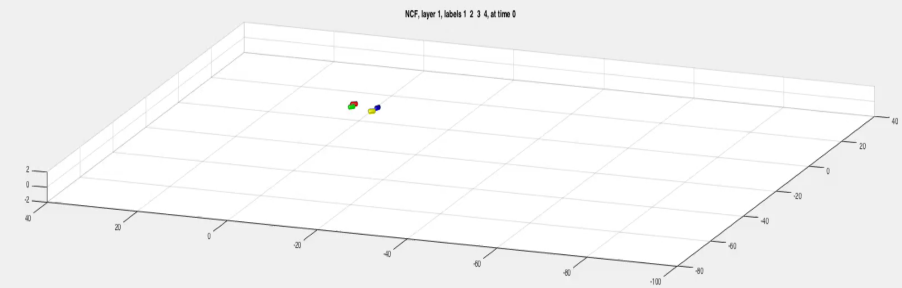
- Maximum growth rate
- The half-growth rate constant in the Monod equation of growth rate
- Constant nutrient concentration in the boundary condition
- Diffusion coefficients for the nutrient
- Friction coefficients
- Viscosity
- Surface tension coefficient

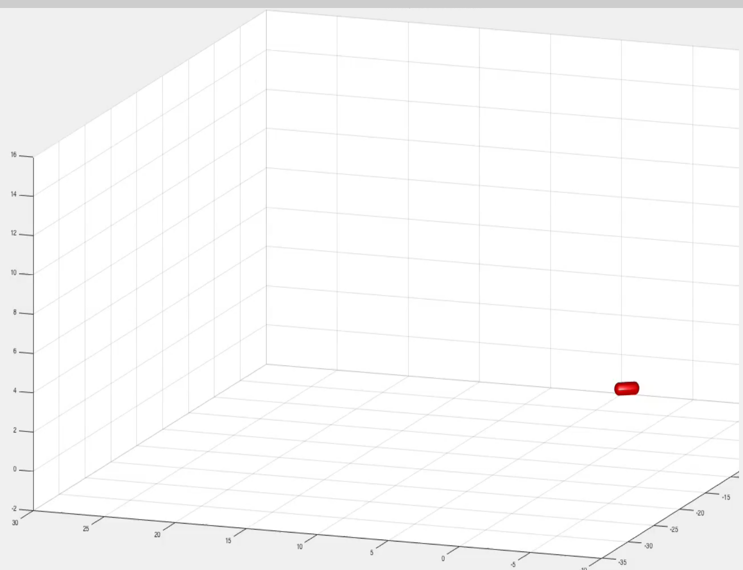
Output of the computational program

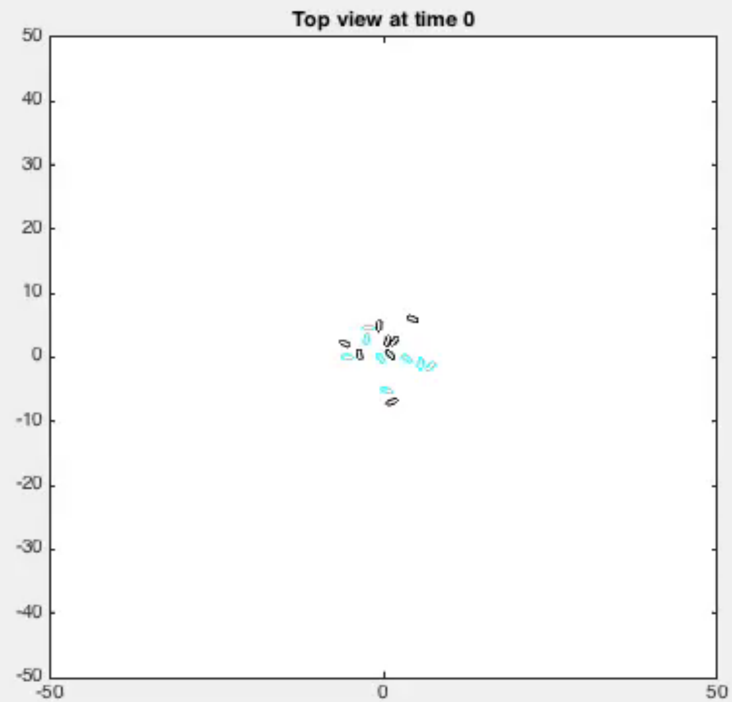
- Cell positions, sizes, and velocities
- Forces exerted on cells
- Nutrient field
- Growth rate

3. Computational Analysis

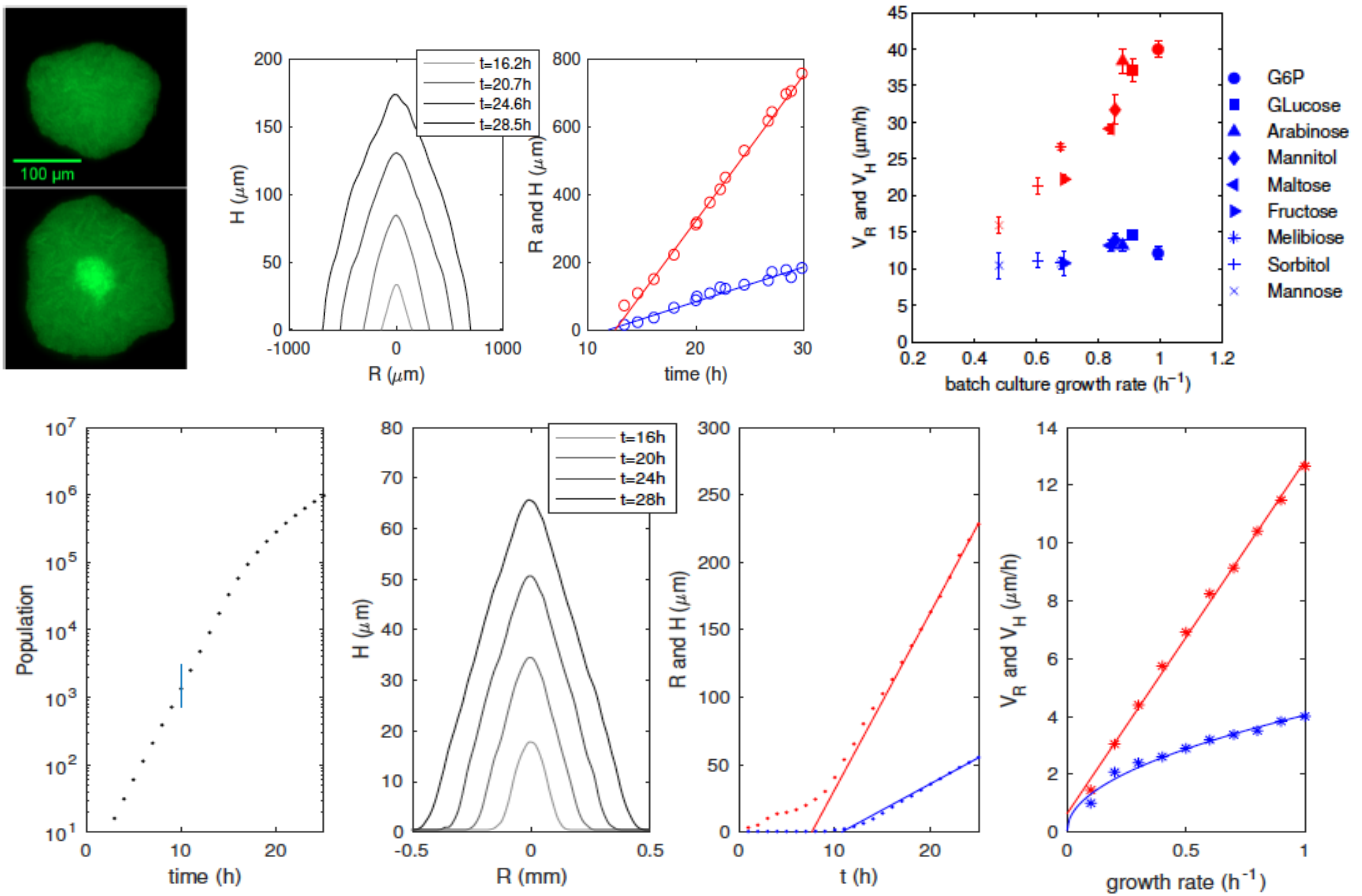

0.2001

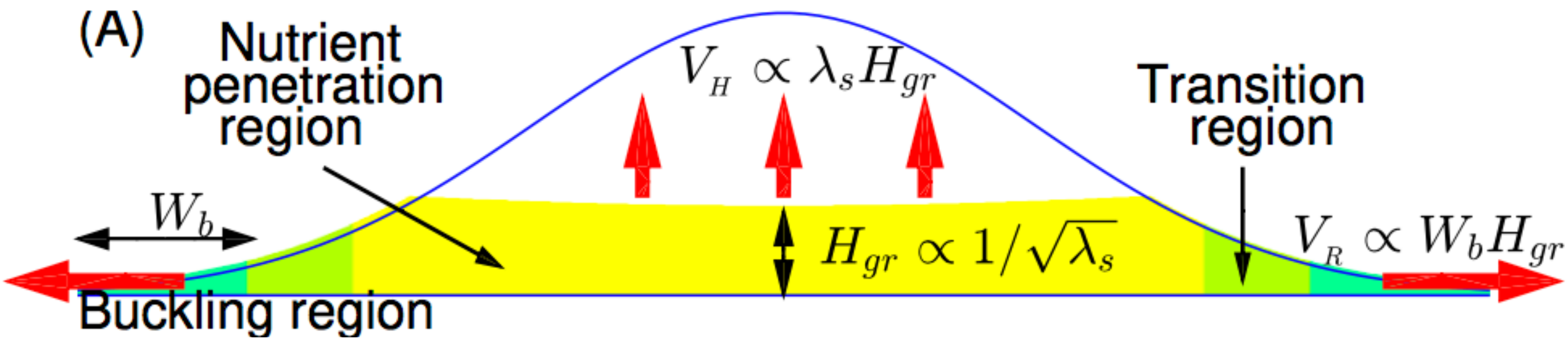

NCF, layer 1, labels 1 2 3 4, at time 0




Top view at time 0

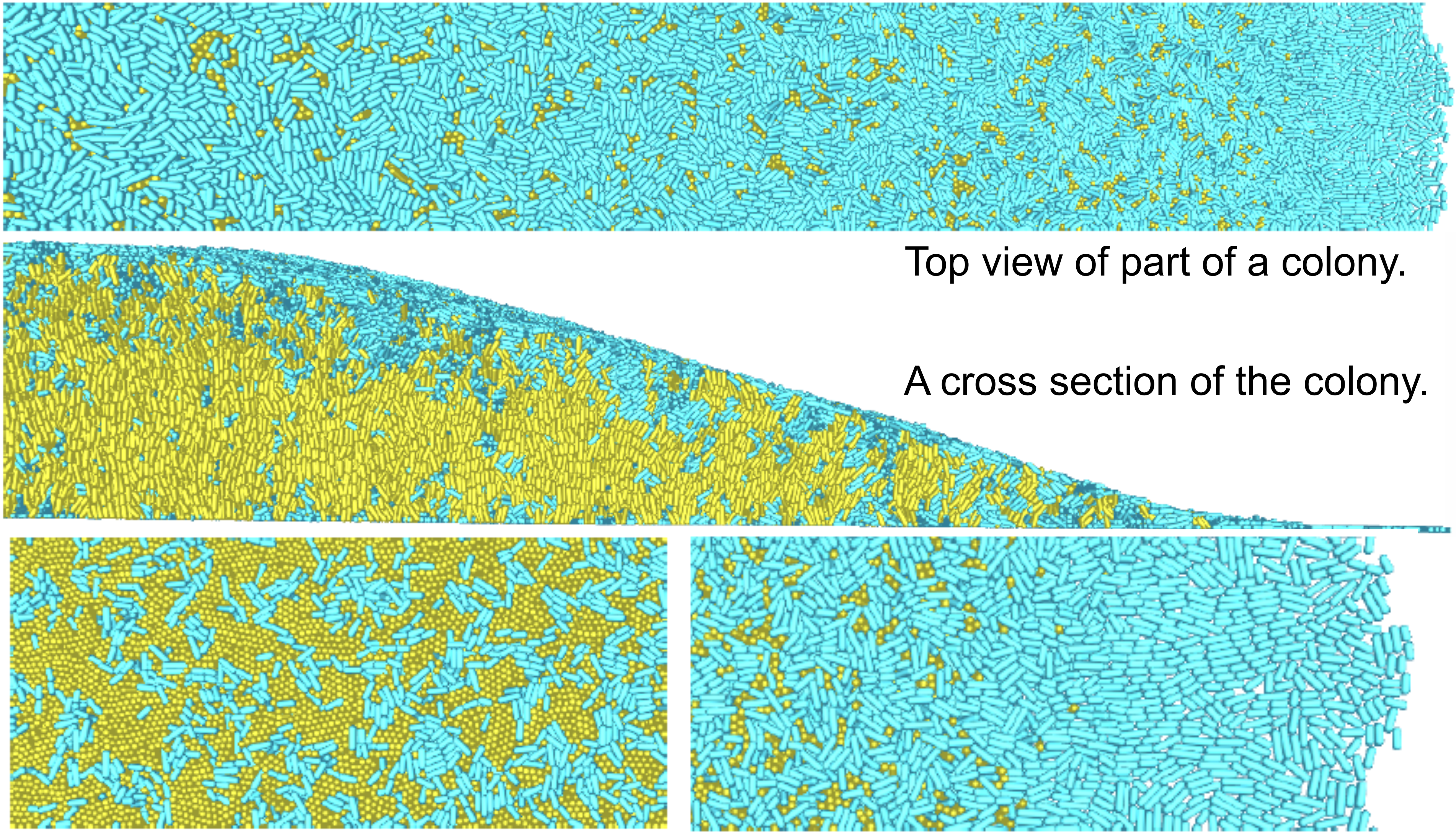
Growth Laws: Experiment (top) and Simulations (bottom)





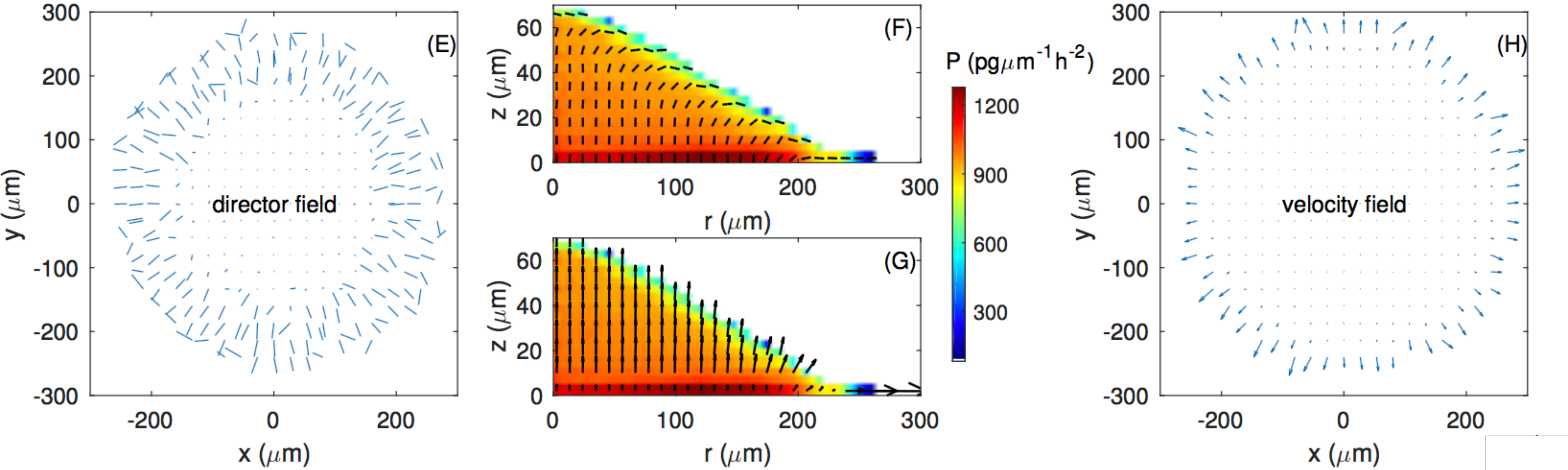
(A)
Nutrient penetration region
$V_H \propto \lambda_s H_{gr}$
Transition region
W_b
$H_{gr} \propto 1/\sqrt{\lambda_s}$
$V_R \propto W_b H_{gr}$
Buckling region

Cyan cells: large angles with the z-axis. Golden cells: smaller angles.

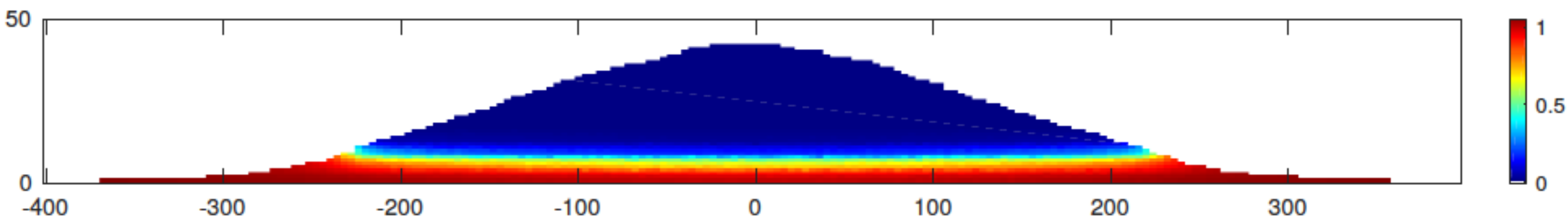


Bottom view of the part of colony close to the center.

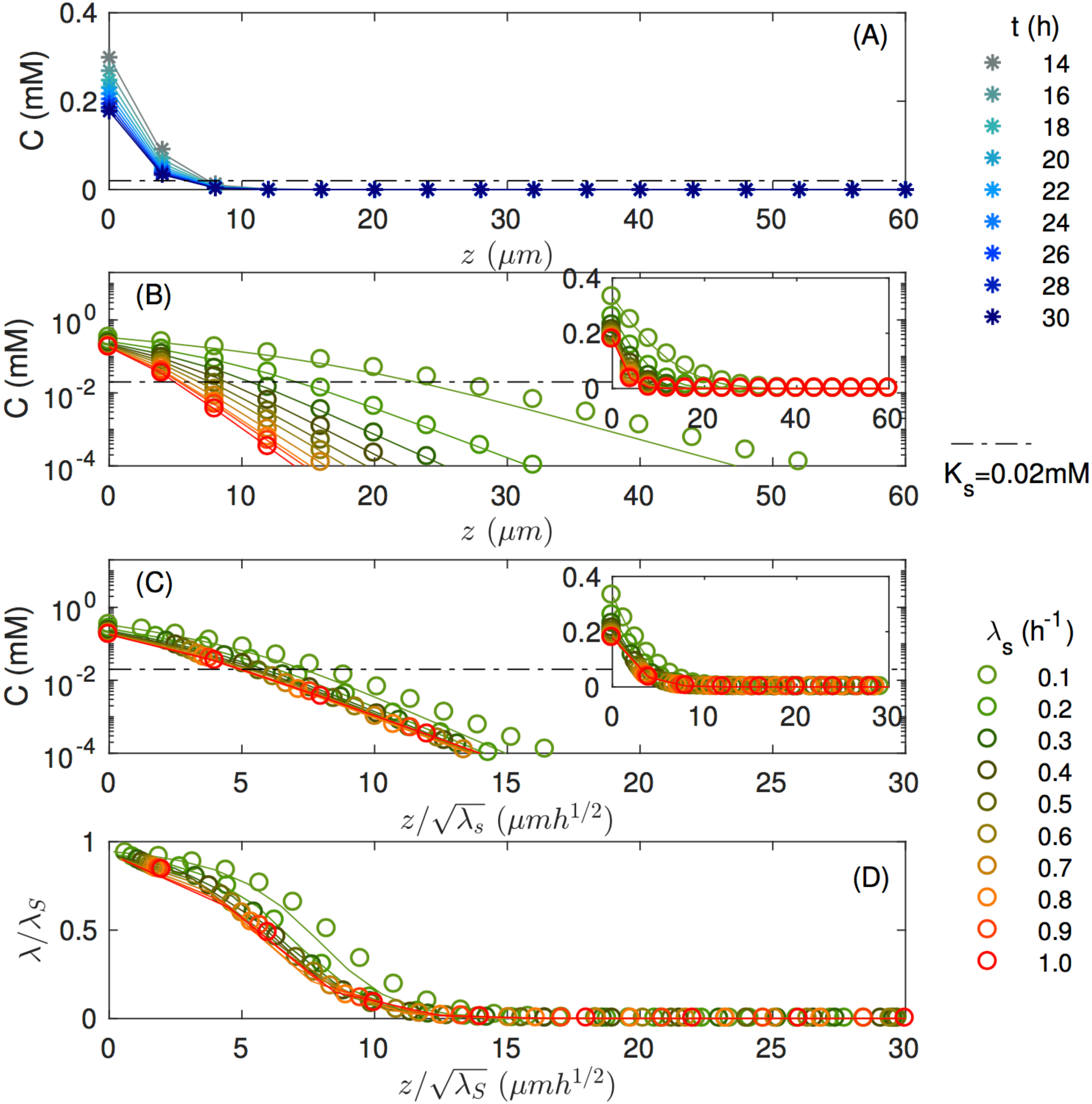
Bottom view of the part of colony close to the periphery.



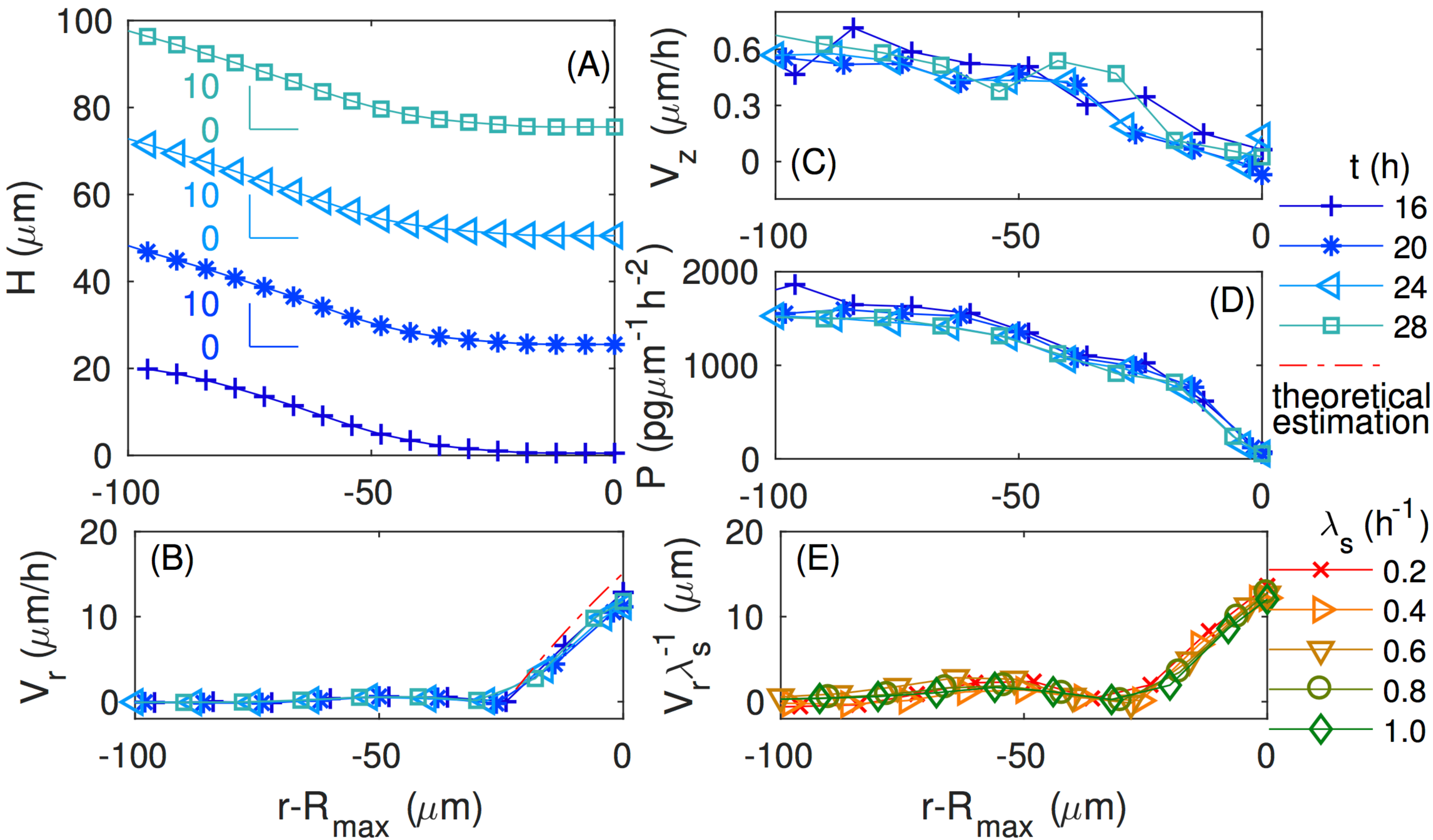
Bottom view of director field (left), velocity field (right), and their azimuthal averages (middle).



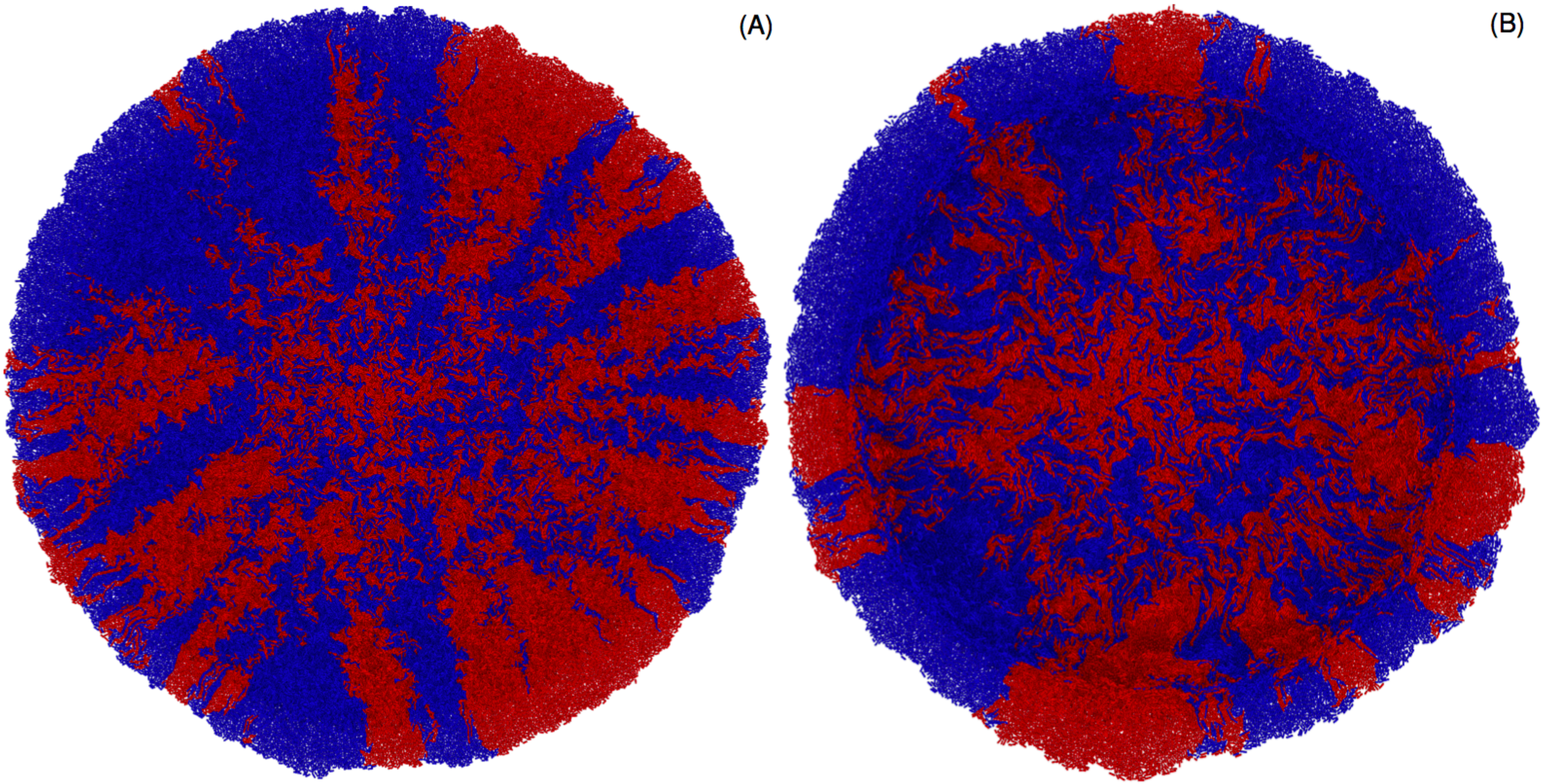
Cross section of the local growth rate, showing that only the bottom few layers are growing.

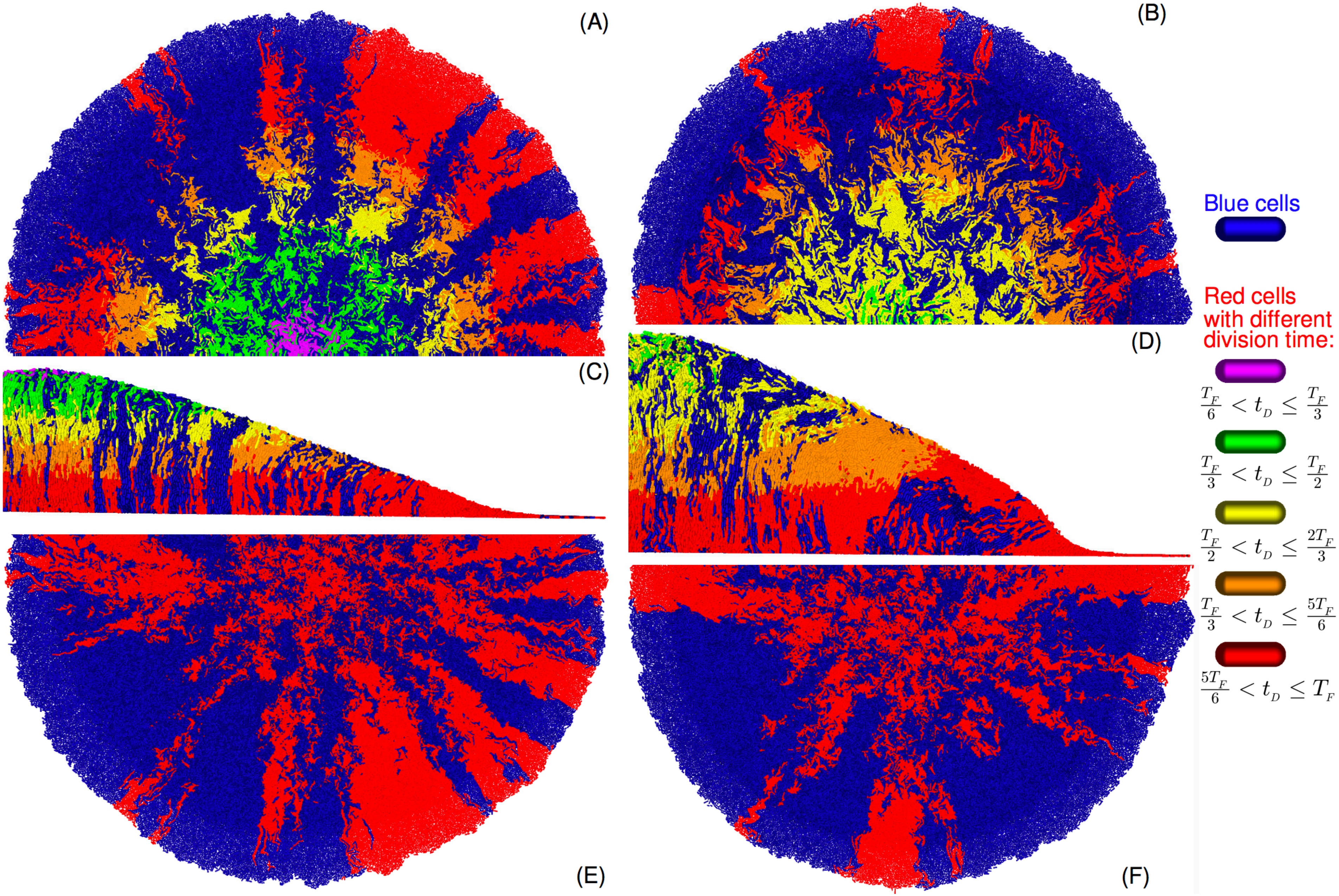


C (mM)
(A)
z (μm)
t (h)
14
16
18
20
22
24
26
28
30
(B)
$z\ (\mu m)$
K_s=0.02mM
(C)
$z/\sqrt{\lambda_s}\ (\mu m h^{1/2})$
$\lambda_s\ (h^{-1})$
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
(D)
λ/λ_S
$z/\sqrt{\lambda_S}\ (\mu m h^{1/2})$



(A)
H (μm)
10
0
(B)
V_r (μm/h)
r-R_max (μm)
(C)
V_z (μm/h)
(D)
P (pgμm^-1 h^-2)
(E)
V_r λ_s^-1 (μm)
r-R_max (μm)
t (h)
16
20
24
28
theoretical estimation
λ_s (h^-1)
0.2
0.4
0.6
0.8
1.0

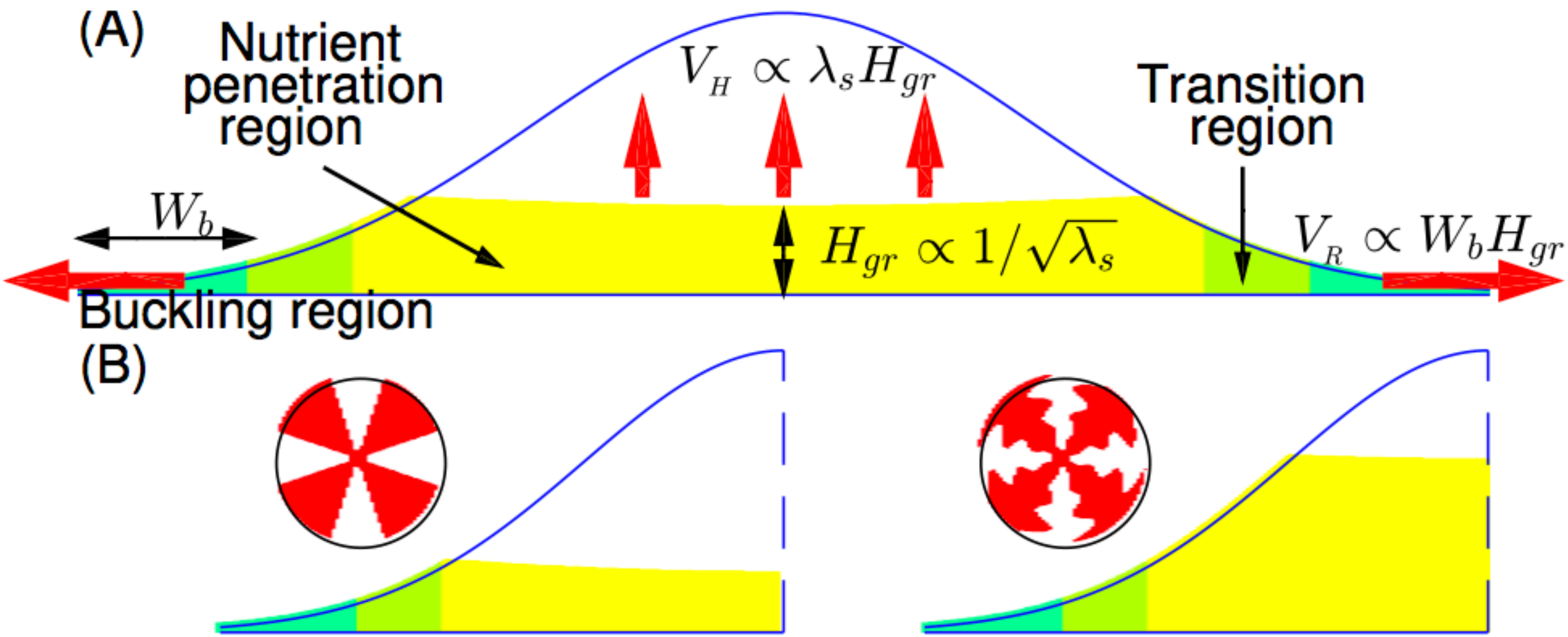

(A)
(B)



(A)
(B)
(C)
(D)
(E)
(F)
Blue cells
Red cells with different division time:
$\frac{T_F}{6} < t_D \leq \frac{T_F}{3}$
$\frac{T_F}{3} < t_D \leq \frac{T_F}{2}$
$\frac{T_F}{2} < t_D \leq \frac{2T_F}{3}$
$\frac{T_F}{3} < t_D \leq \frac{5T_F}{6}$
$\frac{5T_F}{6} < t_D \leq T_F$

5. Conclusions

Summary



(A)
Nutrient penetration region
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W_b
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$V_R \propto W_b H_{gr}$
Buckling region
(B)

Current and Future Work

- Include multiple species of cells; different types of nutrient; oxygen; wastes; etc.
- Parallel computation.
- Derivation of continuum model from cell-cell interactions; other continuum models such as kinetic equation models; roles of fluctuations; etc.
- Explain the experimental observation of the range expansion (i.e., cross feeding with multiple types of cells).
- Biofilms: extracellular matrix, wrinkles, turbulence, etc. Design and conduct new experiment for comparison.

THANK YOU!