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Slide of the Seminar

On Growth and Form of Microorganisms on Liquid Substrates

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ERC Advanced Grant (N. 339032) "NewTURB"
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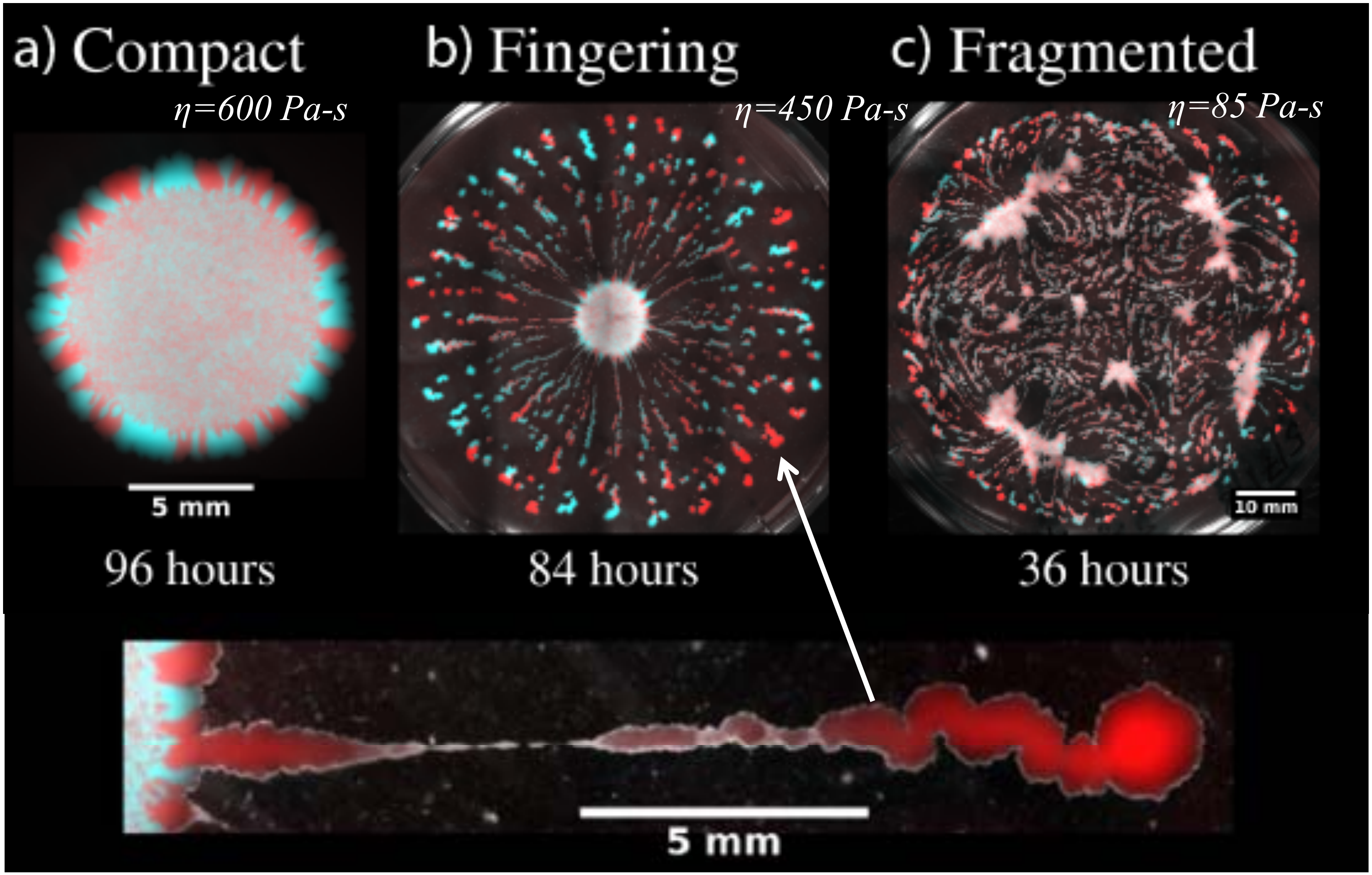
Microbial range expansions of close-packed reproducing yeast cells on <u>liquid</u> substrates



Life probably evolved first in a *liquid* environment

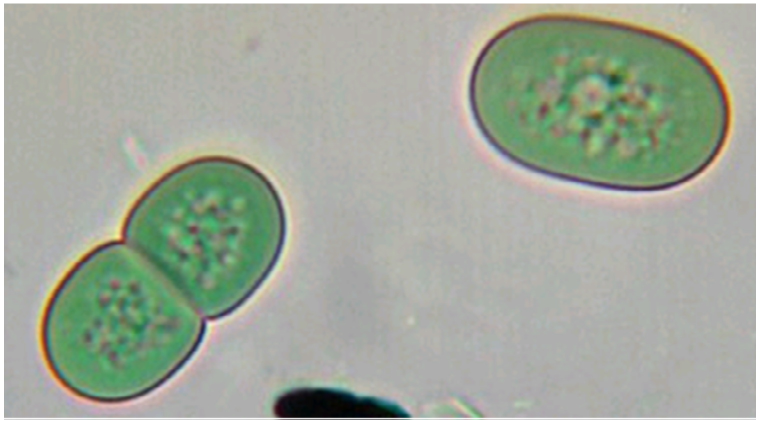
•~2-3 billion years ago, like today, water covered most of the earth

•Fossilized, oxygen-producing cyanobacteria have been dated at ~2 billion years ago.

•Oxygenic cyanobacteria transformed the atmosphere via photosynthesis

•Their spatial growth and evolutionary competition took place in <u>liquid</u> environments at both high and low Reynolds numbers

•These photosynthetic organisms control their height to resist down welling currents and stay close to the ocean or lake surface.



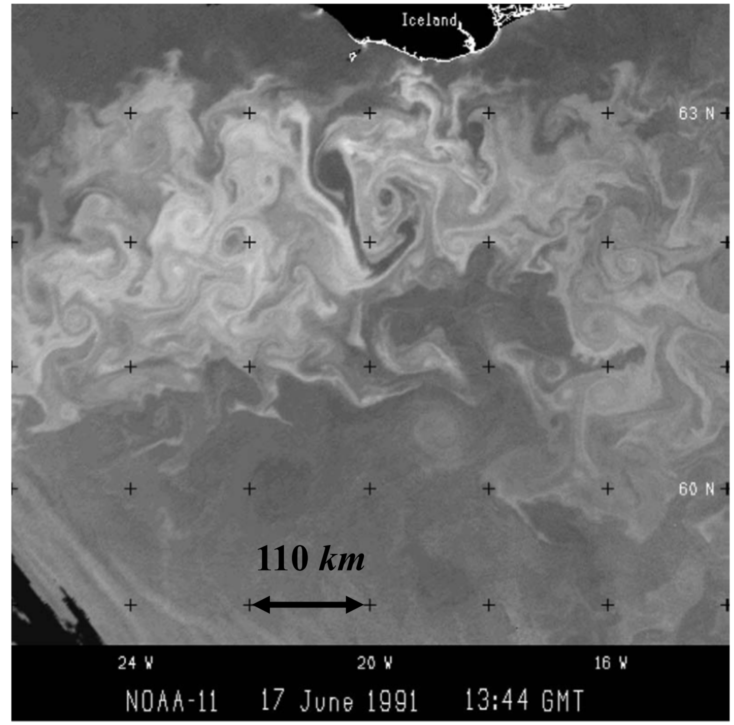
Cyanobacterium *Synechococcus*
www.dr-ralf-wagner.de/Blaualgen-englisch.htm

Bloom of cyanobacteria in Lake Atitlán, Guatemala
NASA Earth observatory

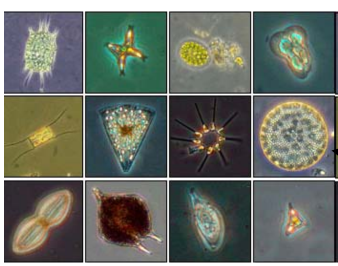
Striated plankton populations in oceanic flows

Phytoplankton blooms at high Reynolds number in the Norwegian Sea and near Iceland

http://visibleearth.nasa.gov/cgi-bin/viewrecord?5278
.see also, Tel. et al. Phys. Rep. **413**, 91 (2005).



A. P. Martin, Prog. Oceanography **57**, 125 (2003)



mixing layer ≈ *25-100* m.

Phytoplankton
(see also zooplankton
& bacterioplankton)

http://earthobservatory.nasa.gov/Experiments/ICE/Channel_Islands/

$\mathrm{Re} = LU/\nu = 10^8 - 10^9$

Large eddy turnover time ≈ 50 days

Small eddy turnover time ≈ 5 minutes

Plankton doubling time ≈ 12-24 hours

Compressible advection of microorganism density *c(x,t)*

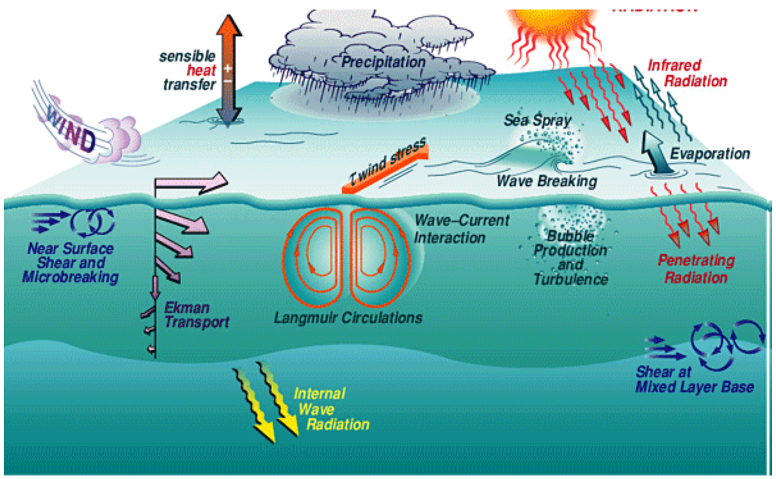
$$\frac{\partial}{\partial t}c(\vec{x},t)+\nabla\cdot[\vec{u}(\vec{x},t)c(\vec{x},t)]=D\nabla^2 c(\vec{x},t)+\mu c(\vec{x},t)[1-c(\vec{x},t)]$$

$$\vec{\nabla}\cdot\vec{u}(\vec{x},t)\neq 0$$

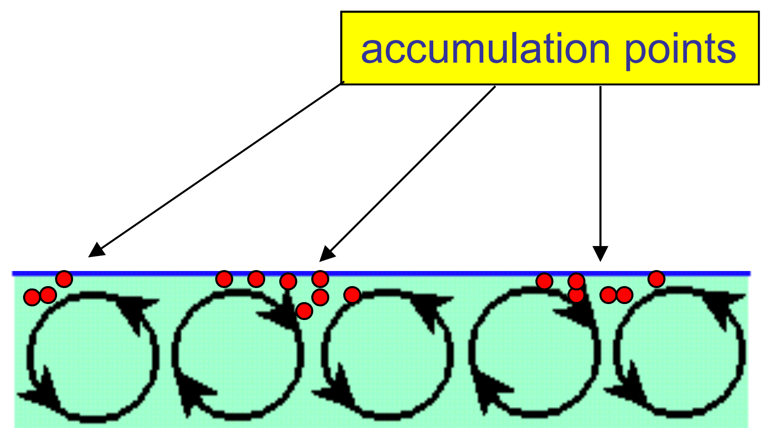
$u(\vec{x},t)$ is an effective $2d$ compressible turbulent velocity field....

μ is the growth rate...

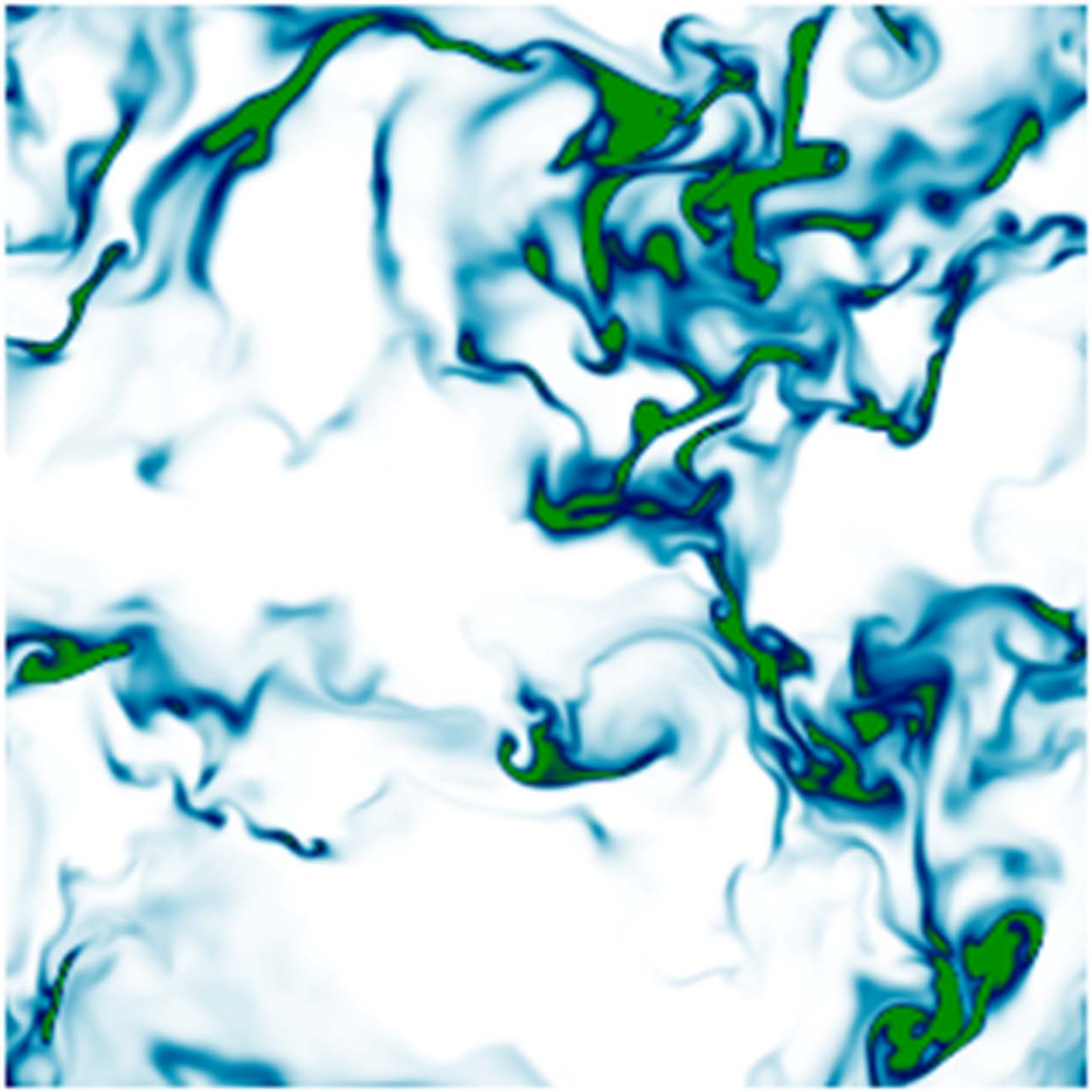
Advection by an effectively compressible two dimensional velocity field results for organisms that actively control their buoyancy to stay close to the ocean surface.



Uop.whoi.edu/projects/projects.htm



Buoyant population dyanamics in Silico (Perlekar, Toschi, Benzi, drn)



$$\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \vec{\nabla} \vec{u} = -\frac{1}{\rho} \vec{\nabla} p + \nu \nabla^2 \vec{u} + \vec{f}$$

project onto a 2d plane $\rightarrow \vec{\nabla} \cdot \vec{u}_{2d} \neq 0$

$$\frac{\partial c}{\partial t} + \nabla \cdot (\vec{u}_{2d} c) = D \nabla^2 c + \mu c (1 - c)$$

Reynolds number

$$Re = \frac{u_{\rm rms} L}{\nu}$$

Schmidt number

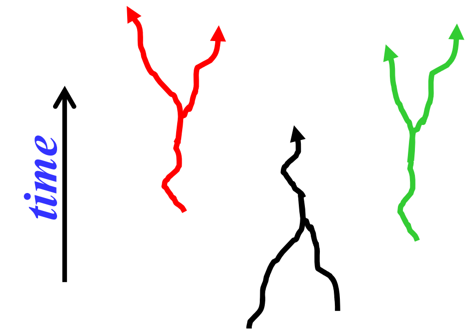
$$Sc = \frac{\nu}{D}$$

Doubling time/eddy turnover time

$$\tau_2 / \tau_{eddy} \sim 1/(\mu \tau_{eddy})$$

Compressible population genetics with two interacting species

Compressible turbulent flow (Re ~10^5) $\kappa = \langle (\vec{\nabla} \cdot \vec{u})^2 \rangle / \langle (\partial_i u_j)^2 \rangle = 0.17$



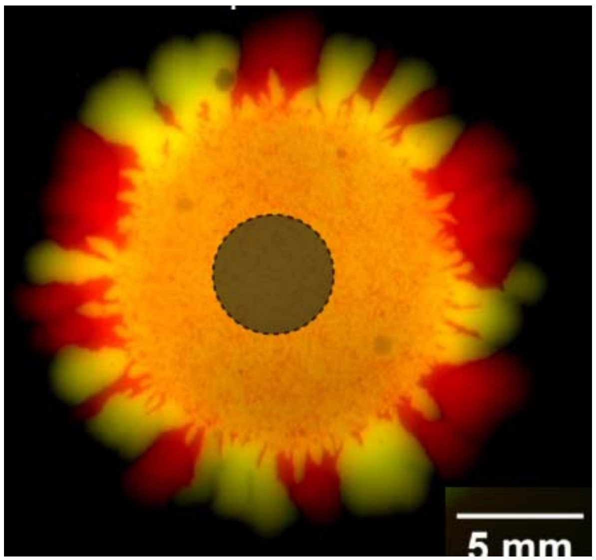
Agent-based simulation:
"Survival of the luckiest"

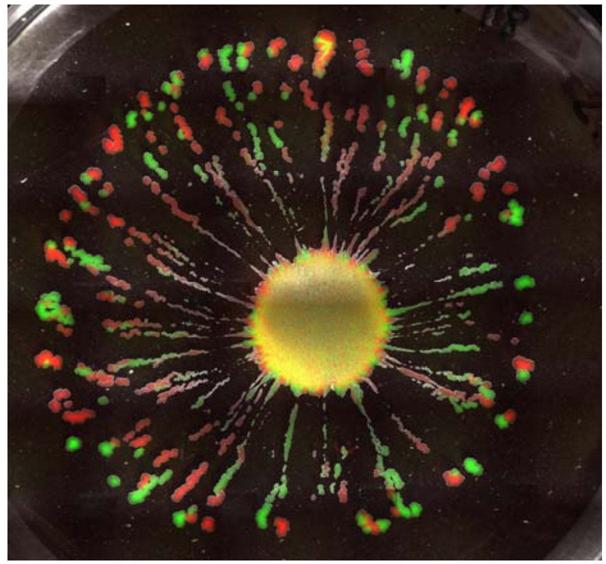
Wanted: simple repeatable experiments that explore how fluid flows affect spatial population genetics….

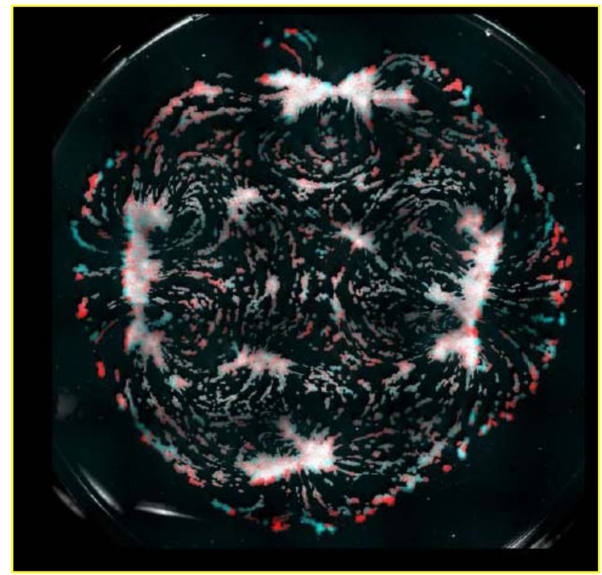
- ***High Reynolds numbers might be hard to achieve in the laboratory, but low Reynolds numbers can also be biologically relevant***
- ***Can we impose a flow such that the doubling time $\tau_2 \ll \tau_{eddy}$, where τ_{eddy} is a eddy turnover time?***

On Growth and Form of Microorganisms on <u>*Liquid*</u> Substrates

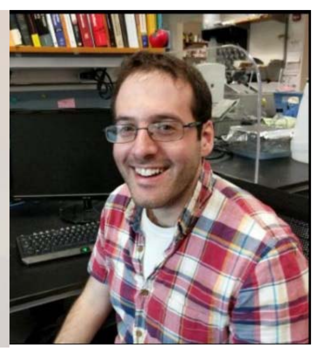
"Microbes on the surface of a highly viscous liquid generate buoyant flows that alter colony morphology and evolutionary dynamics"



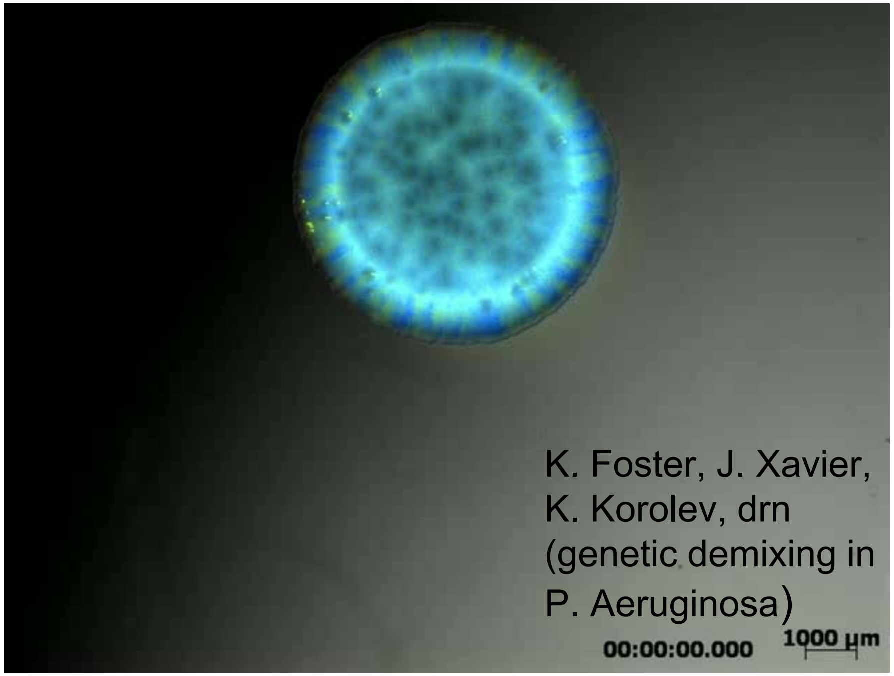


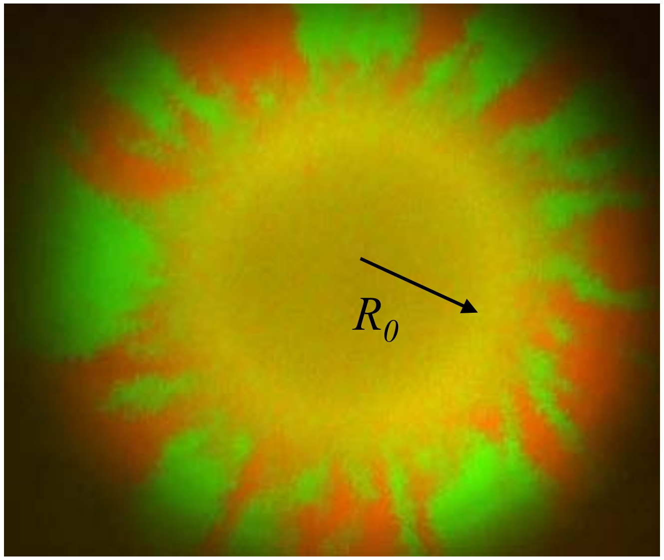


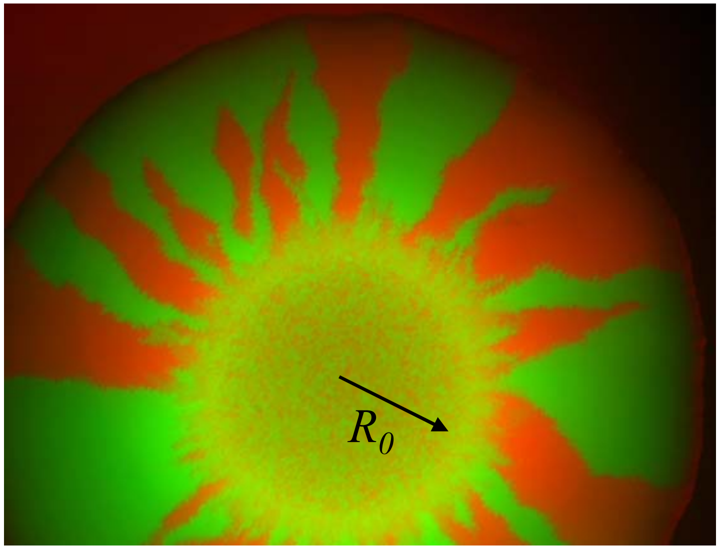
Severine Atis
Bryan Weinstein
Andrew Murray

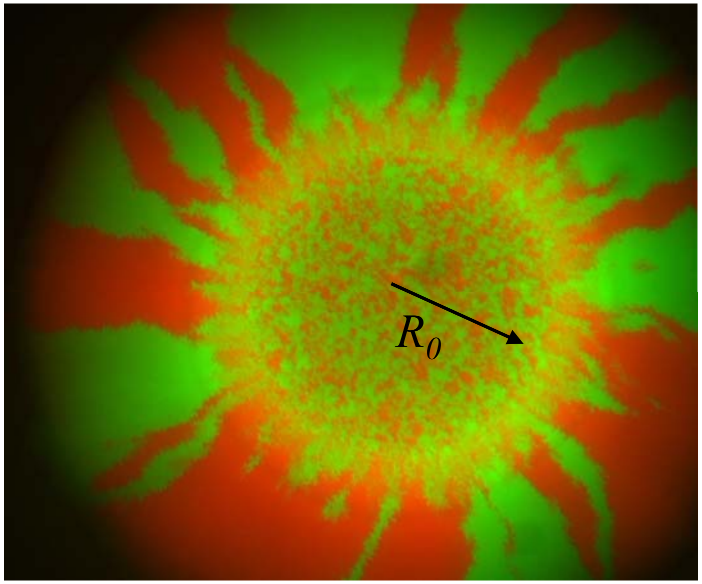


Experiments on hard agar plates: *P. aeruginosa* & *E. coli*



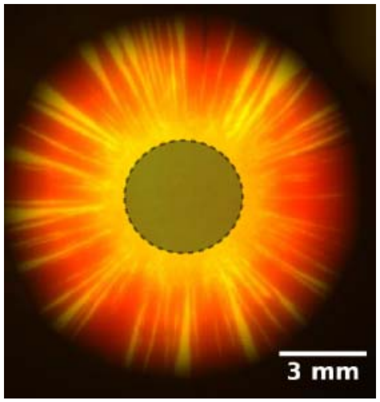






Microorganisms grown on liquid but highly viscous substrates create their own flows (without pumps and syringes!)

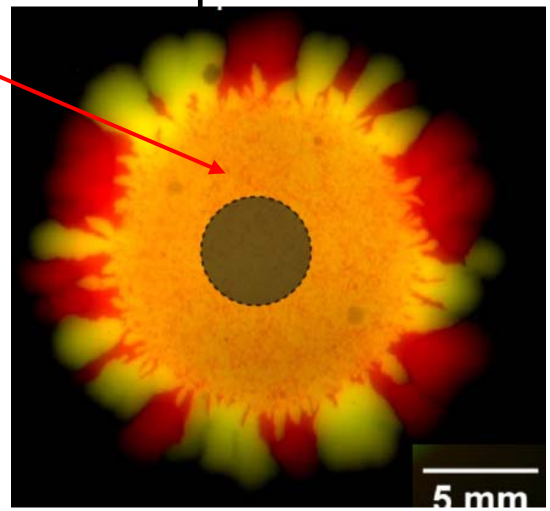
Hard Agar



Yeast on a 1% hard agar YPD plate (viscosity $\eta = \infty$)

Epoch of genetic demixing stretched out….

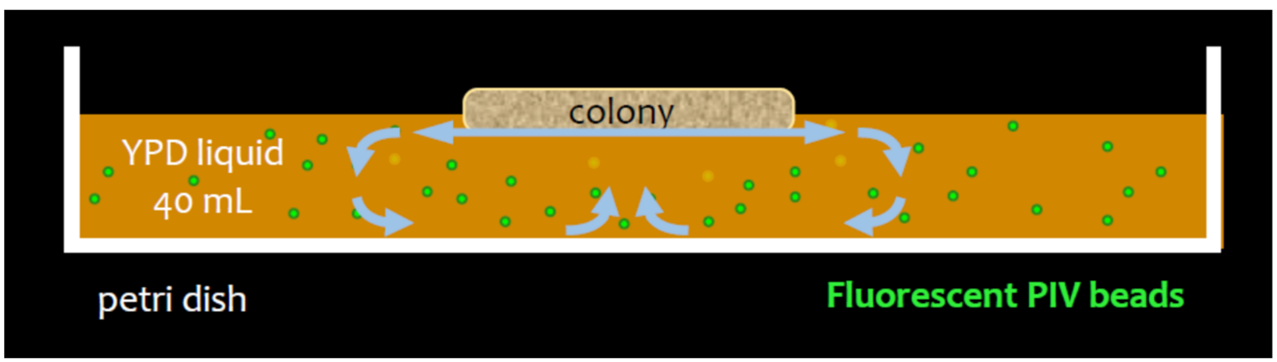
Liquid Media



Yeast on a liquid but highly viscous YPD media with 3% cellulose ($\eta \approx 600$ Pa-s)

Cellulose % (w/v)	Viscosity (Pa·s)
1.8	22 ± 3
2.0	51 ± 6
2.2	81 ± 9
2.4	120 ± 10
2.6	340 ± 50

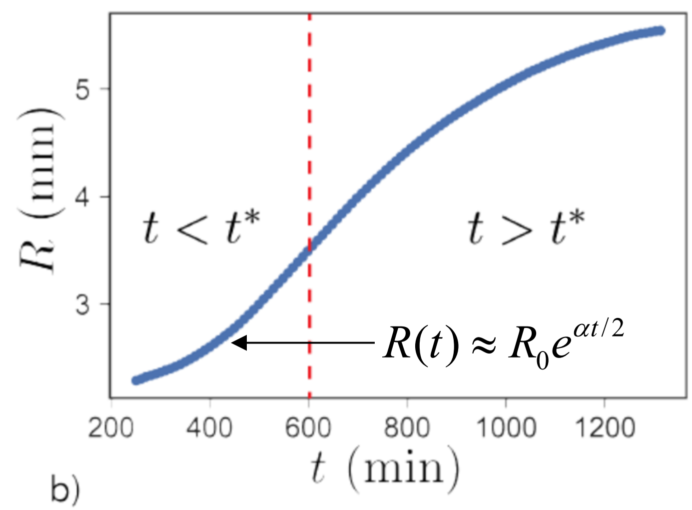
(the viscosity of water is $\eta \approx 10^{-3}$ Pa-s; our viscosities are $10^4 - 10^5$ times larger)



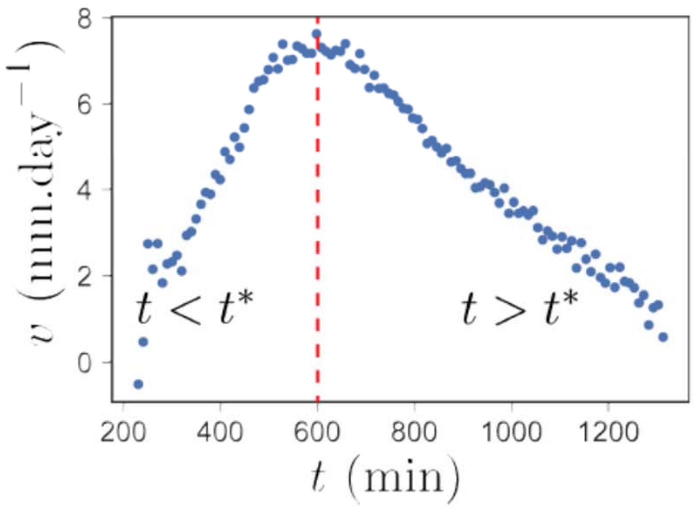
The colony itself generates flows that dilate the colony radially!

We find initial exponential growth for t < t*, followed by a gradually slowing down & genetic demixing at the frontier

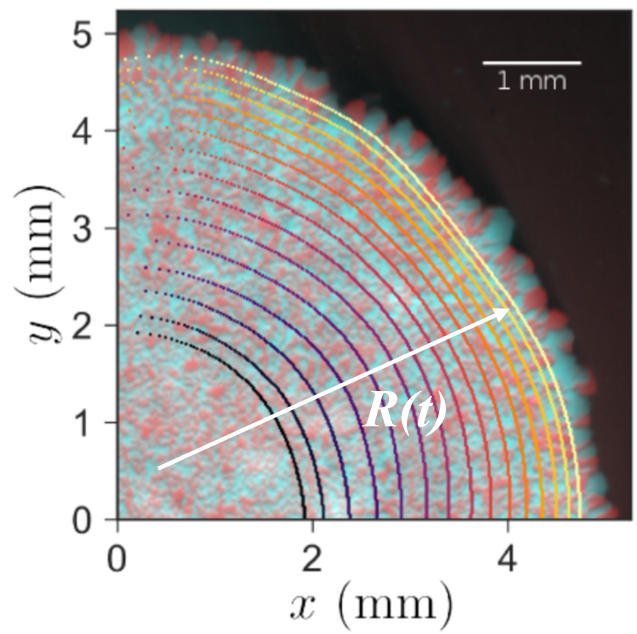
a)



b)



c)



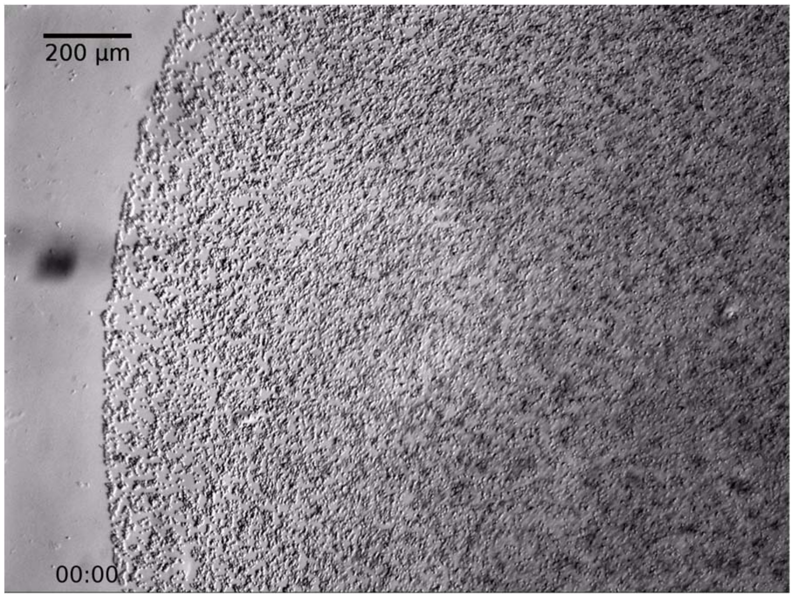
a) Radially averaged yeast colony radius R(t) during the first 24h of growth on a high viscosity liquid substrate with η =600 Pa-sec.

b) The colony front velocity v(t) extracted from R(t), exhibiting: (1) an approximately exponential phase for t < t and (2 a slowly decaying velocity over time for t > t.

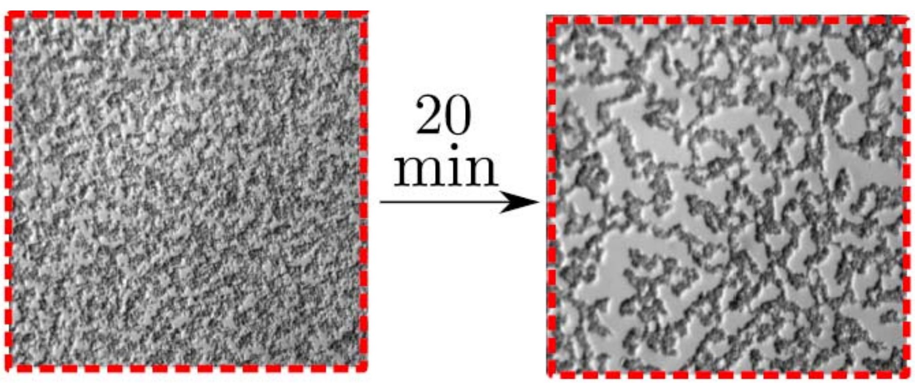
c) Consecutive front spatial positions at 40 min intervals during the first 24h of growth.

As the time since inoculation elapses, microorganisms on liquid substrates can behave like gases, liquids or solids….

Example 1: Early time behavior exhibits gas-liquid phase separation

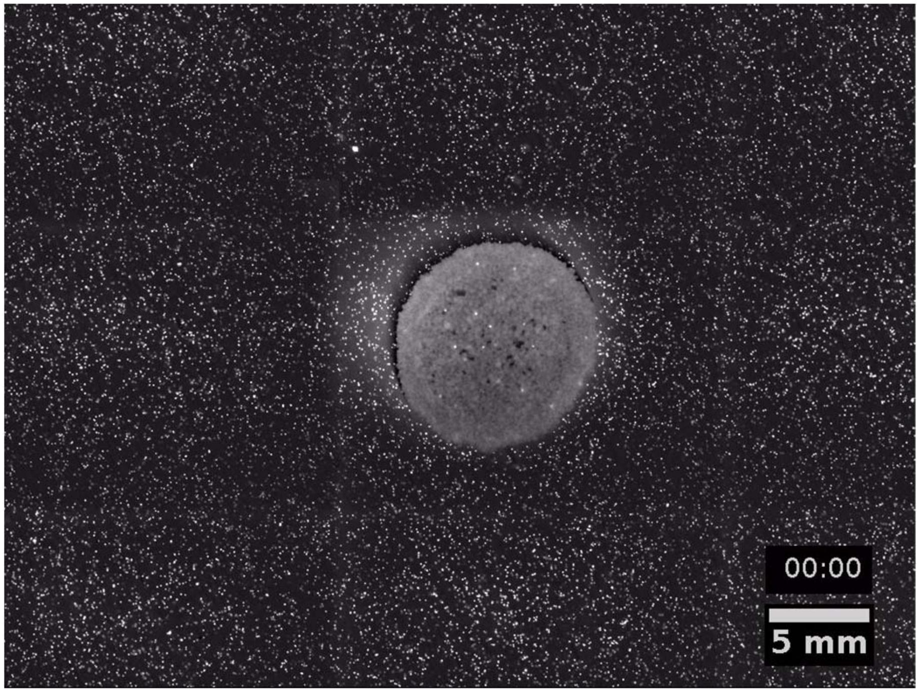


D. Vella and L. Mahadevan, American Journal of Physics 73.9 (2005): 817-825.

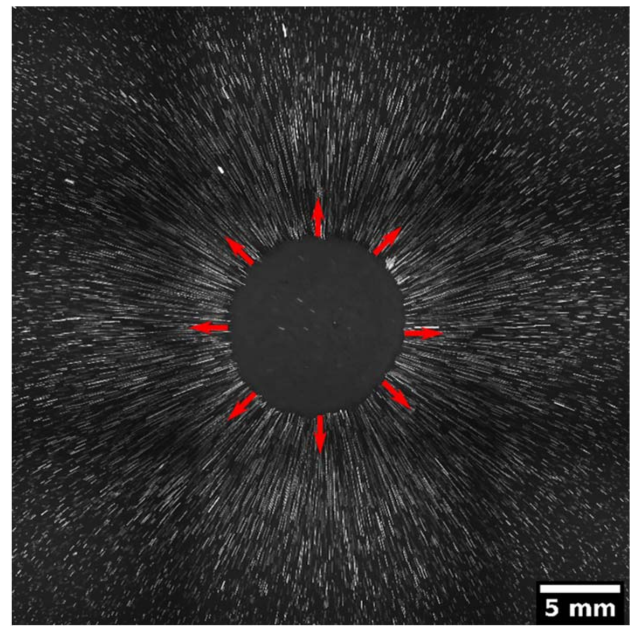


Coarsening or "spinodal decomposition"….

24-48 hours, $\eta \approx 600$ Pa-S



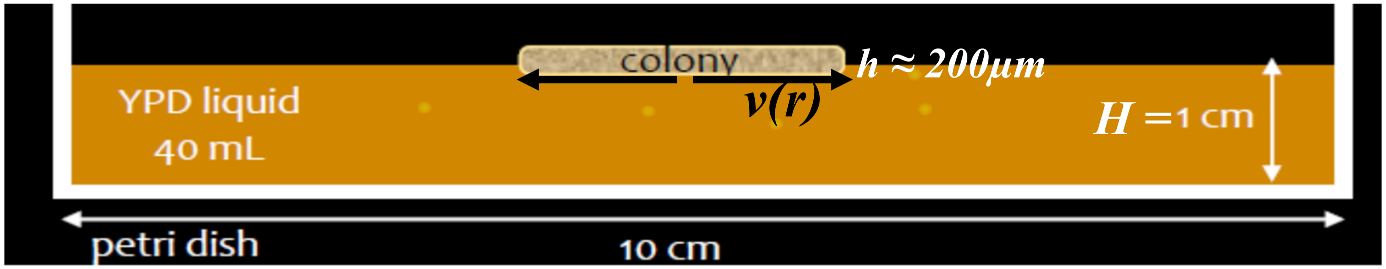
Motion of fluorescent beads around a mature colony reveals that fluid motion is generated beneath the growing colony



One early time mechanism for radial motion is outward pushing when all cells at the interface are actively dividing….

Deformations of features inside colony in a liquid-like regime consistent with a dilational flow (η = 600 Pa-sec)

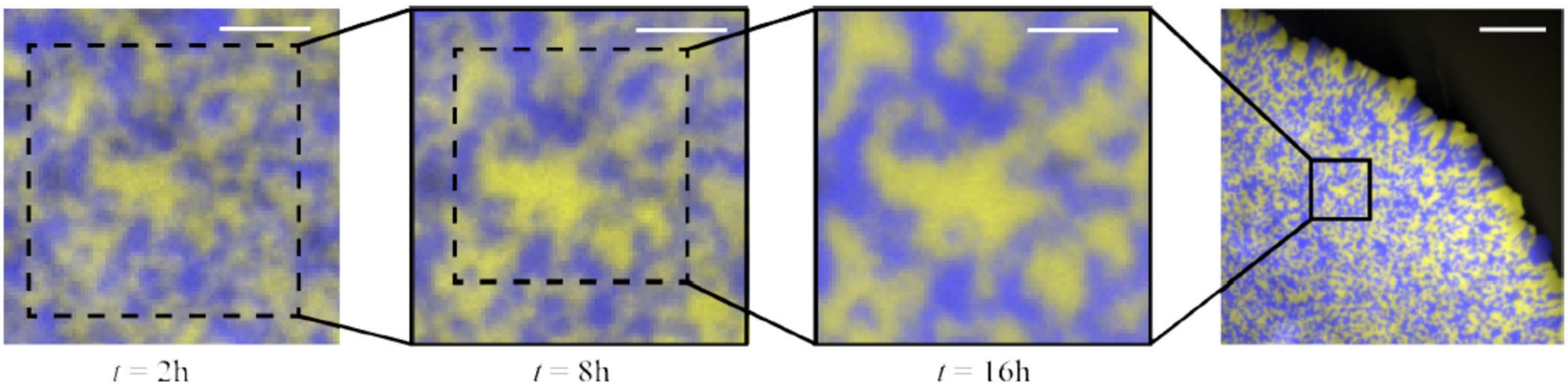
Colony features dilate as if inscribed on an inflating balloon….



Simple model of 2d colony dynamics: $\dfrac{\partial \rho_{2d}}{\partial t} + \vec{\nabla} \cdot (\rho_{2d} \vec{v}_{2d}) = \alpha_1 \rho_{2d}$, ρ_{2d} = cell density

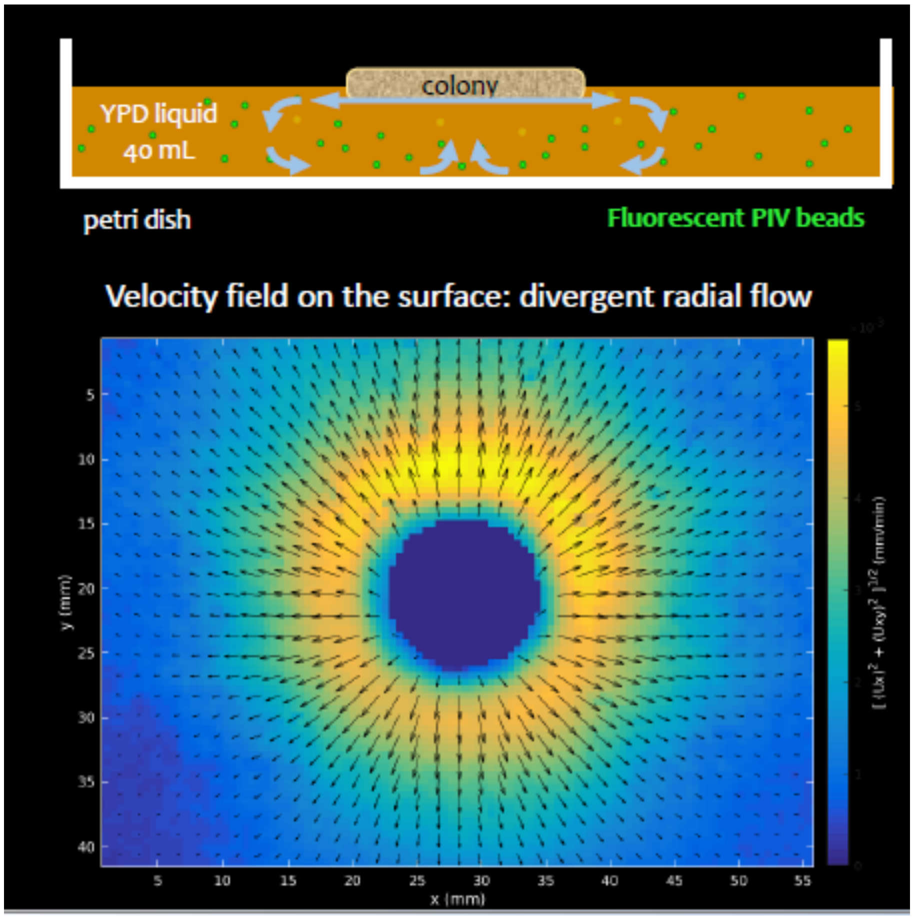
α_1 = growth rate $\rightarrow \vec{\nabla} \cdot \vec{v}_{2d}(r) = \alpha_1$; assume overdamped liquid-like colony dynamics:

$0 \approx -\vec{\nabla} p_{2d} - \gamma \vec{v}(\vec{r}))$; $\gamma = \eta_s / hH$; $\rightarrow$ $\boxed{\vec{v}_{2d}(\vec{r}) \approx \dfrac{1}{2} \alpha_1 r \hat{r}}$ dilational velocity field

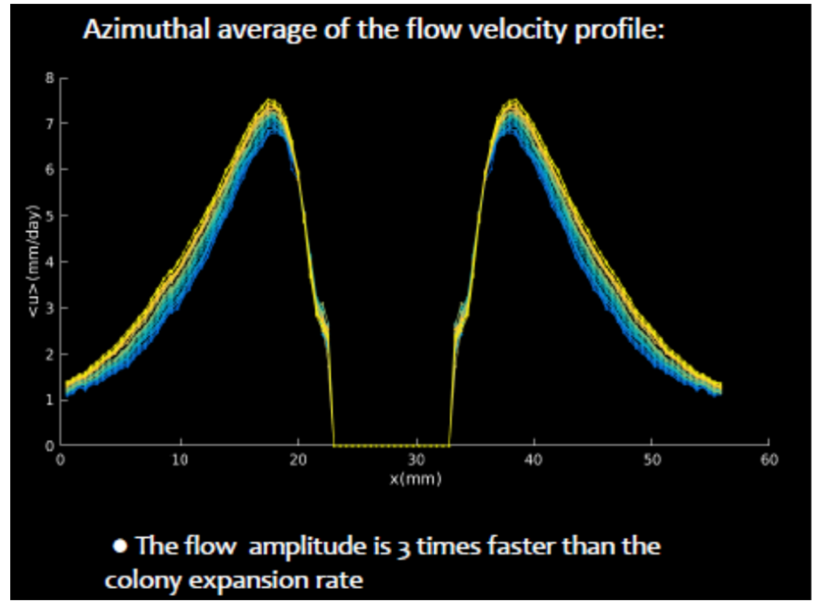


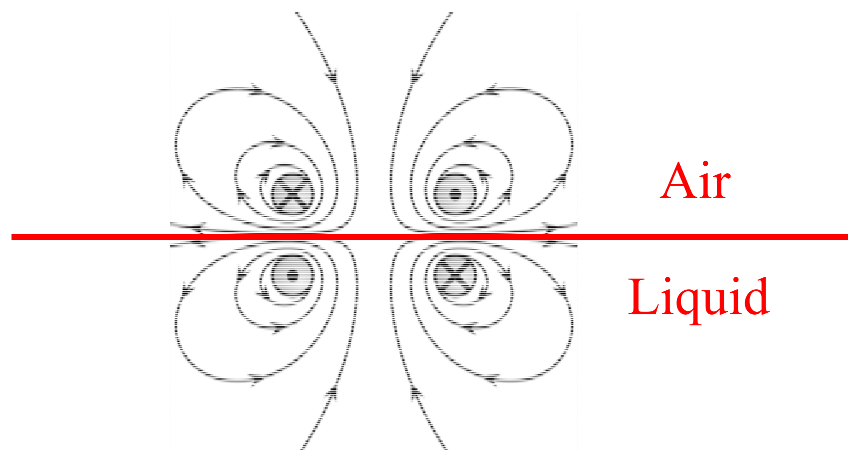
The first three images have the same scale bar = 100 µm. The final picture, with scale bar 500 µm, shows the same feature at the much larger colony scale

In addition to simple outward pushing due to excluded volume interactions, we find a metabolically-induced vortex ring under the colony, enhancing the radial growth rate



$$\Rightarrow \vec{v}(\vec{r}) \approx \frac{1}{2}\alpha_2 r\hat{r}$$

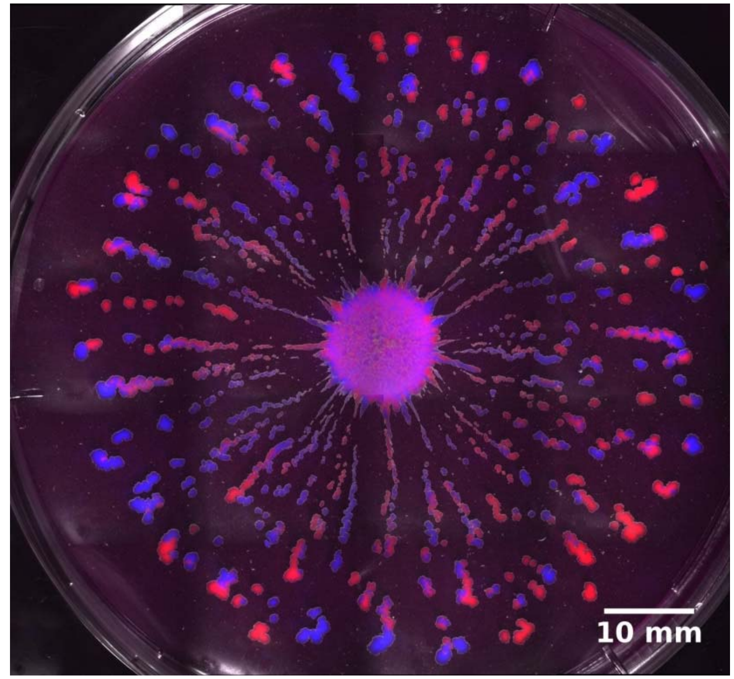




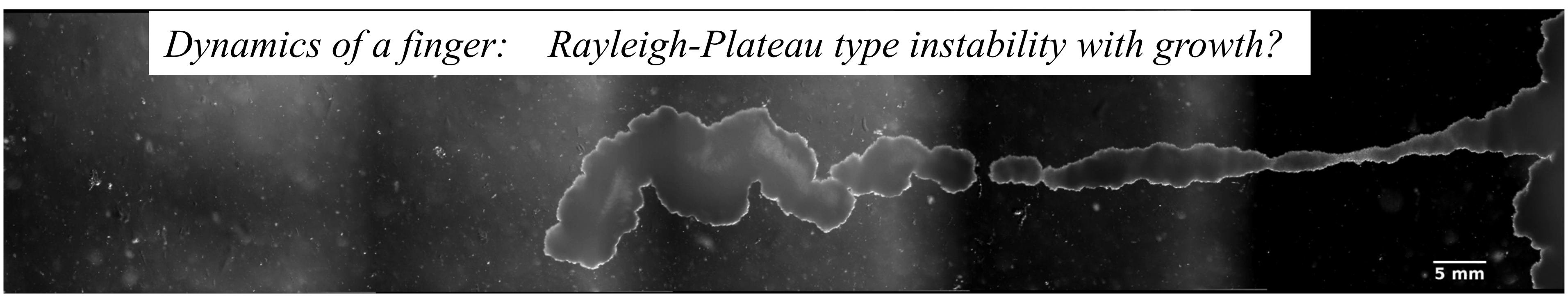
Like an anti-Helmholtz coil!

Liquid-like fingering instabilities

(moderate substrate viscosity η≈450 Pa-s)



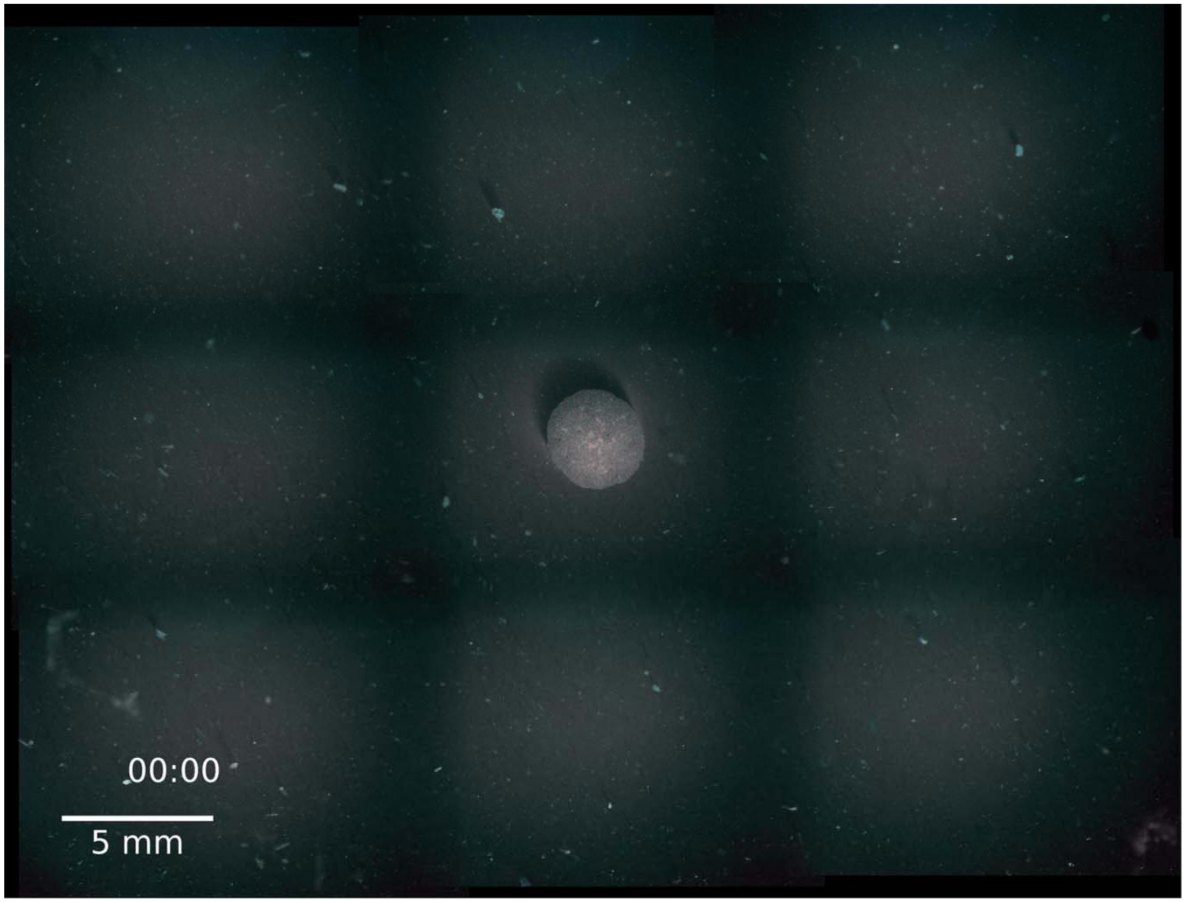
(Happy Bastille Day!)



Dynamics of a finger: Rayleigh-Plateau type instability with growth?

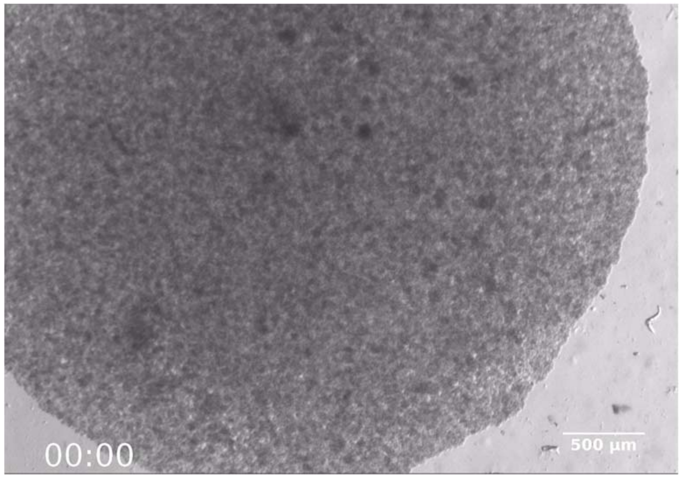
Solid-like colony fragmentation

(low substrate viscosity $\eta \approx 85$ Pa-s)

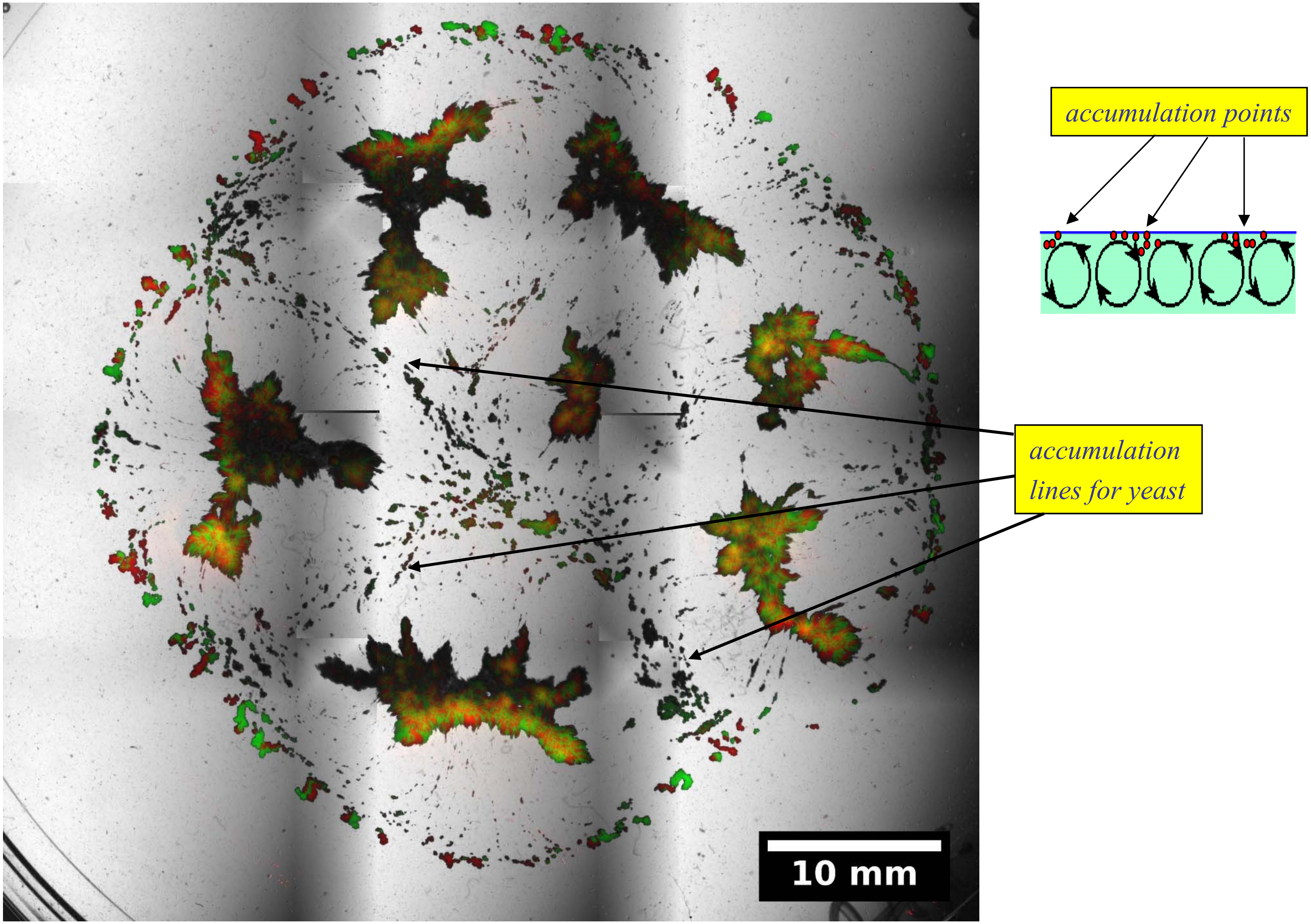


Colony takes over plate in < 1 day!

Zoom in reveals necking dynamics….

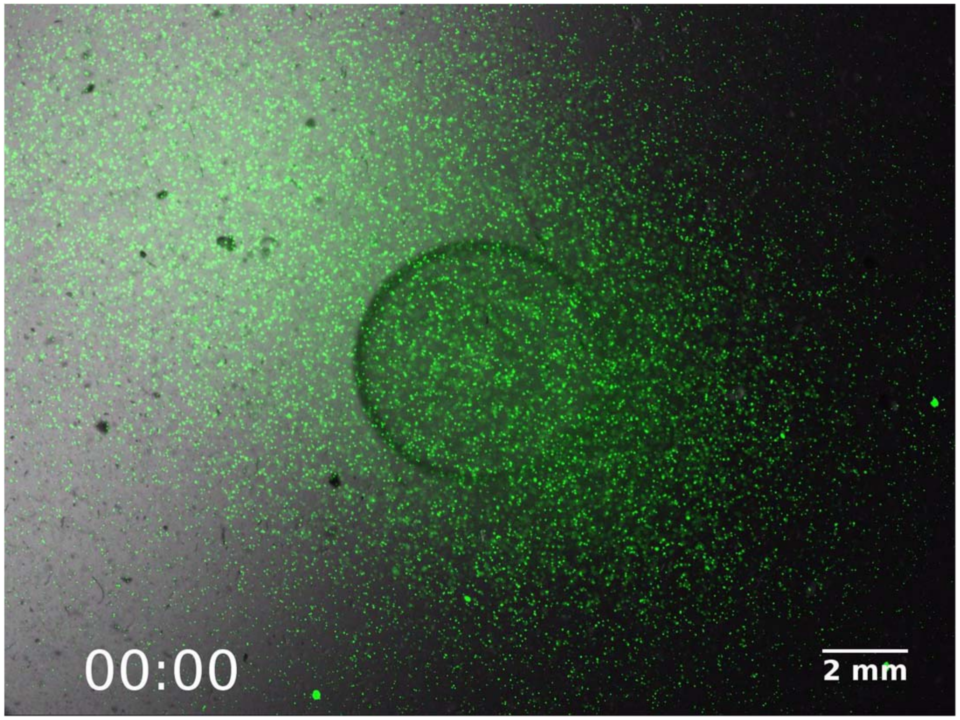


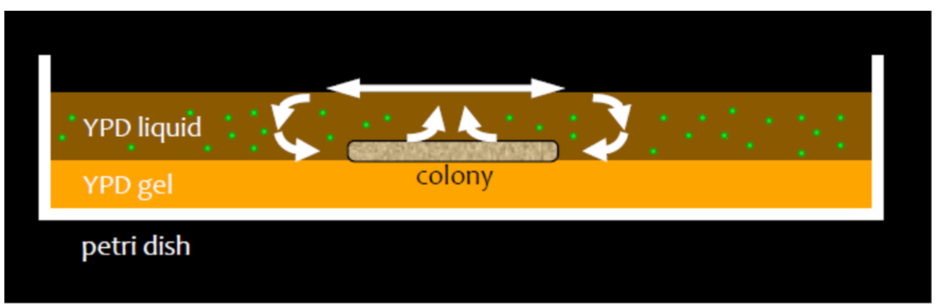
We expect a submerged vortex ring under each colony fragment…



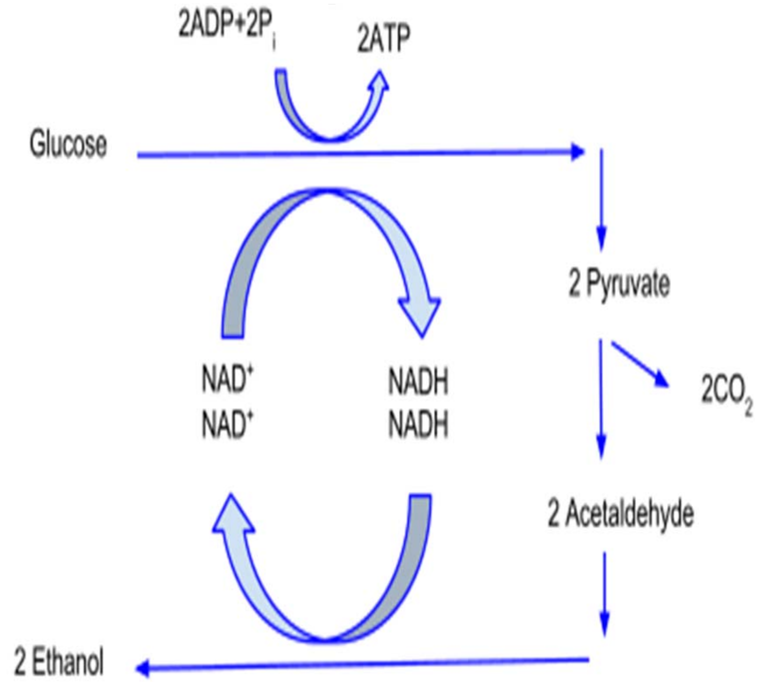
Origin of the enhanced flow beneath colonies growing on liquid substrates

Case I: colony on the <u>bottom</u> of the dish





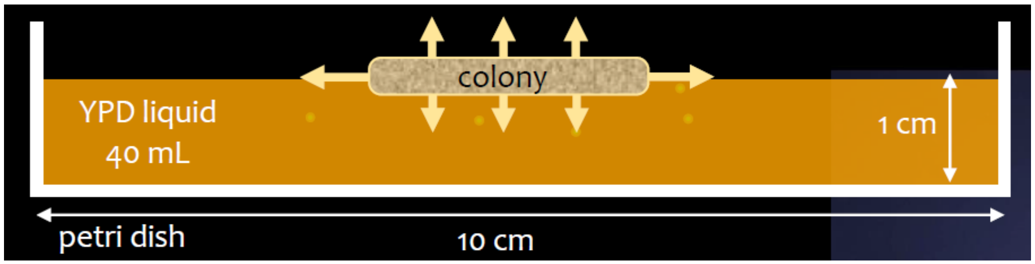
Anaerobic pathway:
dextrose (~3%) → CO_2 + ethanol



wikipedia

Yeast colony on bottom, dextrose-metabolism-induced CO_2 bubbles!!

Origin of the enhanced flow beneath colonies growing on liquid substrates



- Fluid mechanics

Boussinesq approximation (valid in the limit of small density difference)

$$\frac{\partial \vec{v}}{\partial t} + \vec{v}.\nabla\vec{v} = -\frac{1}{\rho_0}\nabla p + \nu\nabla^2\vec{v} + \frac{\rho}{\rho_0}\vec{g}$$

media density:

$$\rho = \rho_0 + \delta\rho = \rho_0(1+\beta c)$$

ρ_0 : fluid density

ν : kinematic viscosity

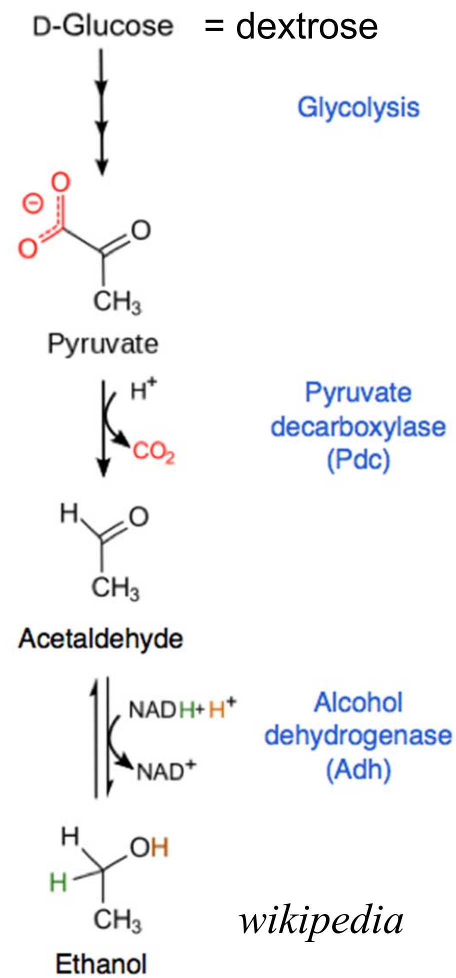
g : gravity

p : pressure

Diffusion equation for the nutrients field

$$\frac{\partial c}{\partial t} + \nabla.(\vec{v}c) = D\nabla^2 c$$

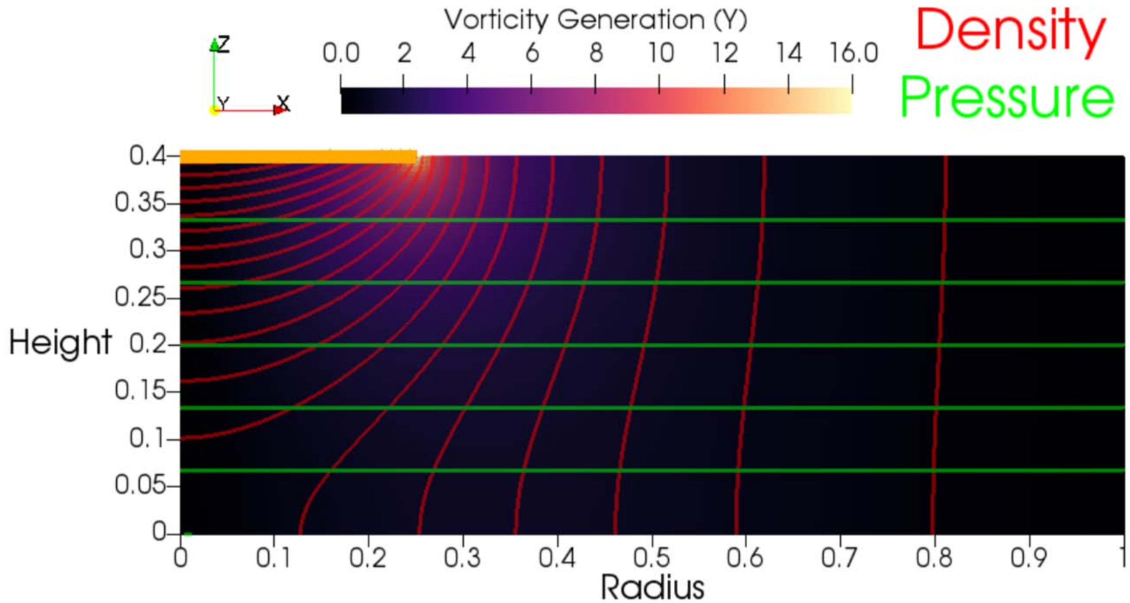
Case II: colony growing at the top of the liquid substrate



wikipedia

Flow simulations

Isobars and isoclines in the <u>absence</u> of flow ($\eta = \infty$)



<u>Boundary conditions:</u>

$$\vec{j_{\text{out}}} = \alpha c \vec{n}$$

$$\vec{j_{\text{diff}}} = \rho_0 \beta D \nabla c$$

$$\vec{j_{\text{out}}} = \vec{j_{\text{diff}}}$$

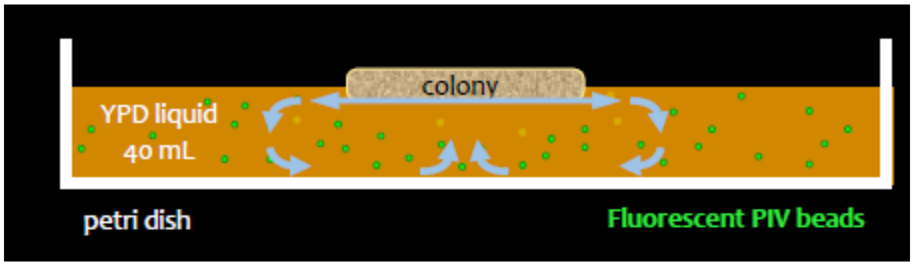
$$\boxed{\nabla c . \vec{n} = \frac{\vec{\alpha c}}{\rho_0 \beta D} . \vec{n}}$$

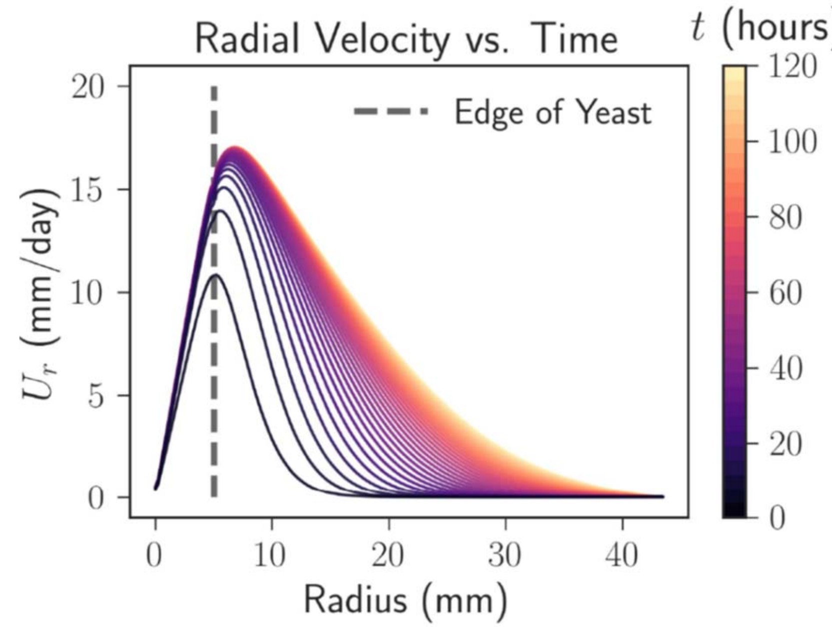
<u>Vorticity equation:</u> $\vec{\omega} = \nabla \times \vec{u}$

$$\frac{\partial \vec{\omega}}{\partial t} + (\vec{u}.\nabla)\vec{\omega} = (\vec{\omega}.\nabla)\vec{u} + \boxed{\frac{1}{\rho^2}(\nabla \rho \times \nabla p)} + \nu \nabla^2 \vec{\omega}$$

A thresholdless baroclinic instability generates a ring of vorticity beneath the colony….

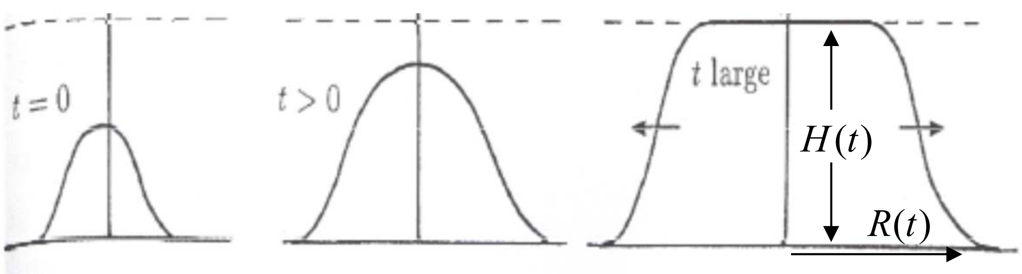
Lubrication approximation for growth with radial stretching in liquid colonies





$$\frac{\partial H(\vec{r},t)}{\partial t} + \vec{\nabla} \cdot \left[H(\vec{r},t)\vec{v}(\vec{r}) \right] = D\nabla^2 H(\vec{r},t) + \mu H(\vec{r},t)\left[1 - H(\vec{r},t)\right]$$

$\vec{v}(\vec{r}) \approx \frac{1}{2}\alpha r\hat{r}$; α contains effects of both colony pushing & a metabolically generated vortex ring



$R(t) \approx R(0)e^{\frac{1}{2}\alpha t}$, exponential growth accompanied by colony thinning

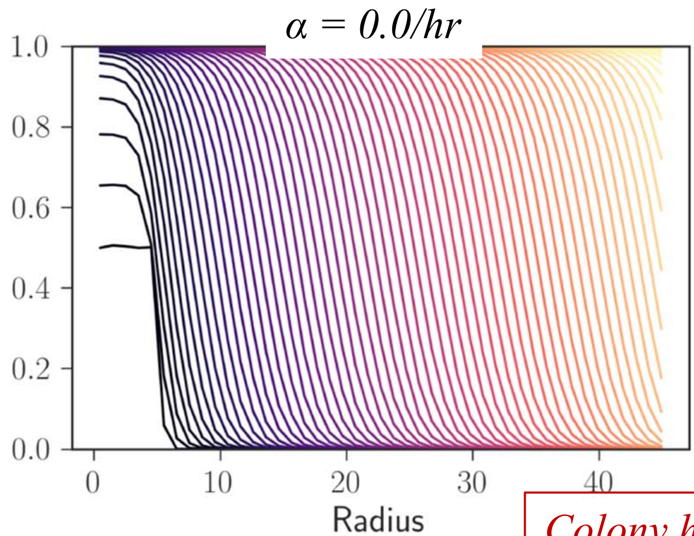
$$H(t) \approx \frac{e^{(\mu-\alpha)t}H(0)}{1 + \frac{\mu H(0)}{H_0(\mu-\alpha)}\left[e^{(\mu-\alpha)t} - 1\right]},$$

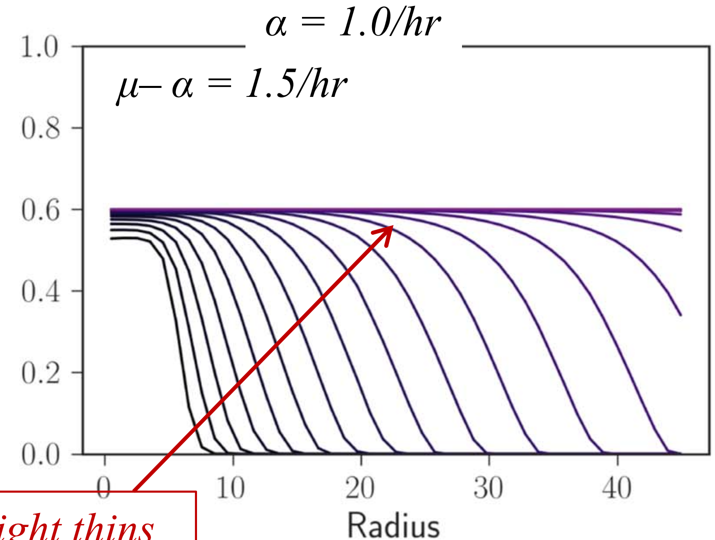
$$\lim_{t\to\infty} H(t) = H^* = H_0(1-\alpha/\mu), \ \alpha < \mu$$

$$\lim_{t\to\infty} H_0(t) = 0, \ \alpha < \mu$$

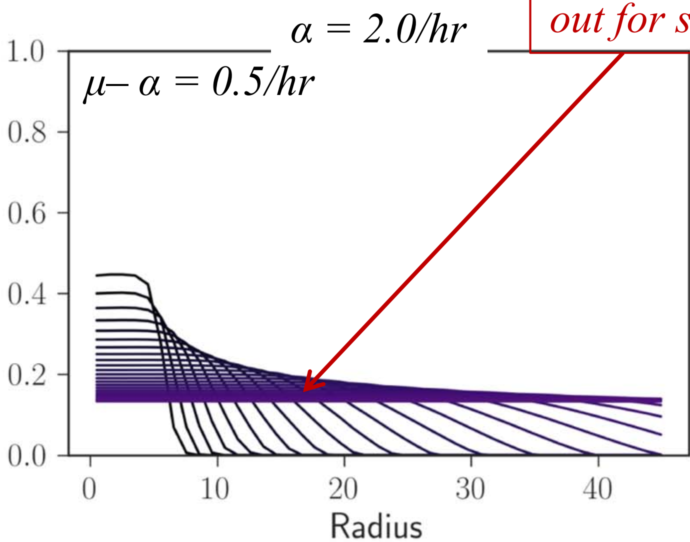
Radial height profiles with different radial flows v(r)=αr/2

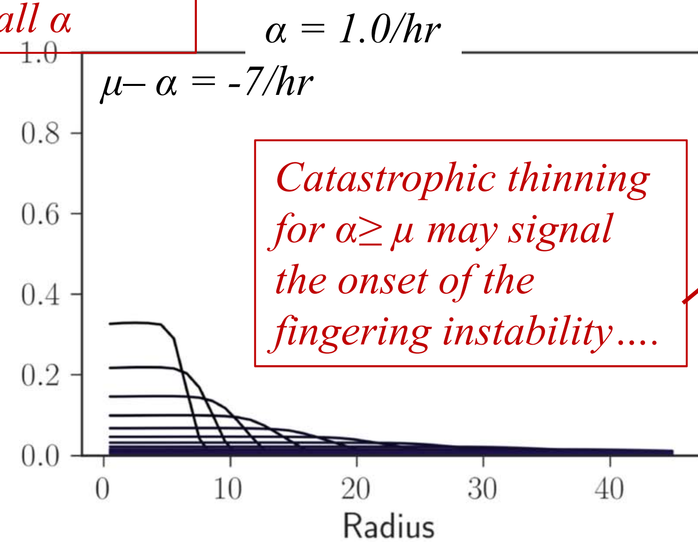
variable $\mu - \alpha$; equal time intervals



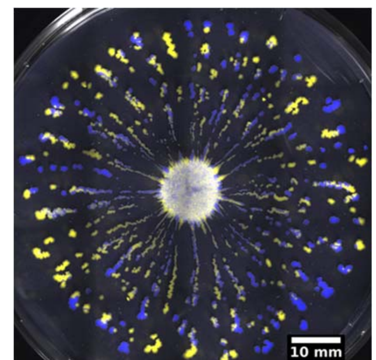


Colony height thins out for small α

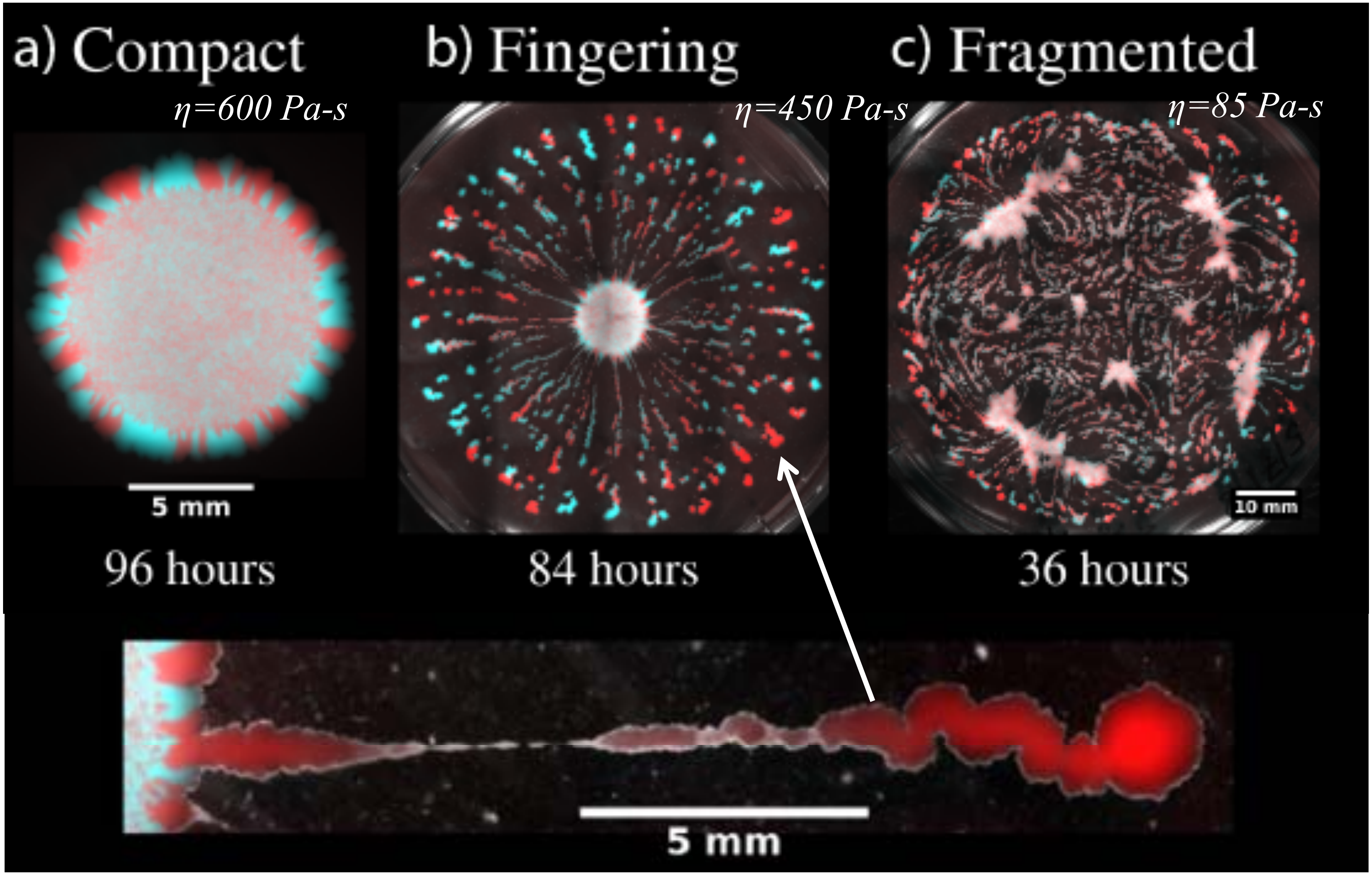




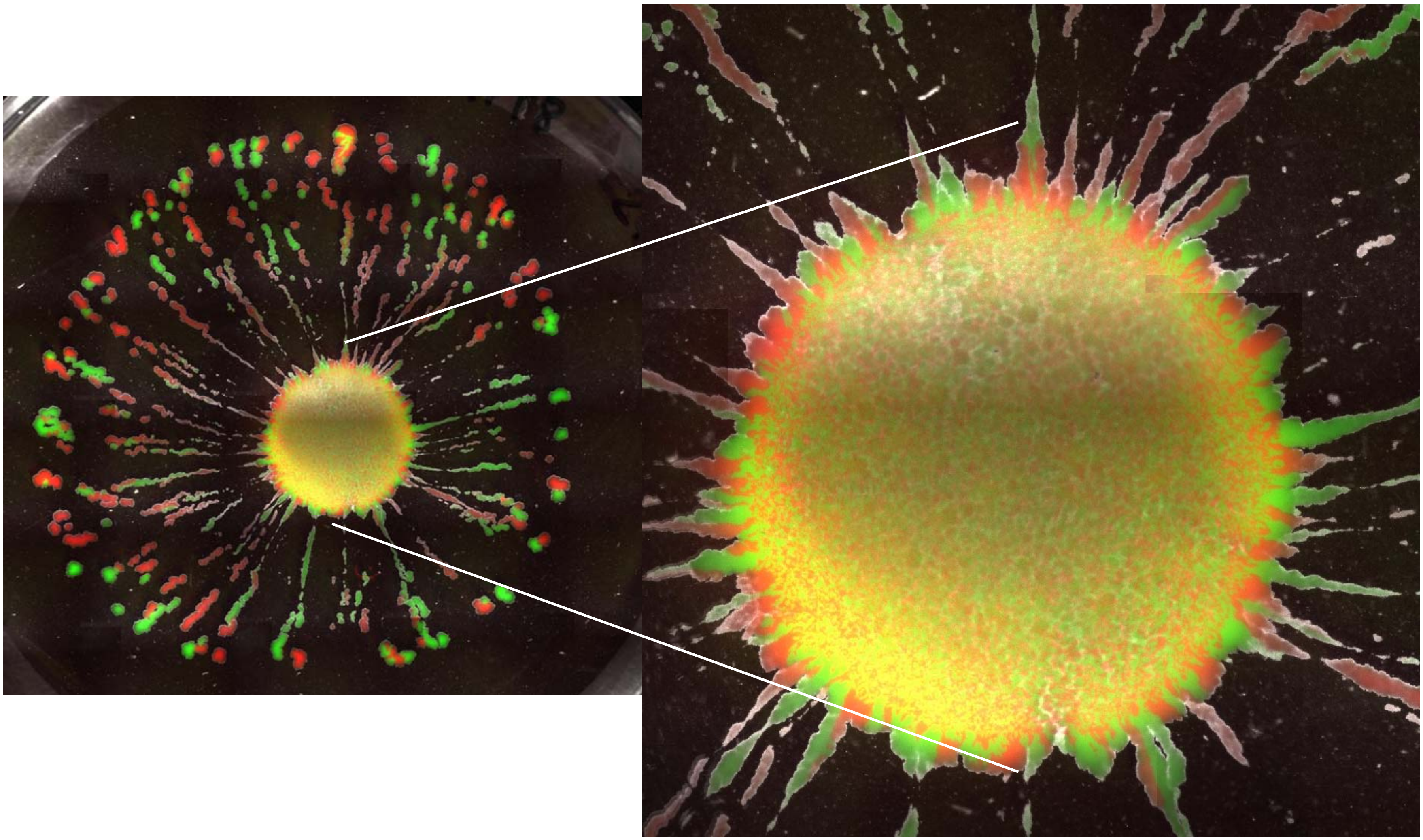
Catastrophic thinning for $\alpha \geq \mu$ may signal the onset of the fingering instability….



Summary: Range expansions on liquid substrates exhibit three qualitatively different viscosity-dependent morphologies



Could the fingering instability be due to the discreteness of the ~5μ yeast cells?



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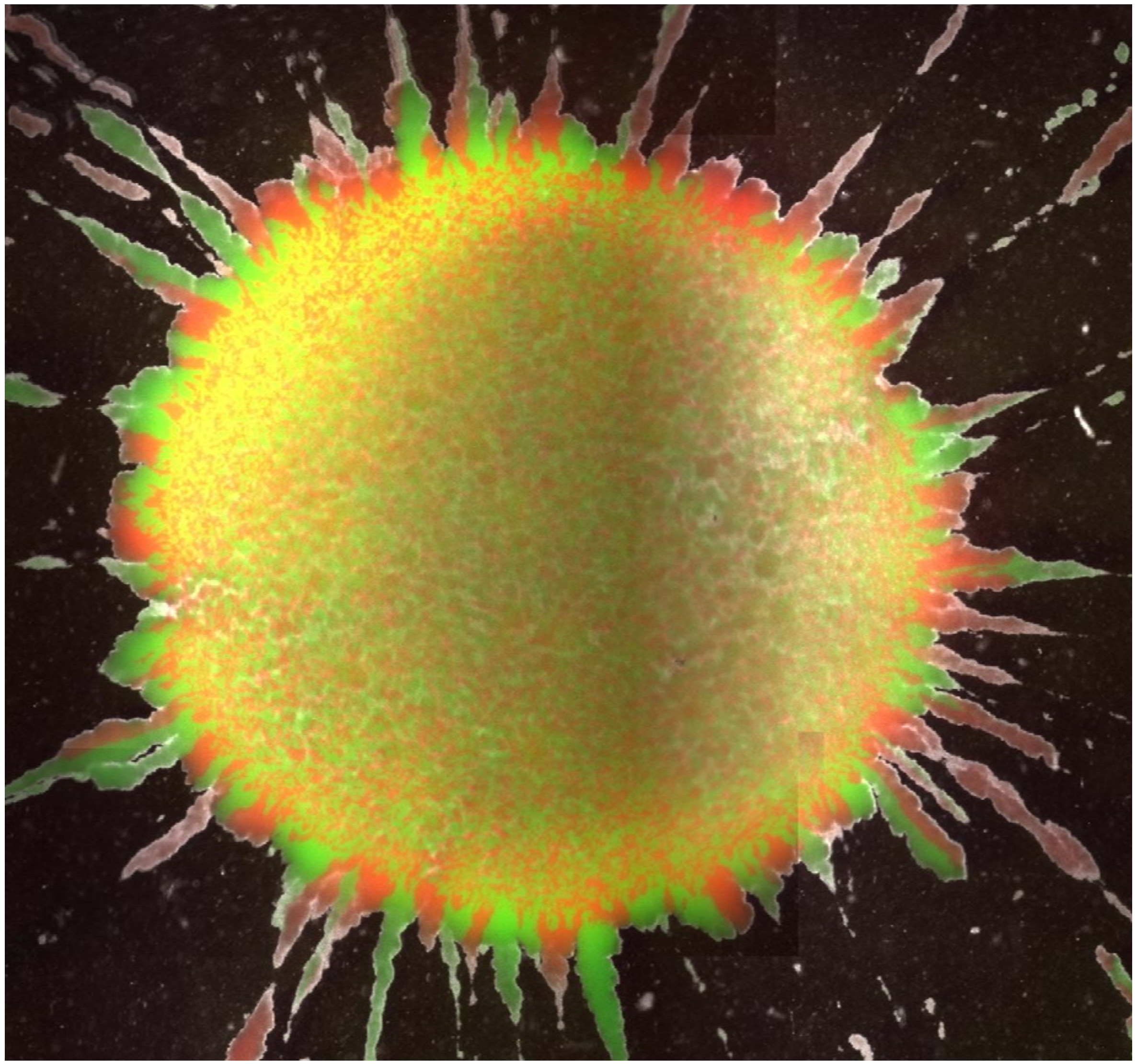
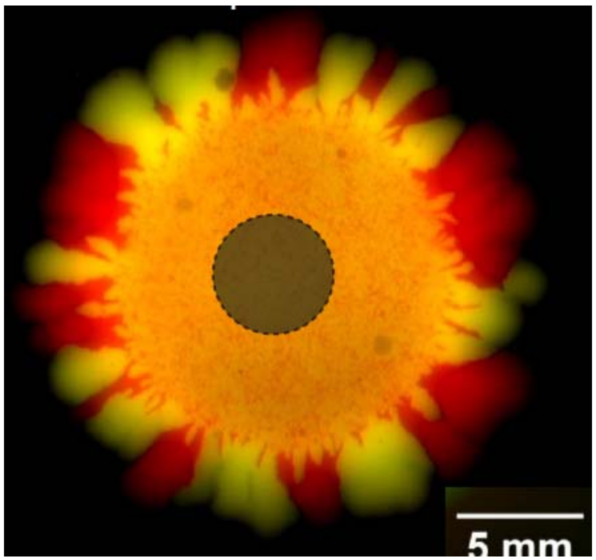
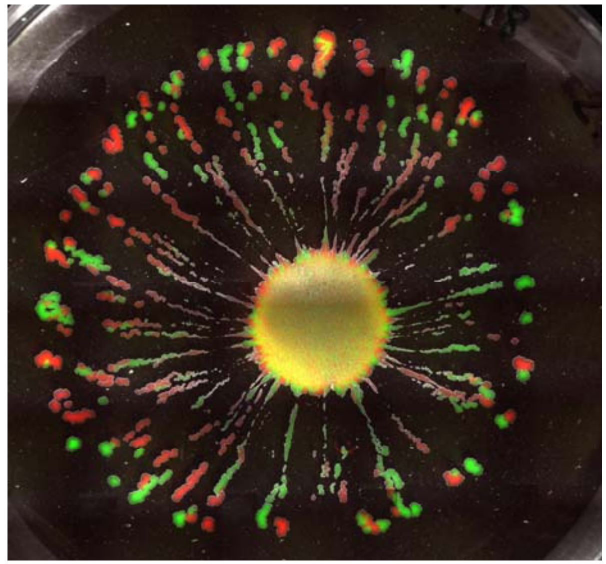
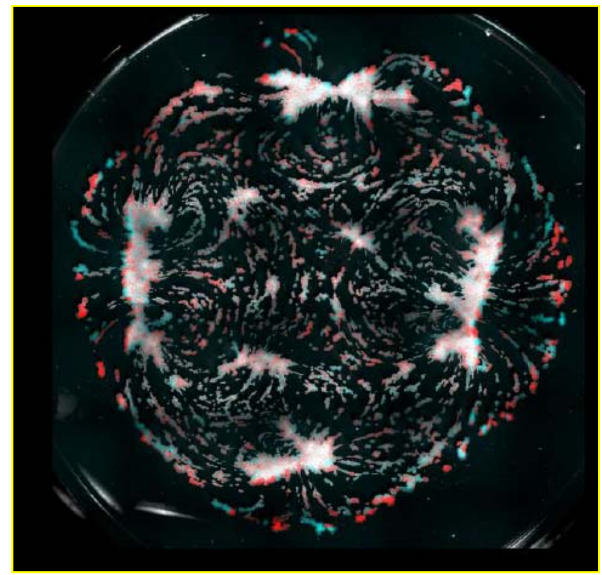


On Growth and Form of Microorganisms on *Liquid* Substrates

"Microbes on the surface of a highly viscous liquid generate buoyant flows that alter colony morphology and evolutionary dynamics"







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