Microbial Biofilms: Structure, Transport, and Dynamics

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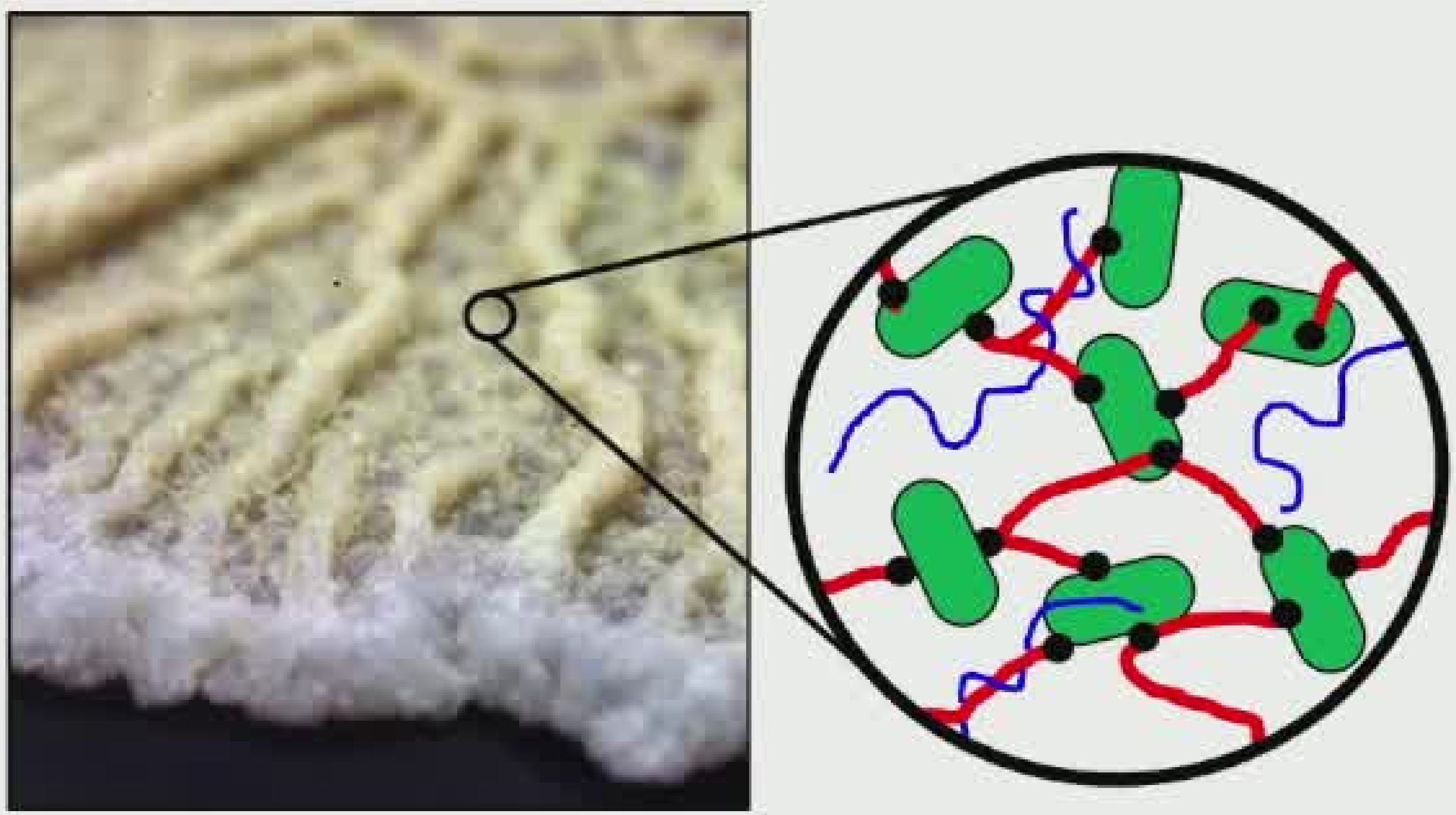
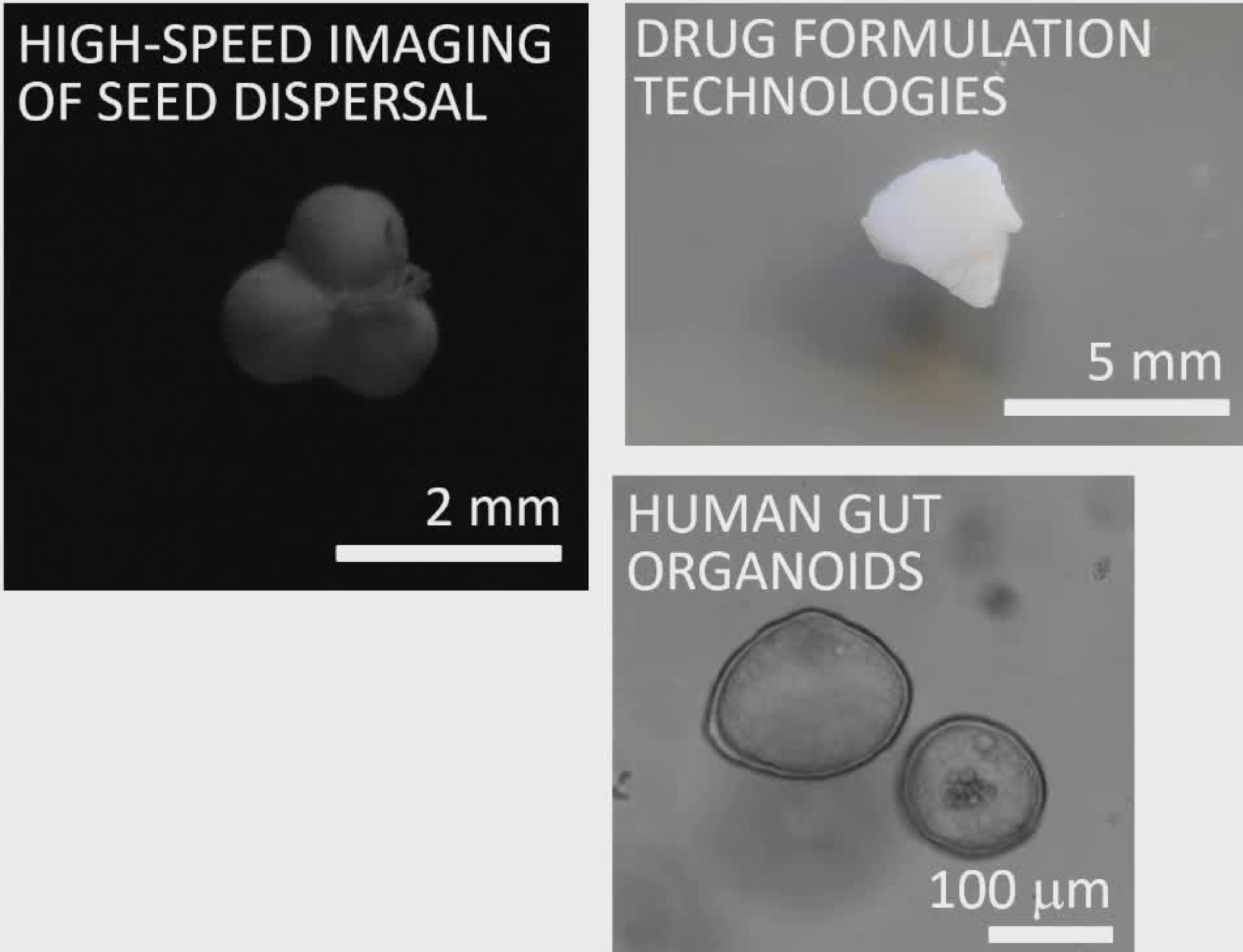
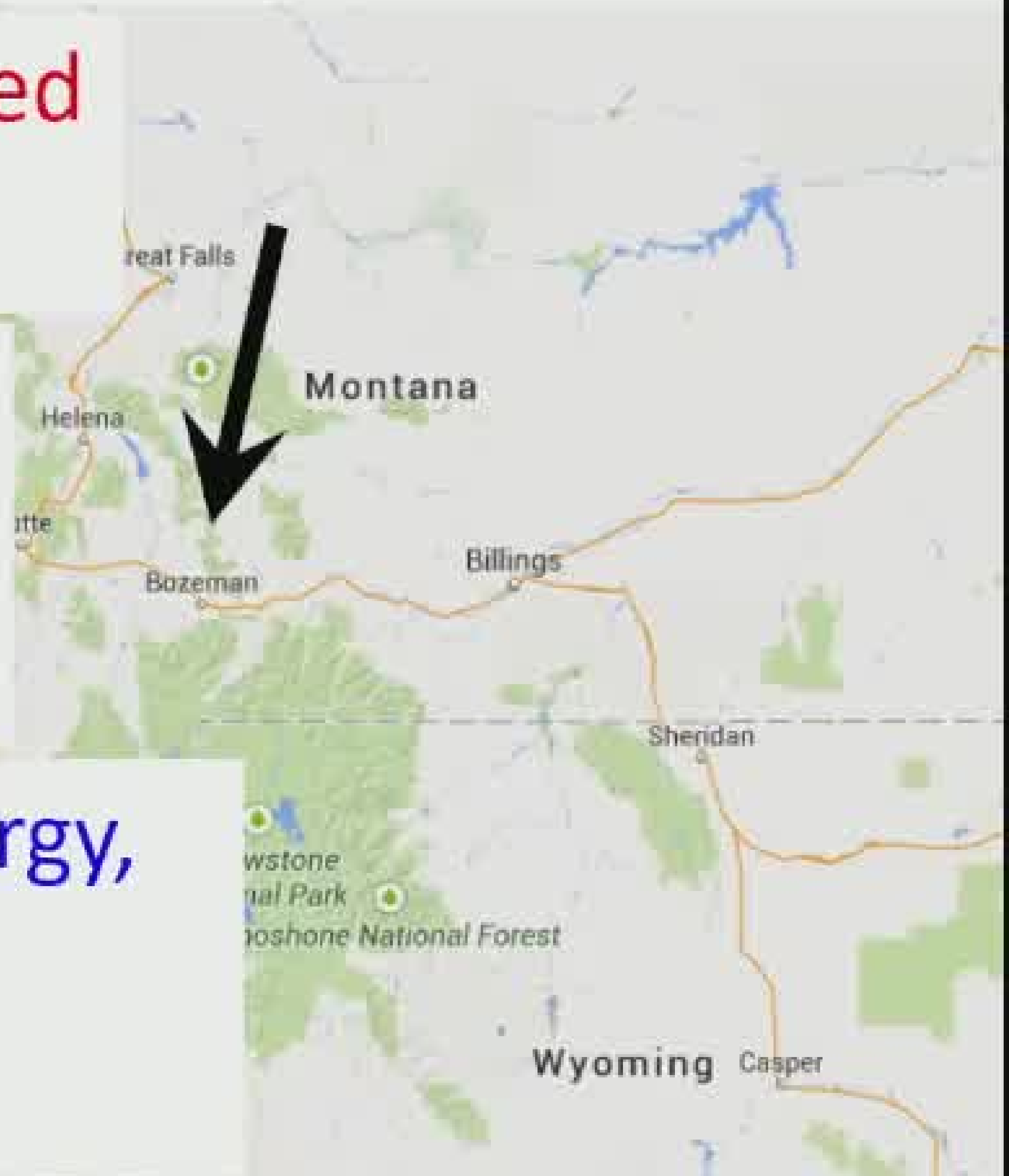



HIGH-SPEED IMAGING OF SEED DISPERSAL
2 mm
DRUG FORMULATION TECHNOLOGIES
5 mm
HUMAN GUT ORGANOIDS
100 μm

Center for Biofilm Engineering
Montana State University, Bozeman, MT

Established in 1990 as an NSF-sponsored Engineering Research Center.



Currently "self-sustaining" with federal research grants and 35+ industrial members.

Research areas: control strategies, energy, health & medical, industrial systems, standardized methods, water systems

Key data: $6.8 million research grants in 2018, 38 faculty from eight disciplines, 62 undergraduates, 52 graduate students.

CBE Industrial Membership

Consumer Products

Church & Dwight
Colgate-Palmolive
Johnsc
Kimbe
Masco
Procte
Reckit
Sherwin Williams

Testing Laboratories

WuXi AppTec

Energy

ExxonMobil
BP

Specialty Chemicals

BASF
BCG Solutions
Novozymes A/S
Sani-Marc
Sample6 Technologies
Sealed-Air

US Gov't Programs/Labs

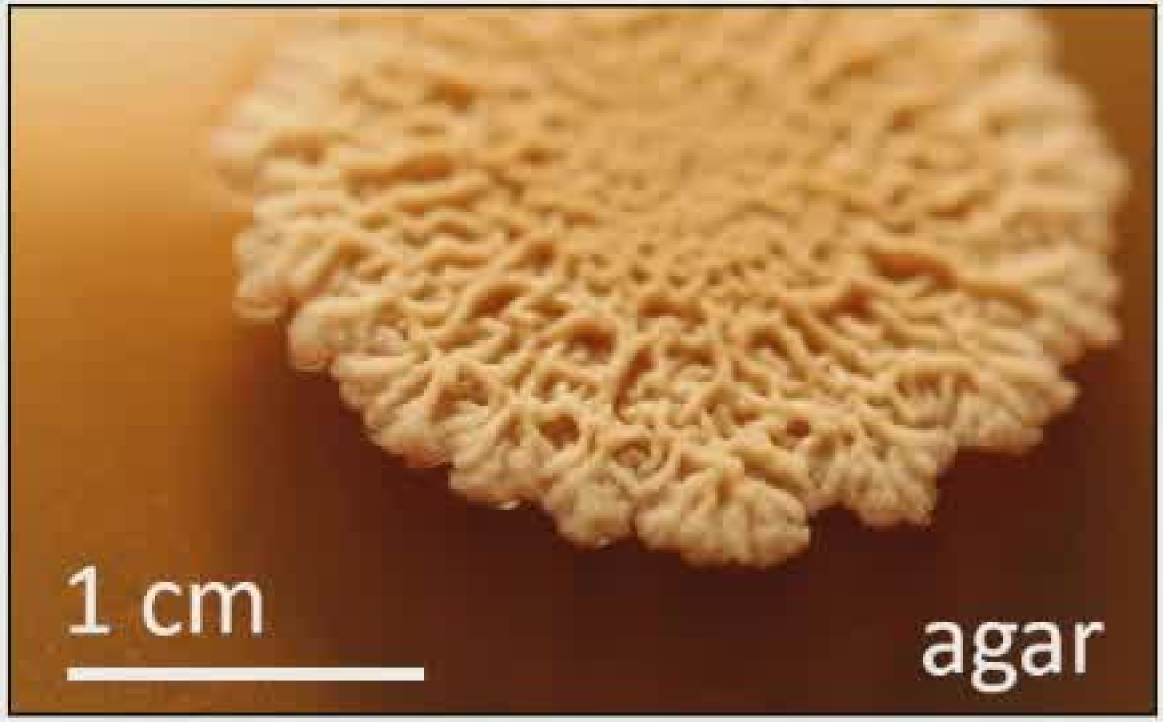
NASA

Health Care/Biomedical

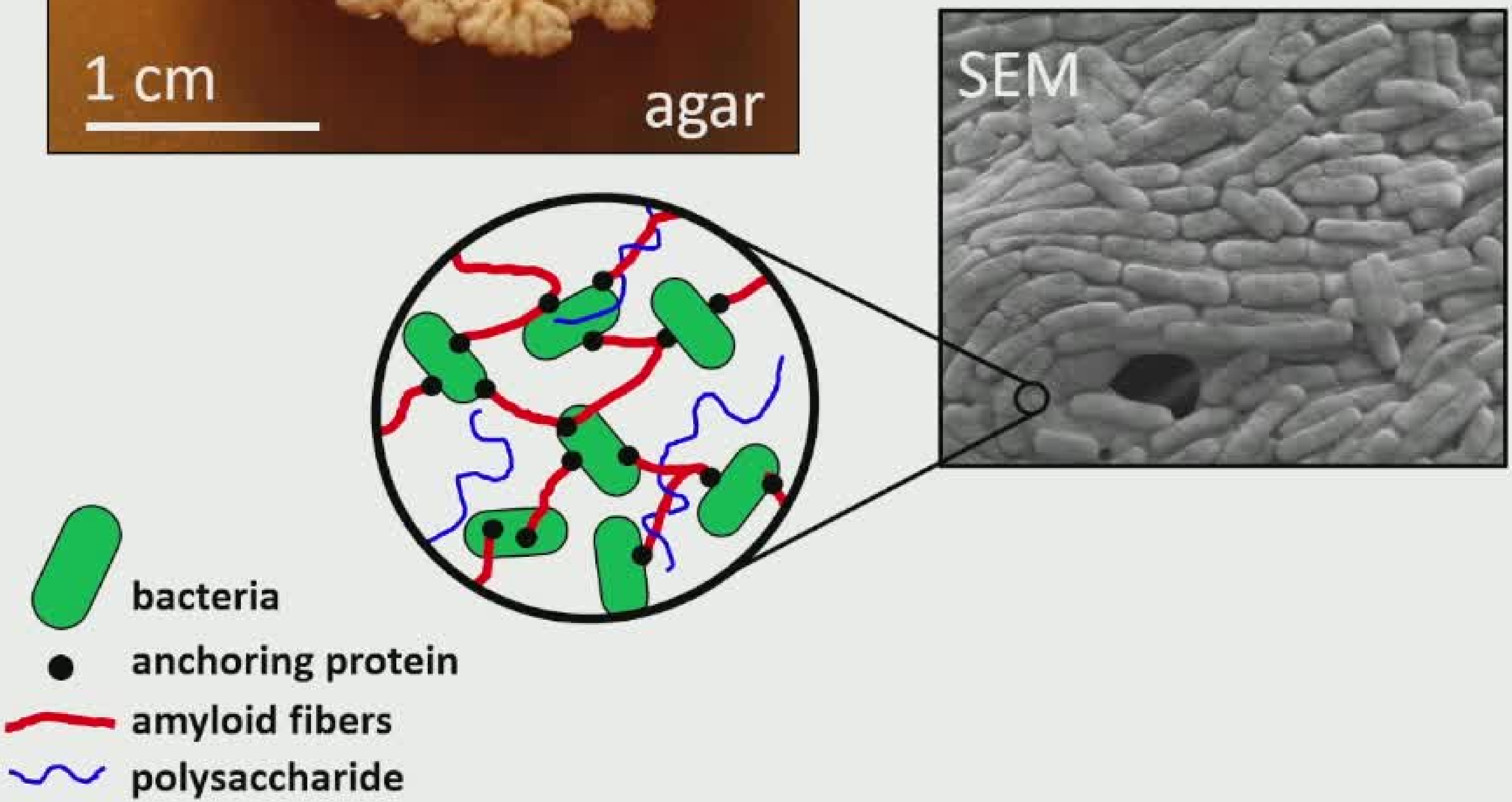
Steris
3M
Bard Access Systems
ICU Medical
Kane Biotech
KCI
Next Science
Semprus Biosciences
W.L. Gore

PROBLEM: Naturally-formed biofilms are complex

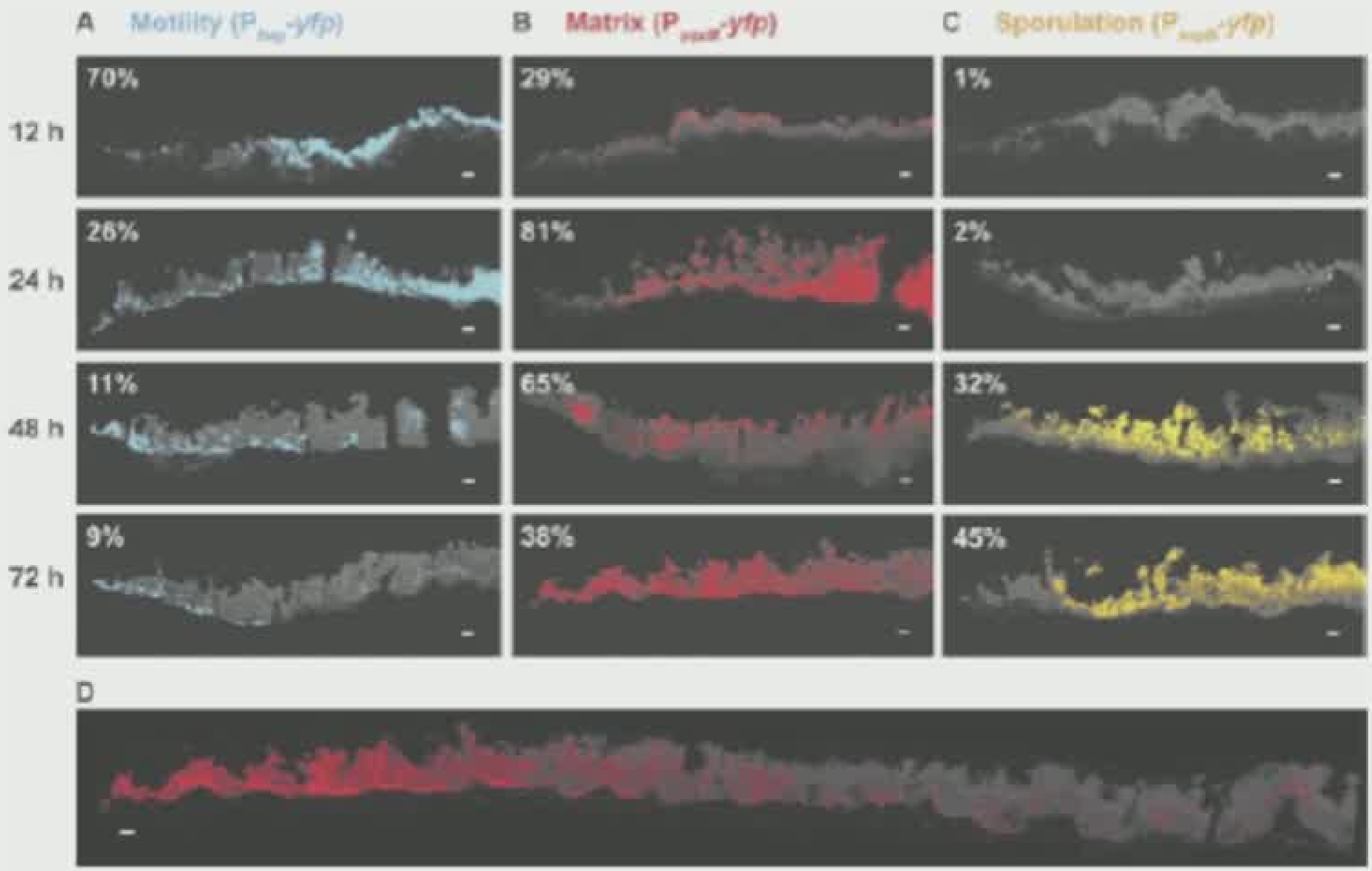
Focus on *Bacillus subtilis* as model organism



- soil microbe
- easy to manipulate genetically
- not a pathogen
- primary matrix components identified



Bacillus subtilis biofilms exhibit multiple phenotypes



Vlamakis Aguilar Losick Kolter, Gen Dev (2008)

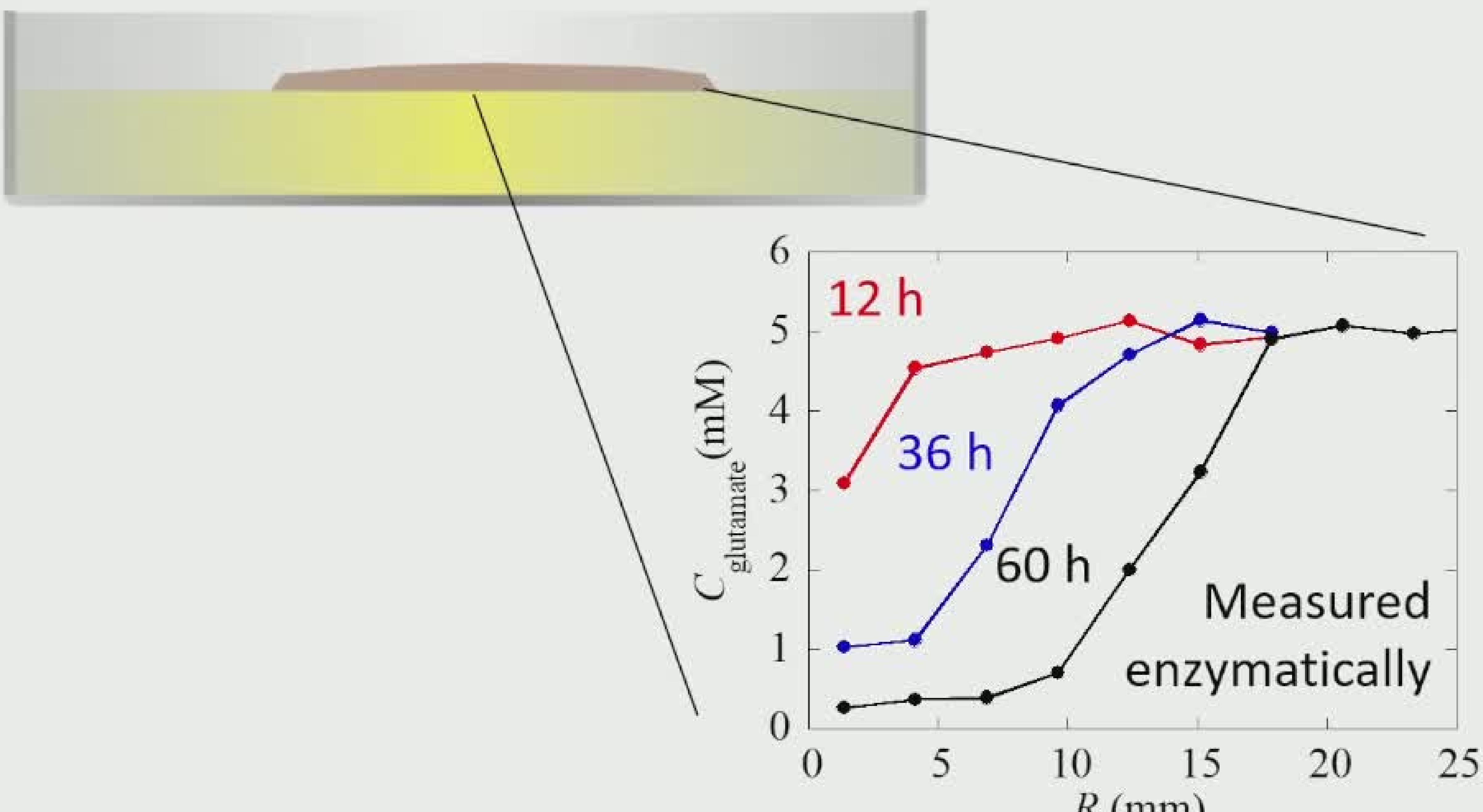
OUTLINE: Understand the structure, dynamics, and mechanics of model biofilms

1. Spreading
2. Wrinkling
3. Mechanics
4. 3D Printing

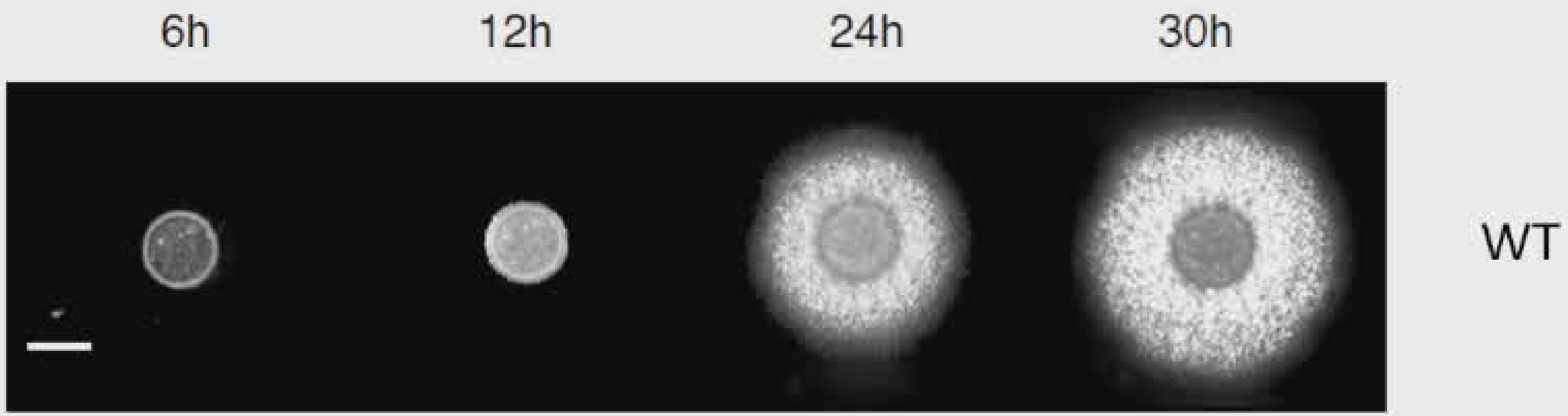
Understand

Engineer

Diffusion →
Starvation and the build up of waste



Observation: Matrix production appears to help spreading



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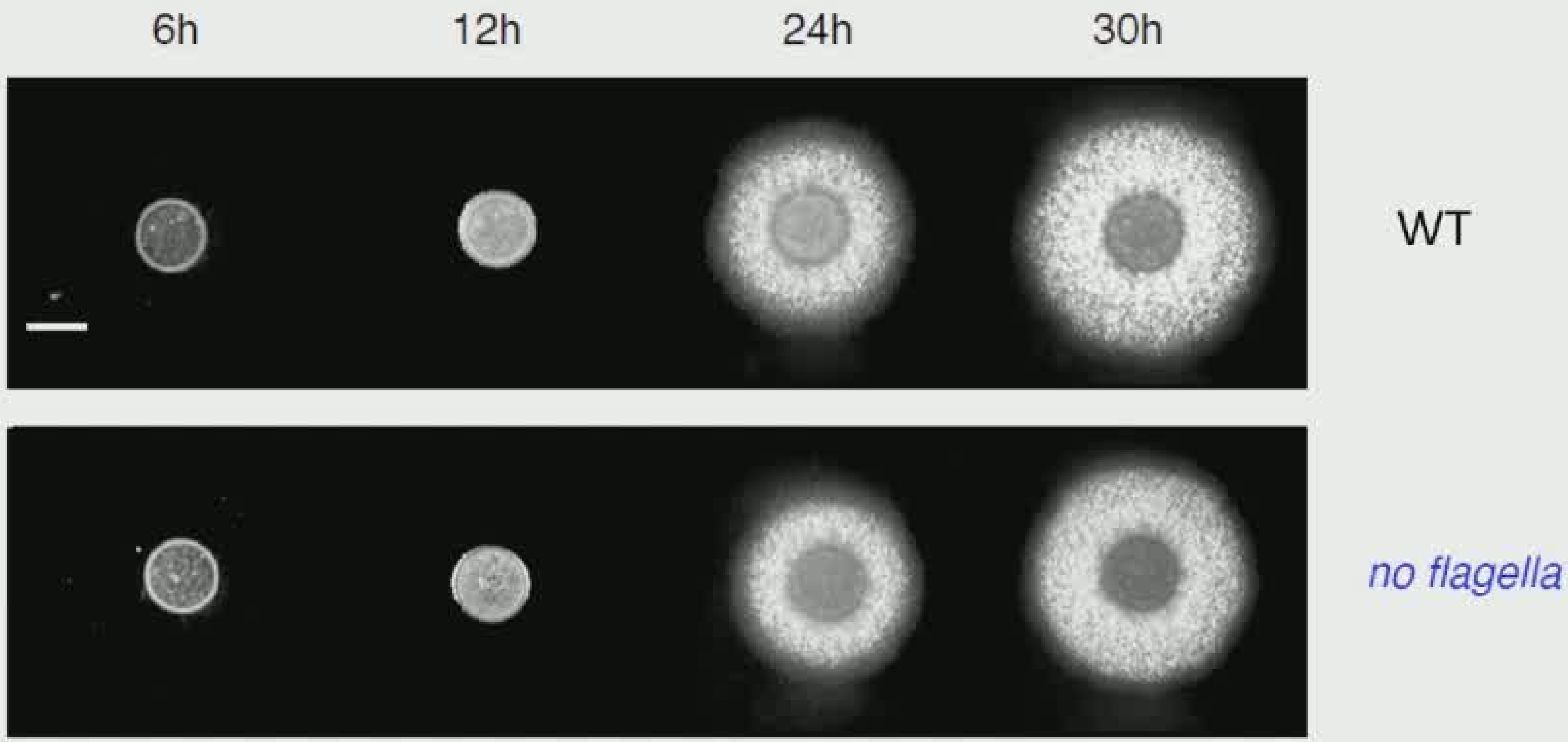

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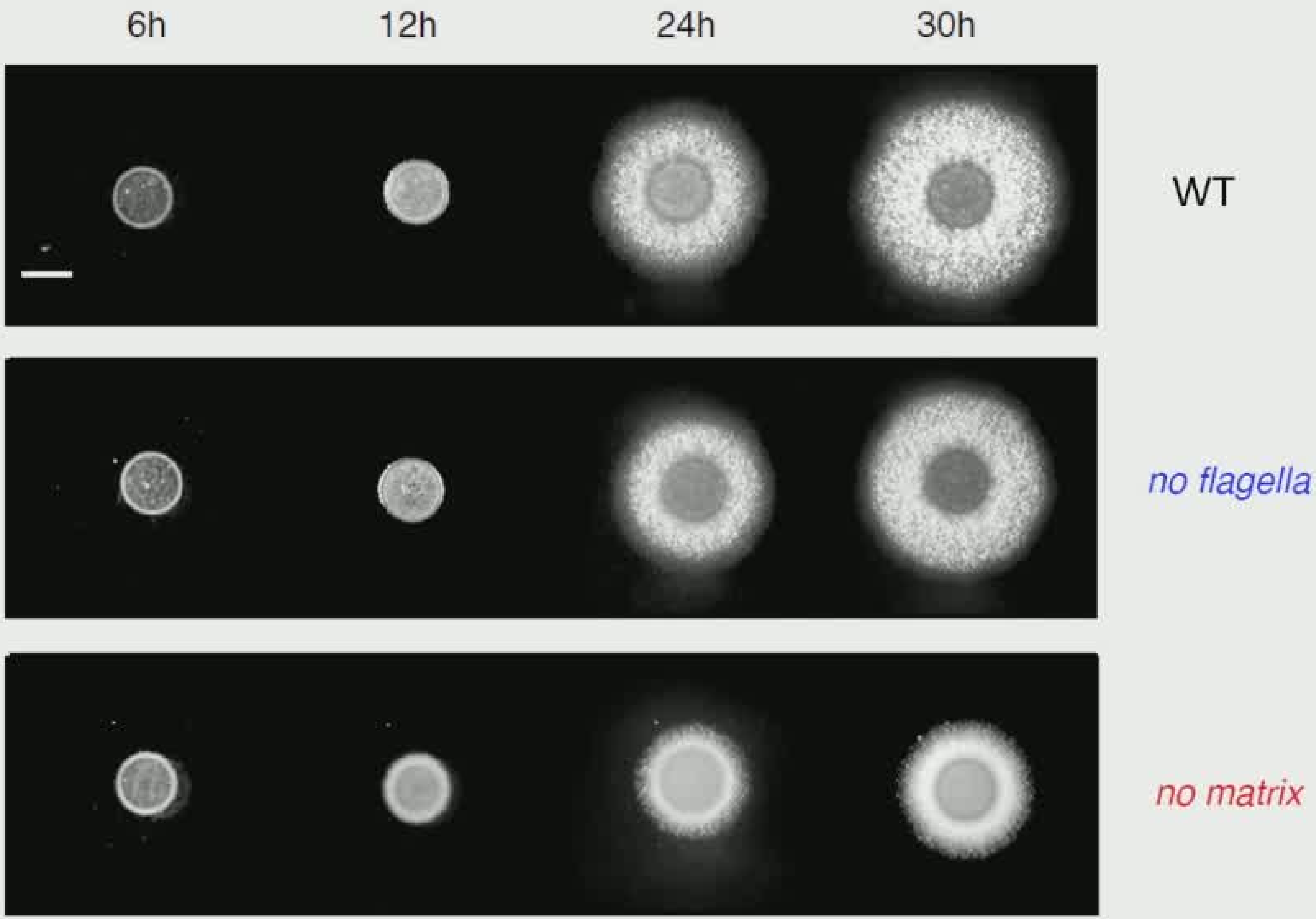
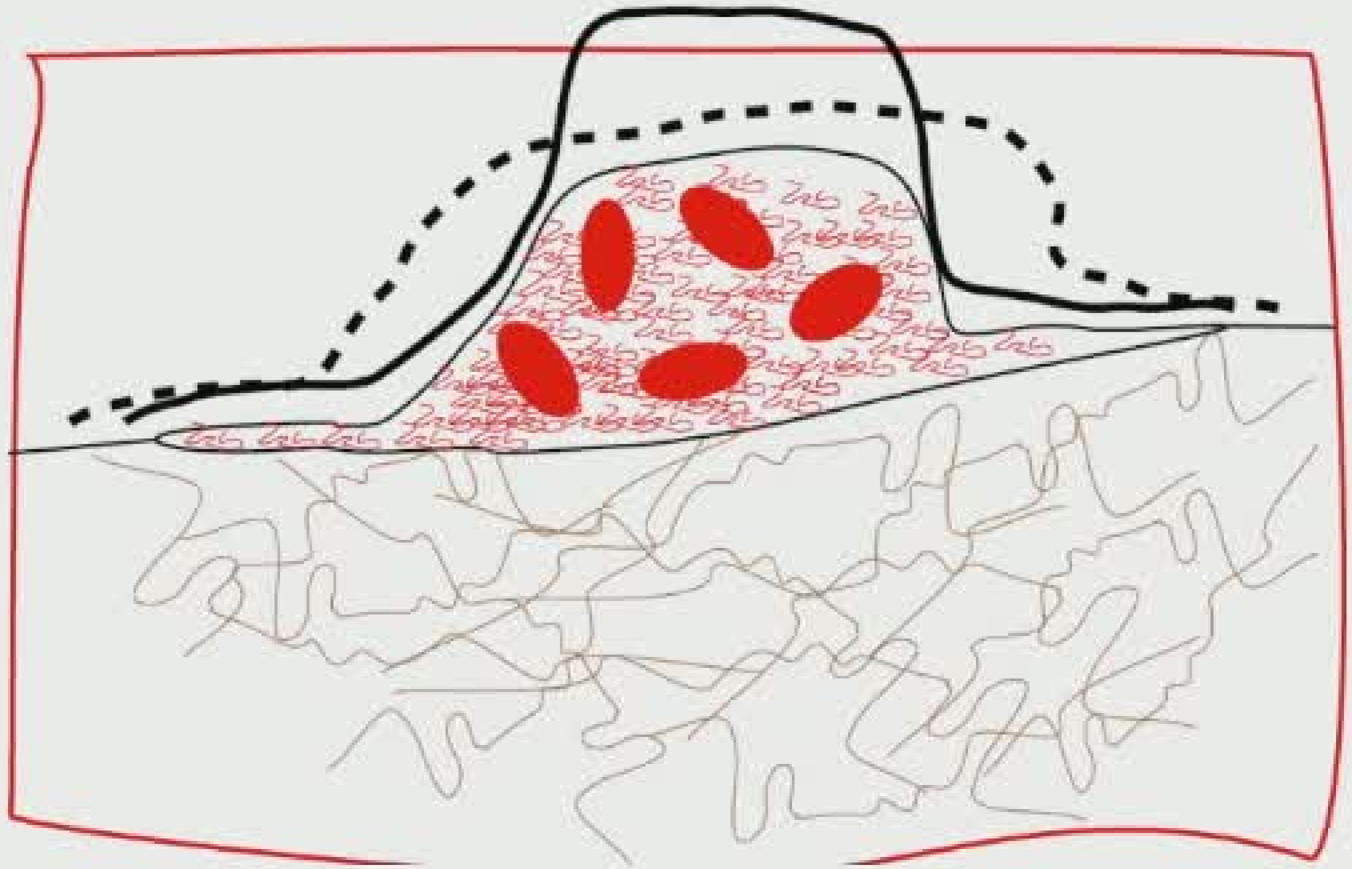
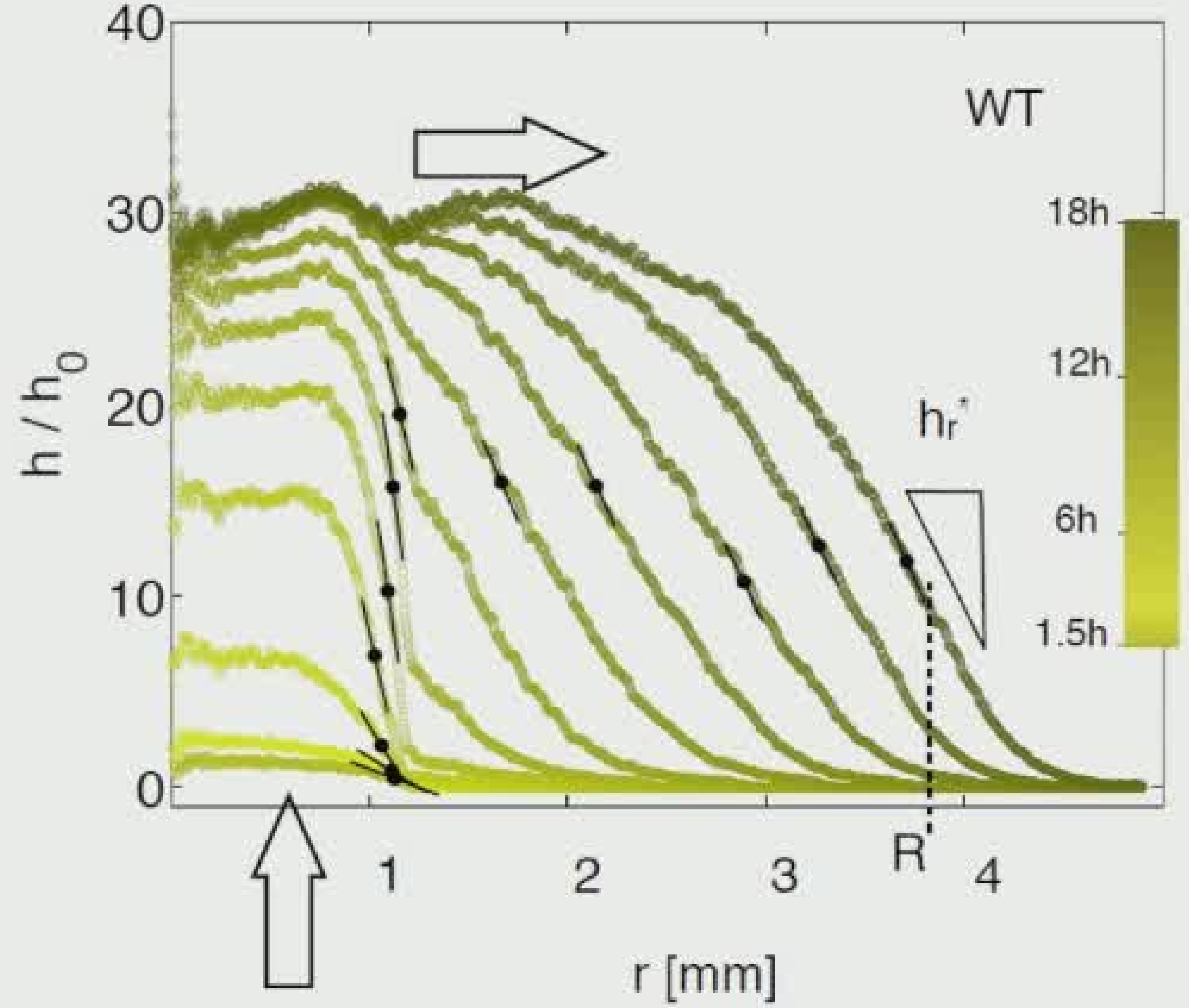


<u>Hypothesis</u>: Osmotic spreading is driven by matrix production



Observe a swelling to spreading transition driven by osmotic pressure predicted by thin film equation



A. Seminara, T. E. Angelini, J. N. Wilking, H. Vlamakis, S. Ebrahim, R. Kolter, D. A. Weitz, M. P. Brenner, *PNAS* (2012).

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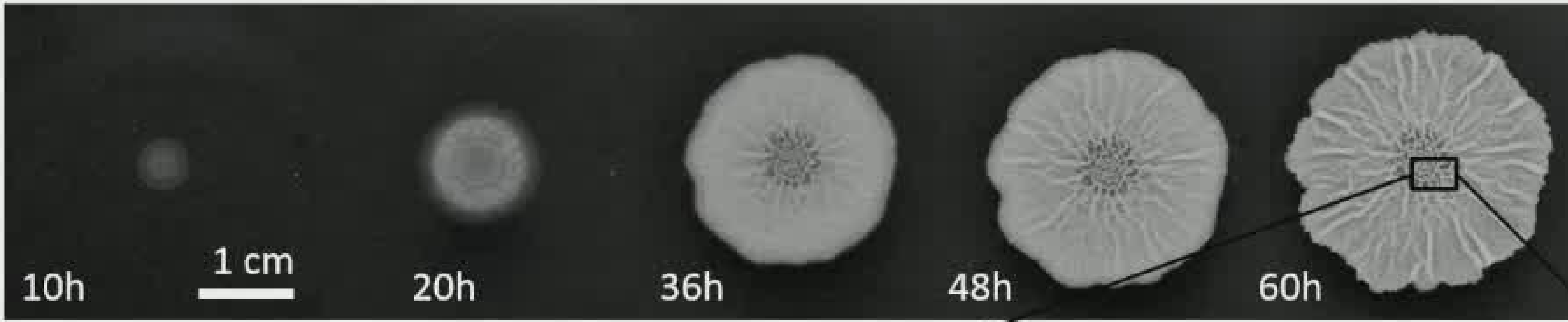
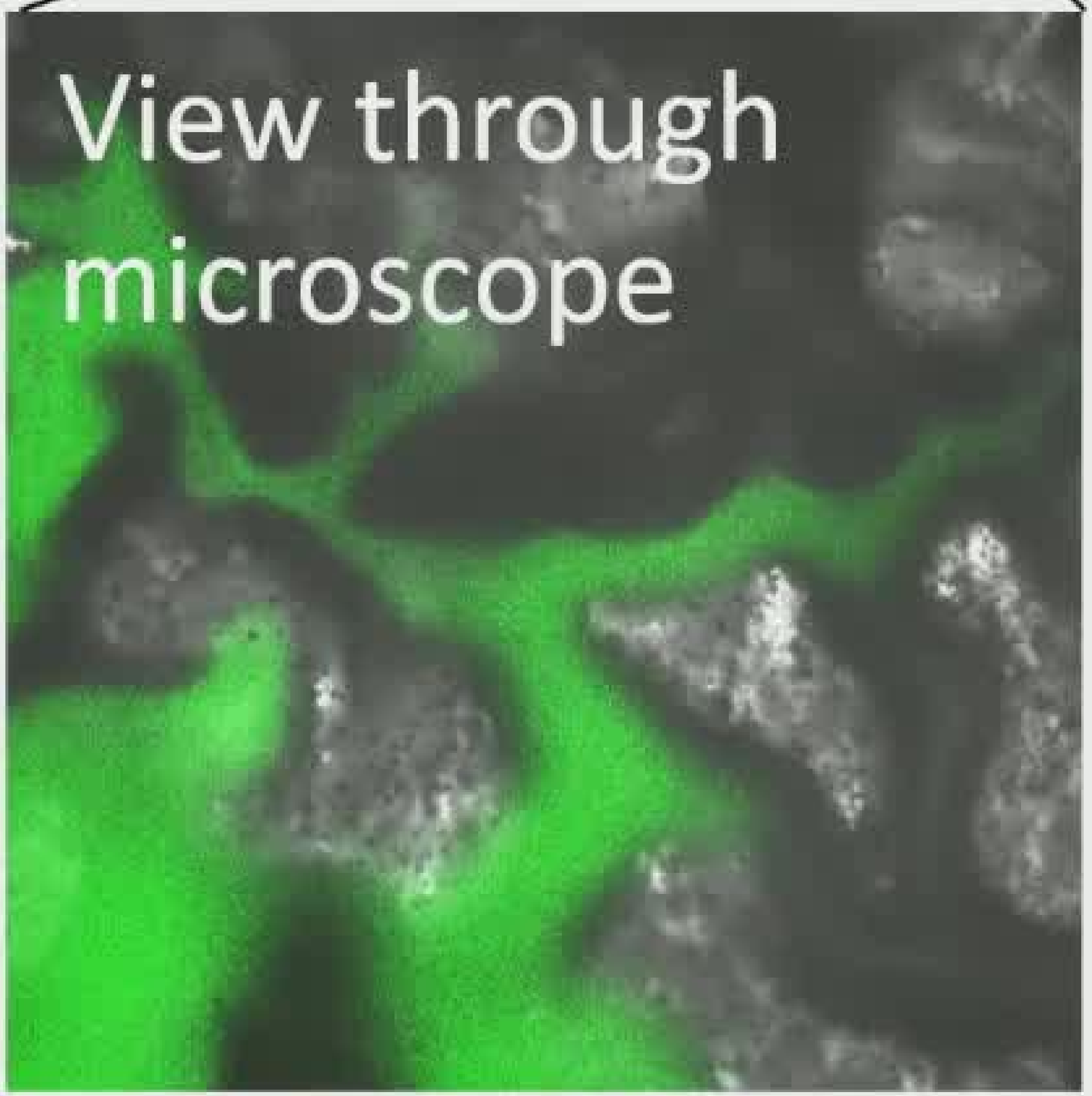
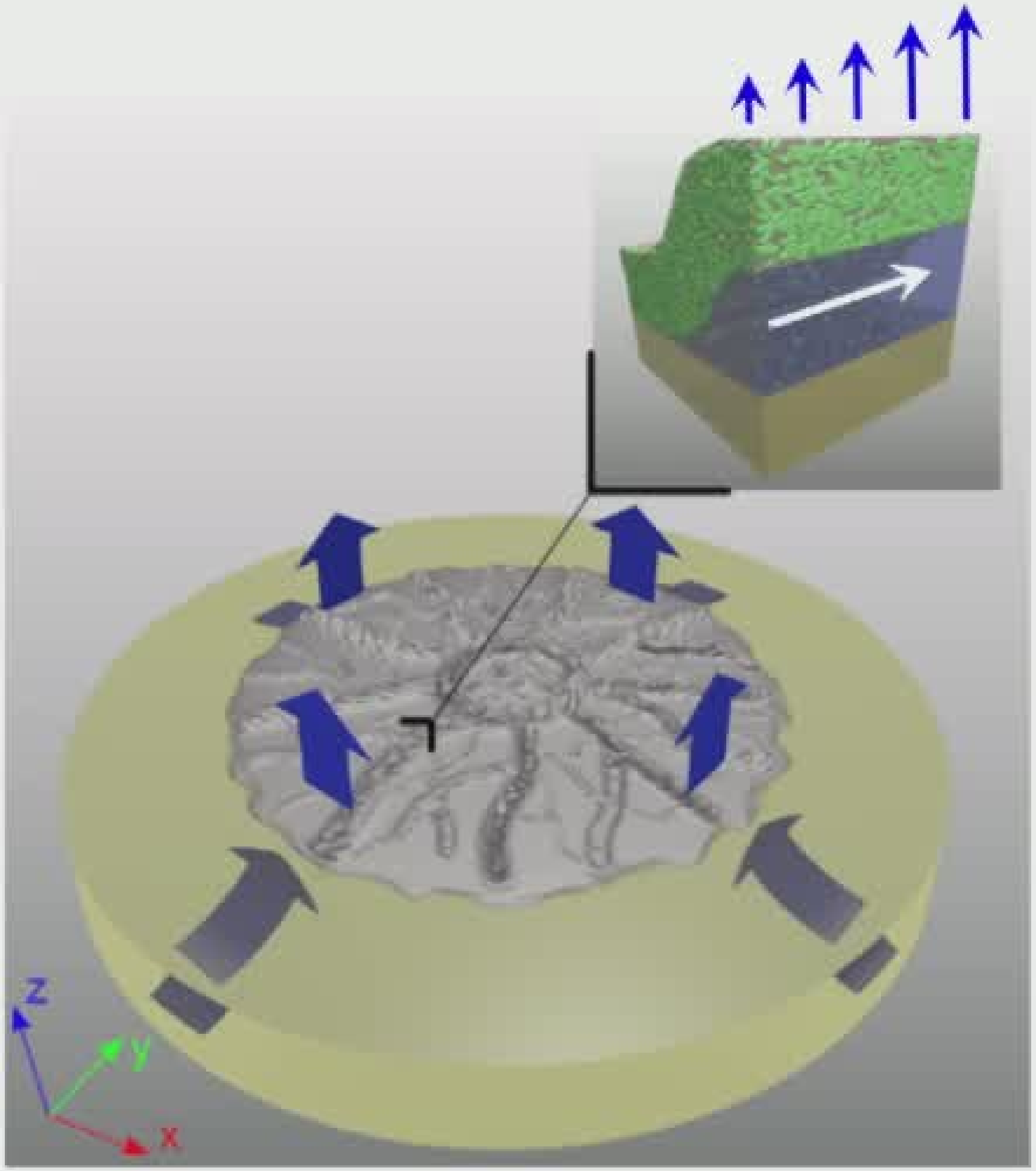
"What's going on with those wrinkles?"





Hypothesis: Differential evaporation drives liquid in the xy-plane



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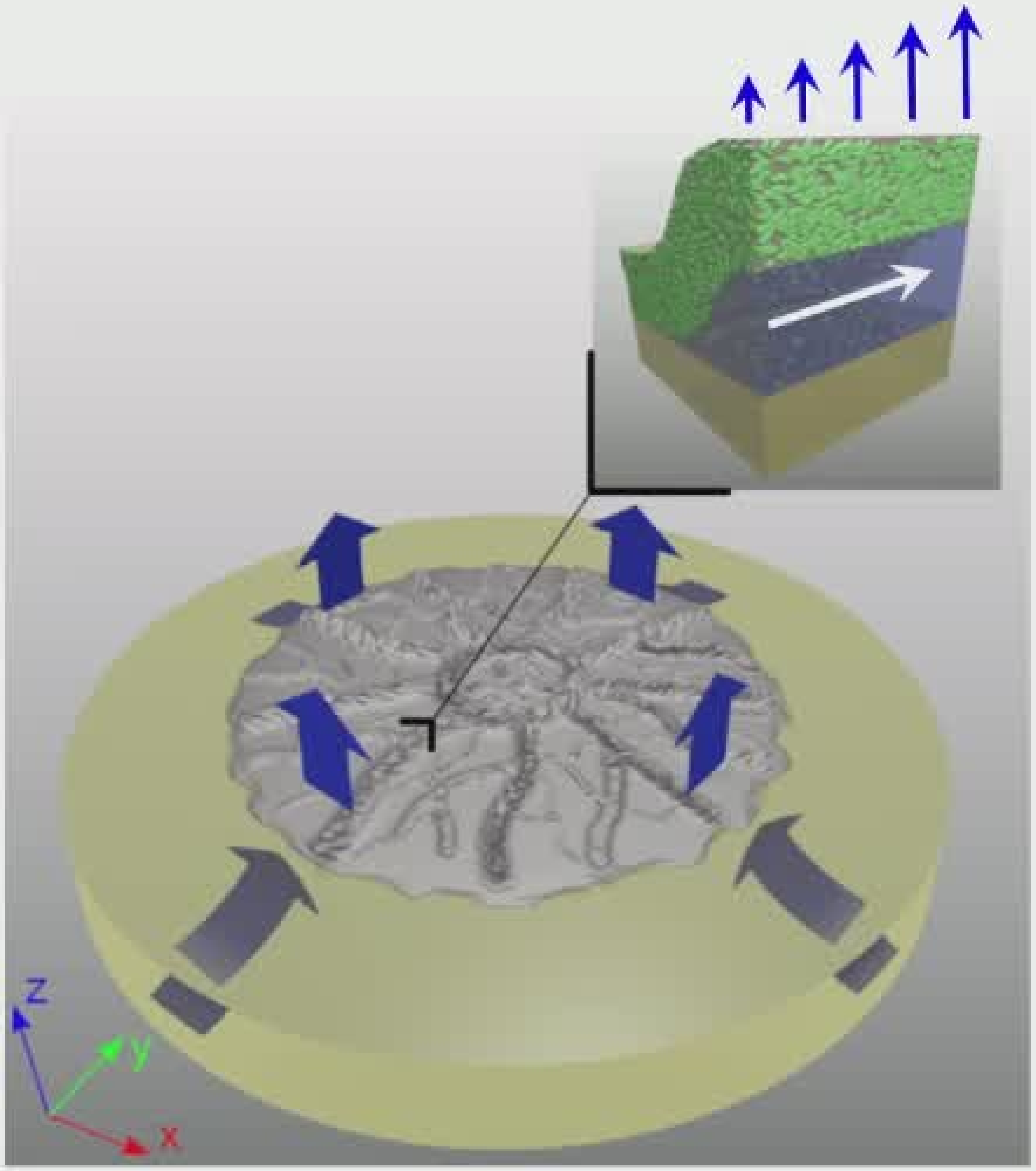
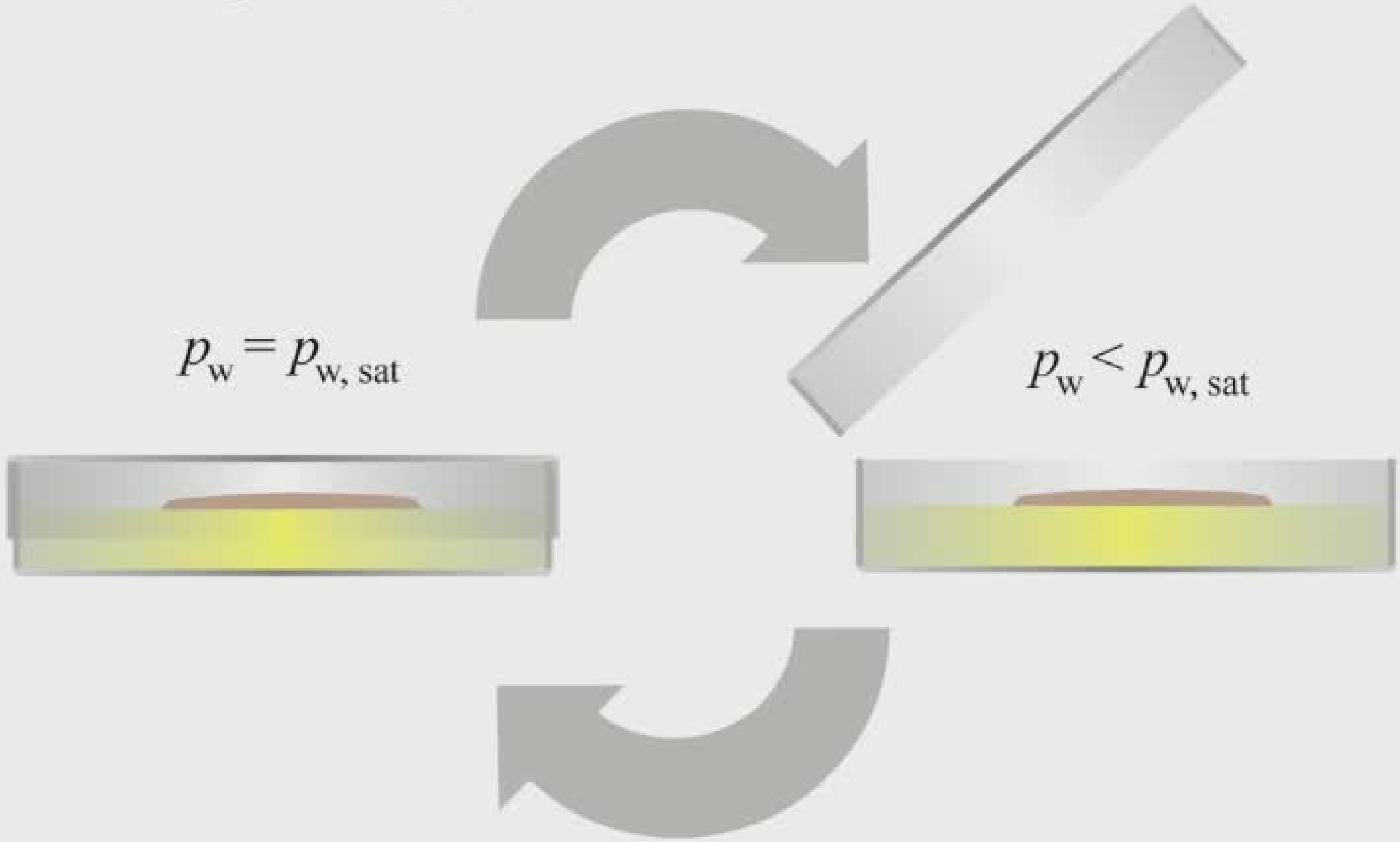
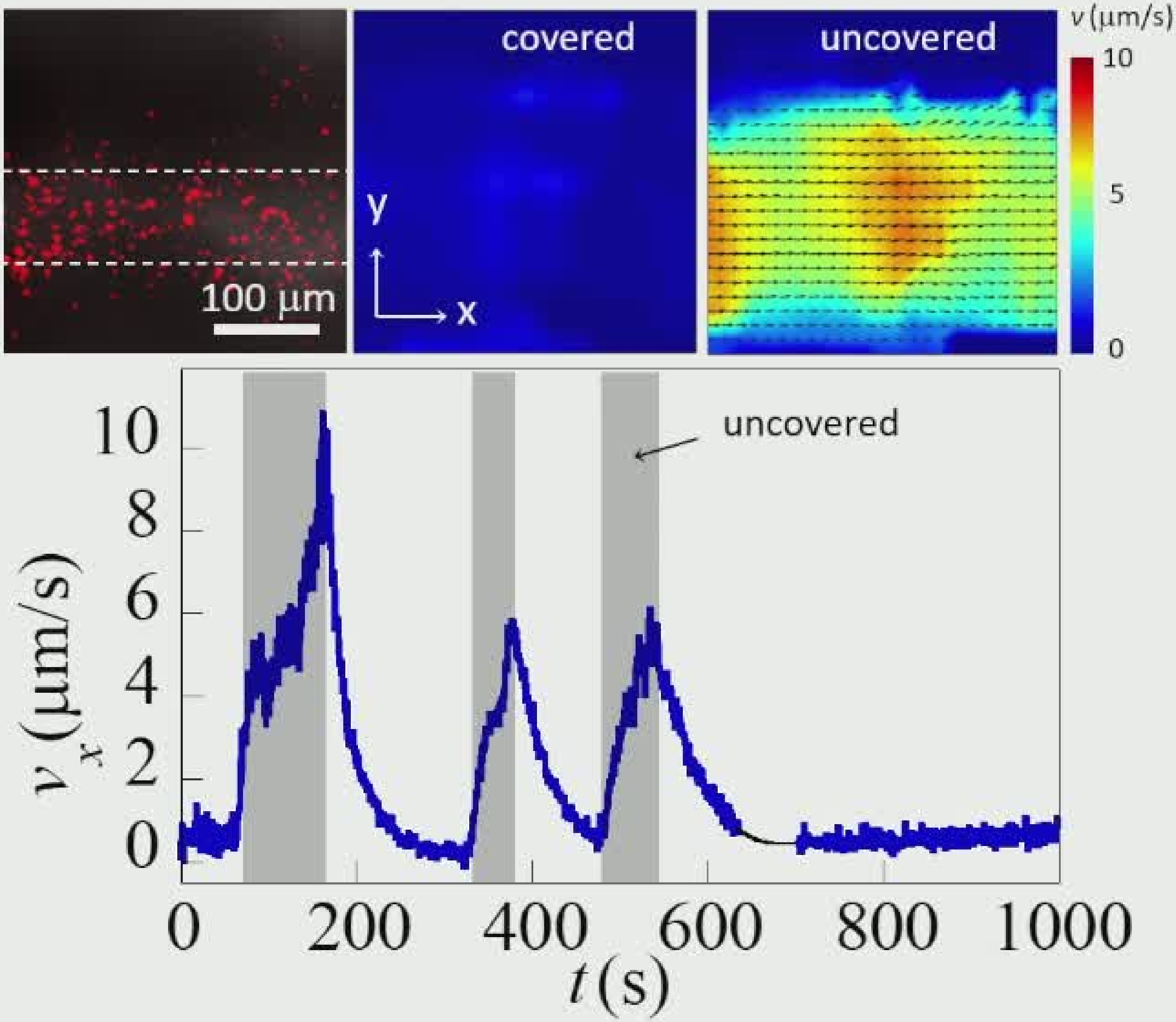


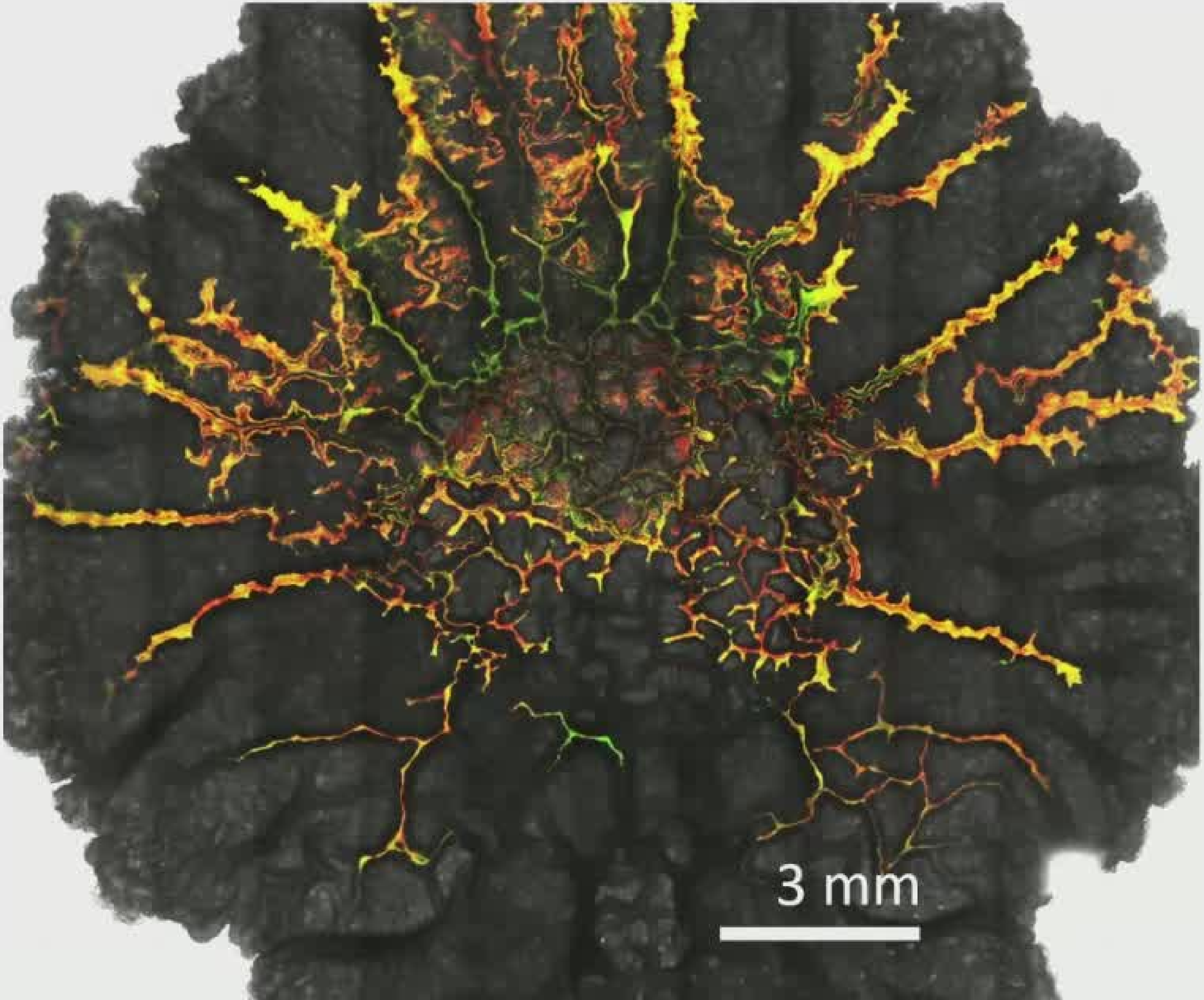
Can flow be turned on and off by controlling evaporation?



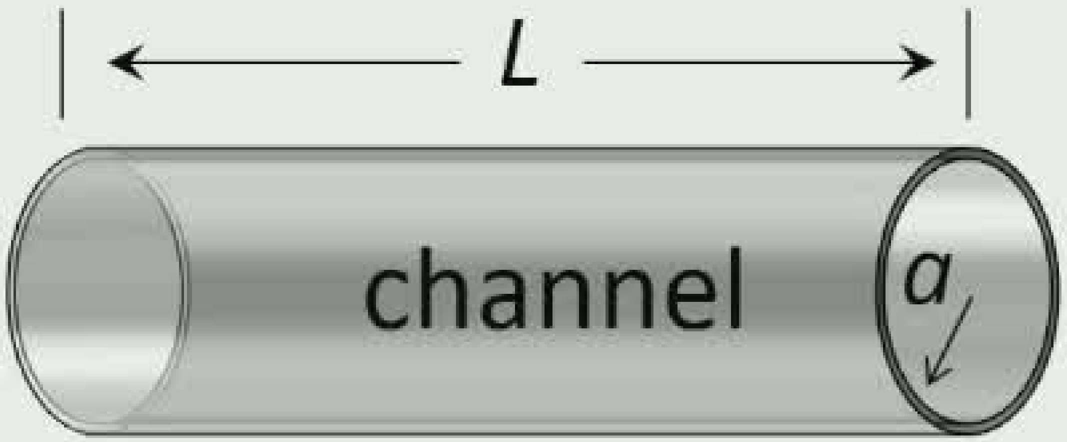
Evaporation drives flow in channels (*xy*-plane)



Interconnected network of channels mapped with fluorescent colloids



Channels allow for much faster transport.



Poiseuille flow:

$$\Delta P_P = 8\eta LV / a^2$$

V = 10 μm/s,
L = 1 cm,
a = 50 μm

$\Delta P_P \approx 0.3$ Pa

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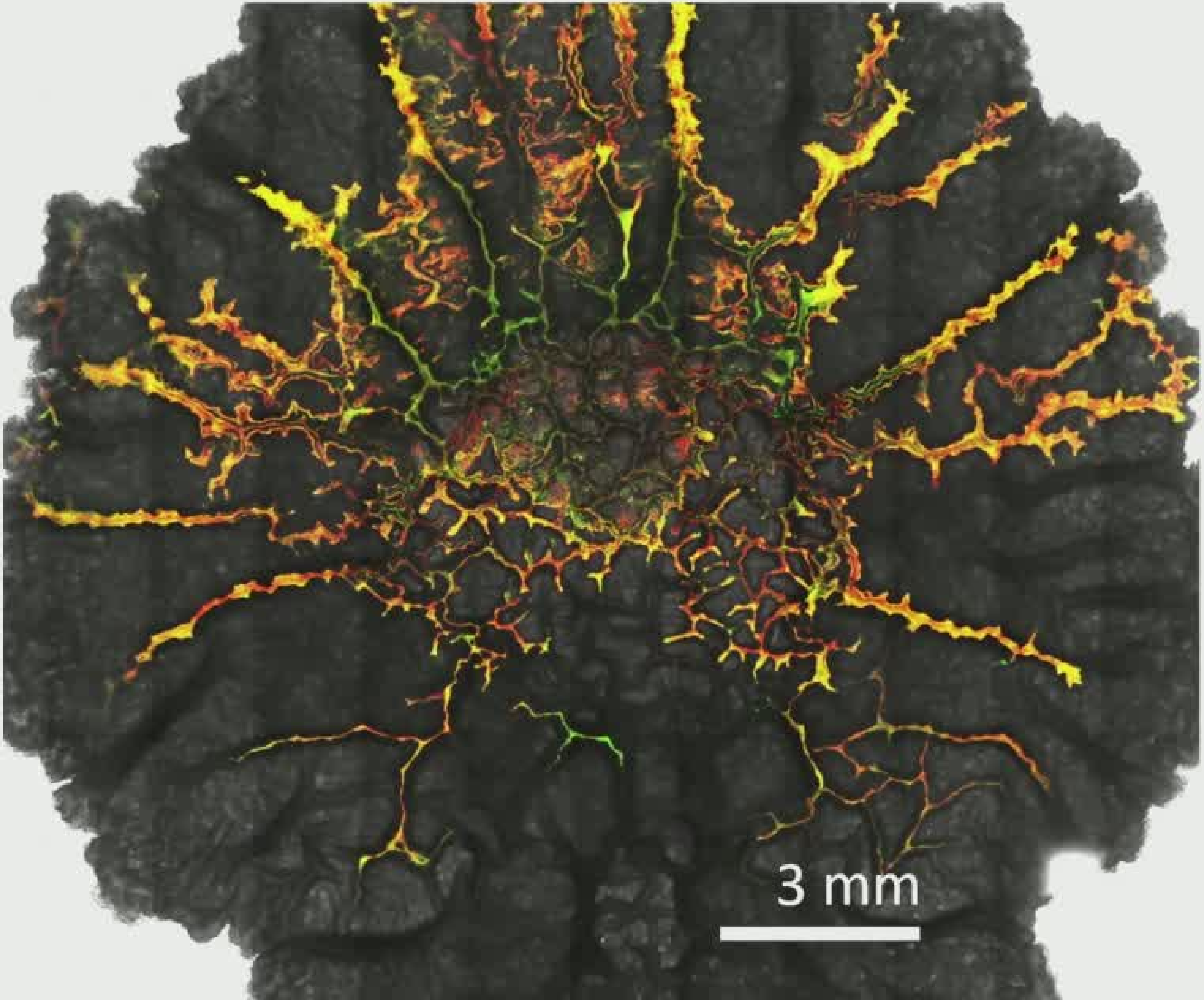

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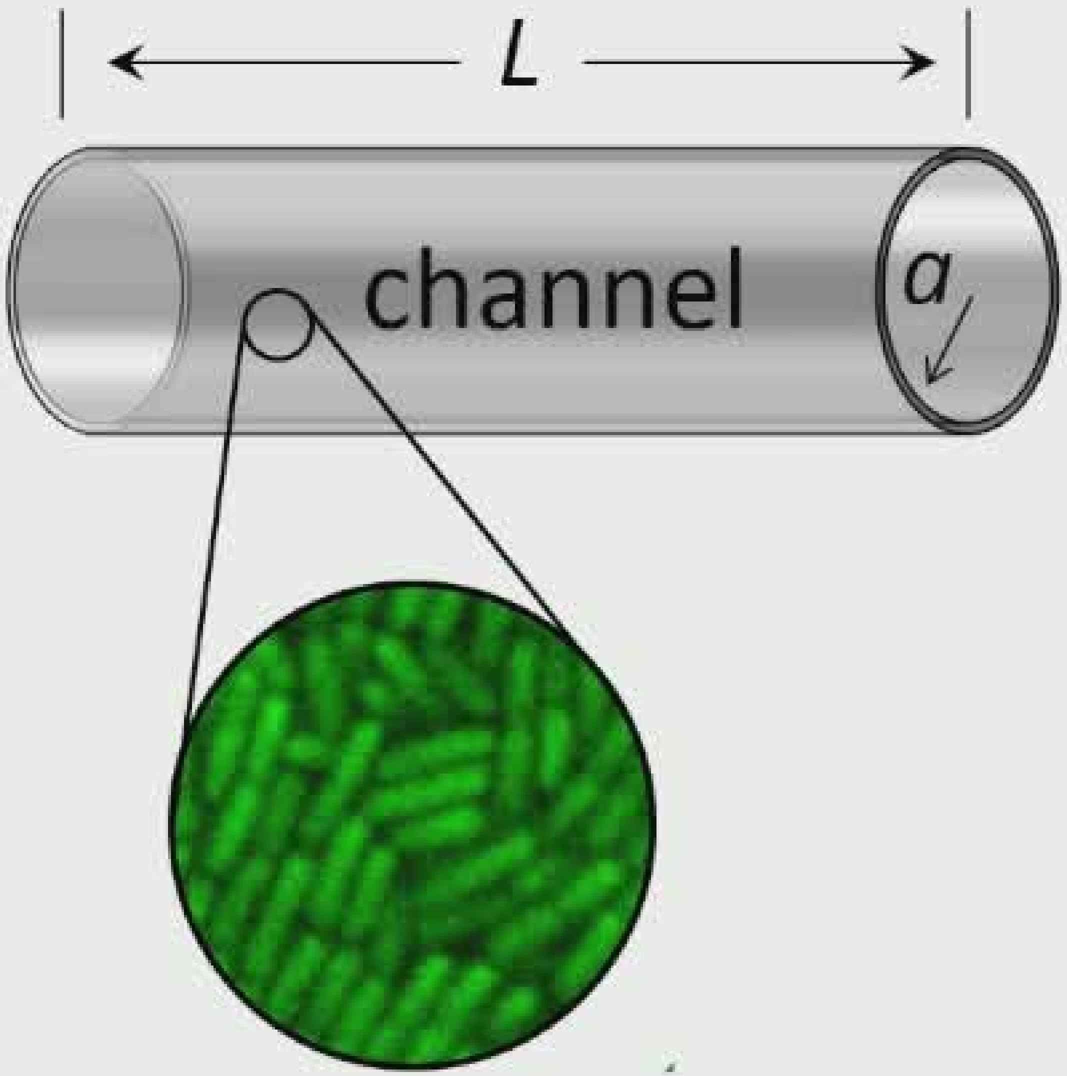


Poiseuille flow:

$$\Delta P_P = 8\eta LV / a^2$$

V = 10 μm/s,
L = 1 cm,
a = 50 μm

$\Delta P_P \approx 0.3$ Pa

Darcy flow:

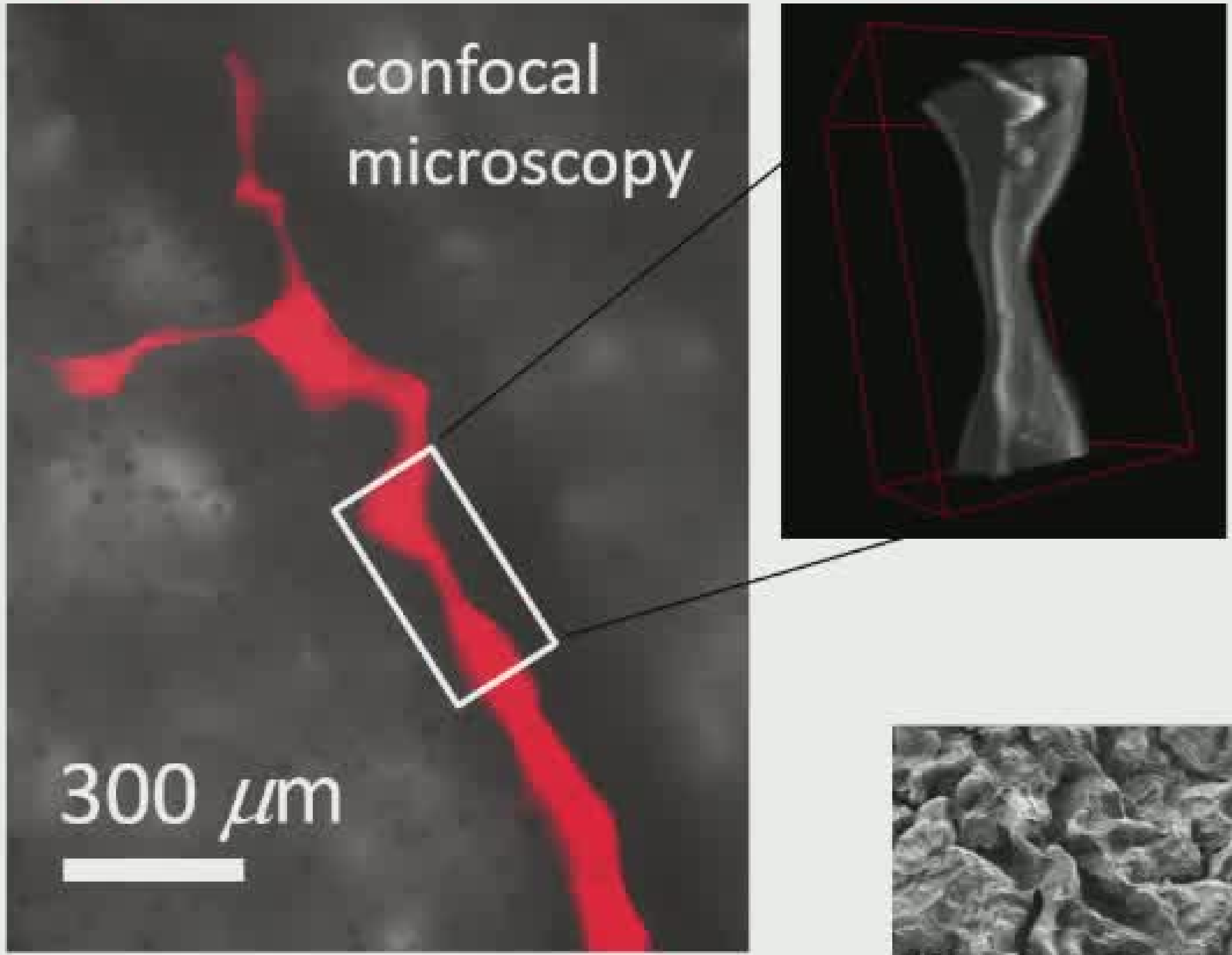
$$\Delta P_D = \eta LV / k$$

V = 10 μm/s,
L = 1 cm,
$k \approx 8$ nm^2

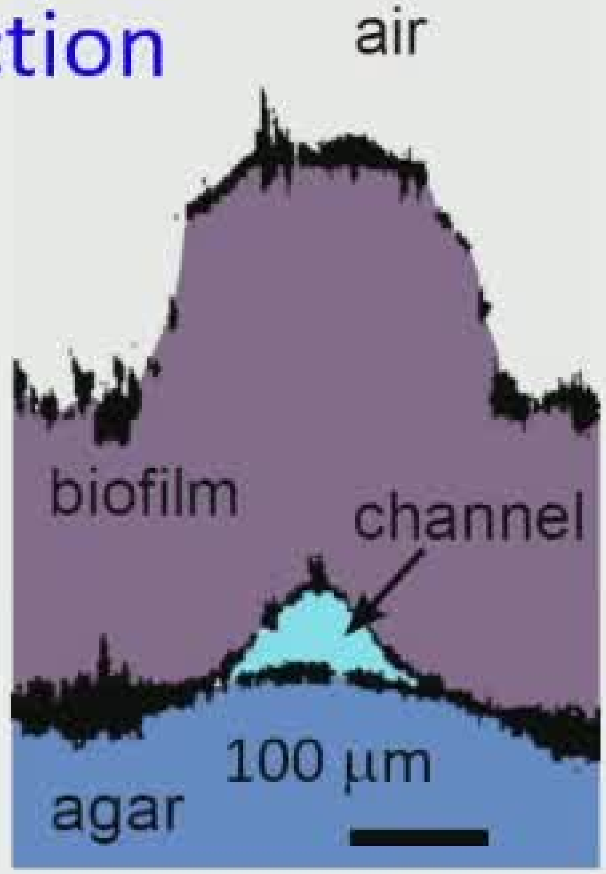
$\Delta P_D \approx 10^7$ Pa

Channel Structure

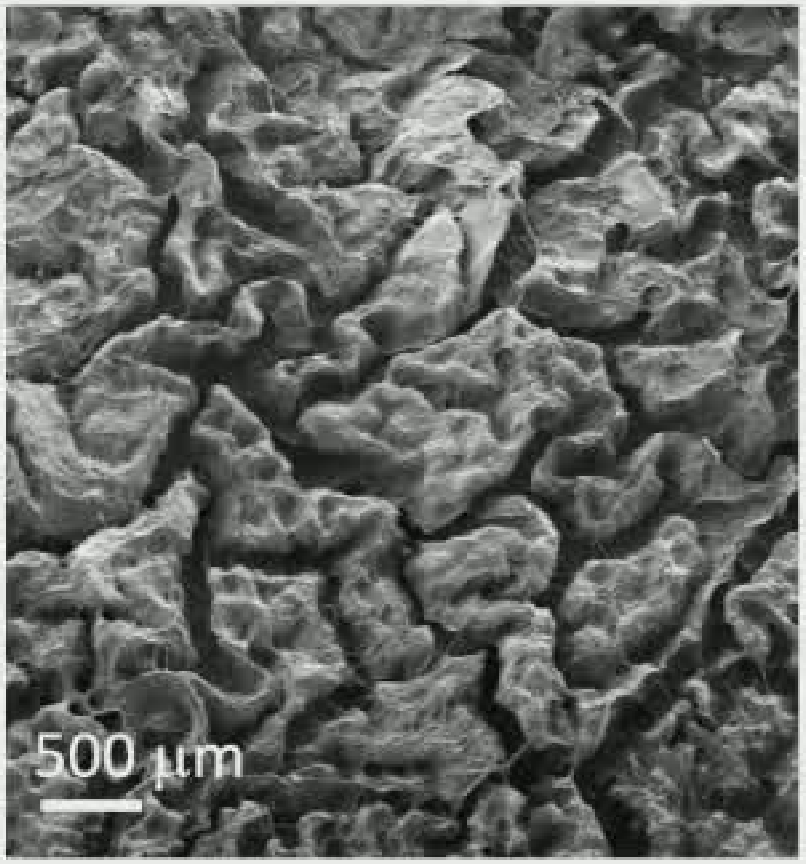
Inject cross-linkable resin

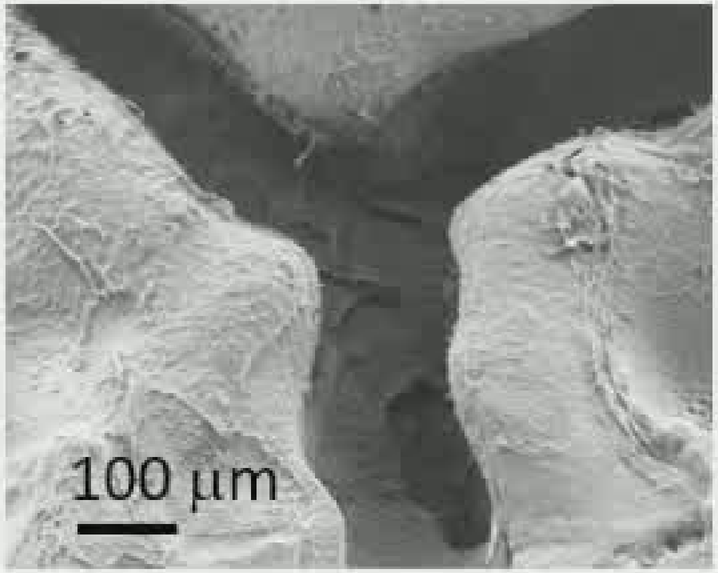


Construct channel cross-section



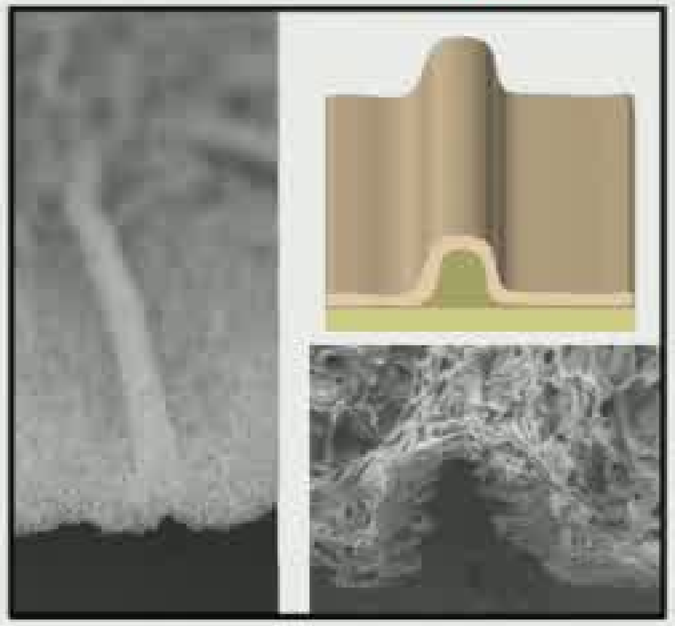
Freeze-dry& SEM biofilm bottom



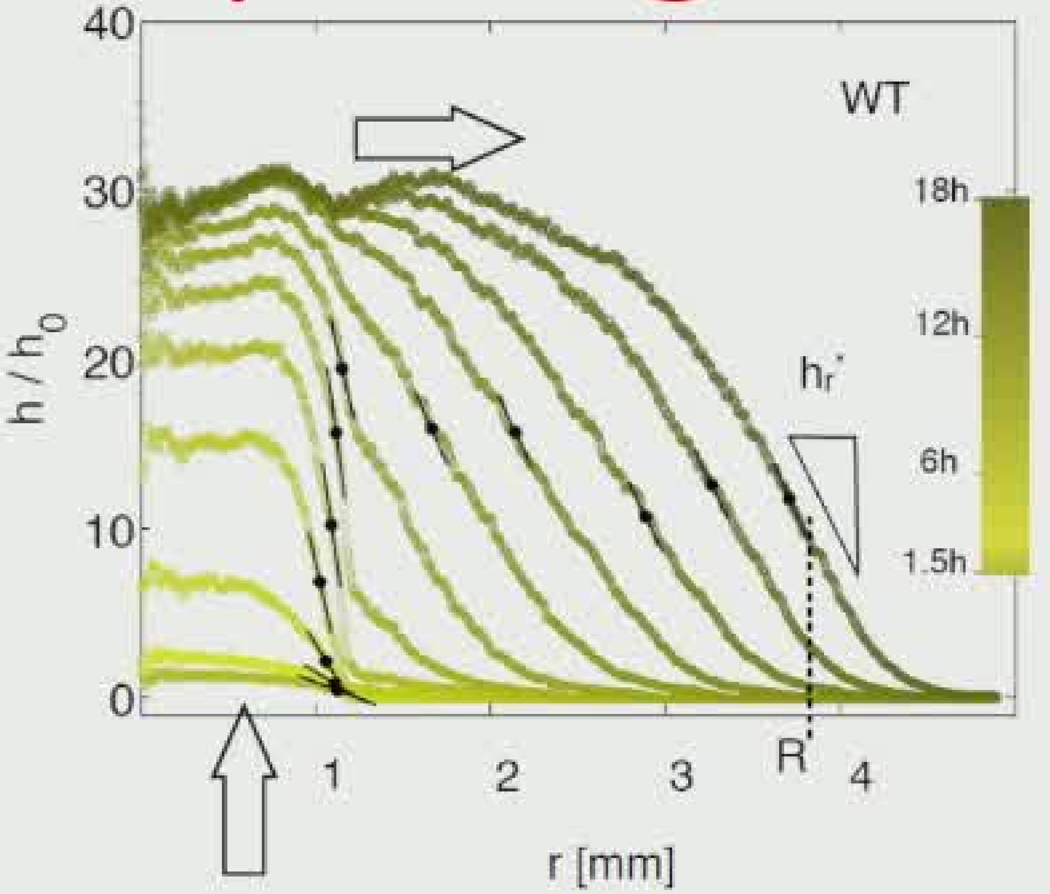


Mechanical properties are important for biofilm physiology…

Wrinkling

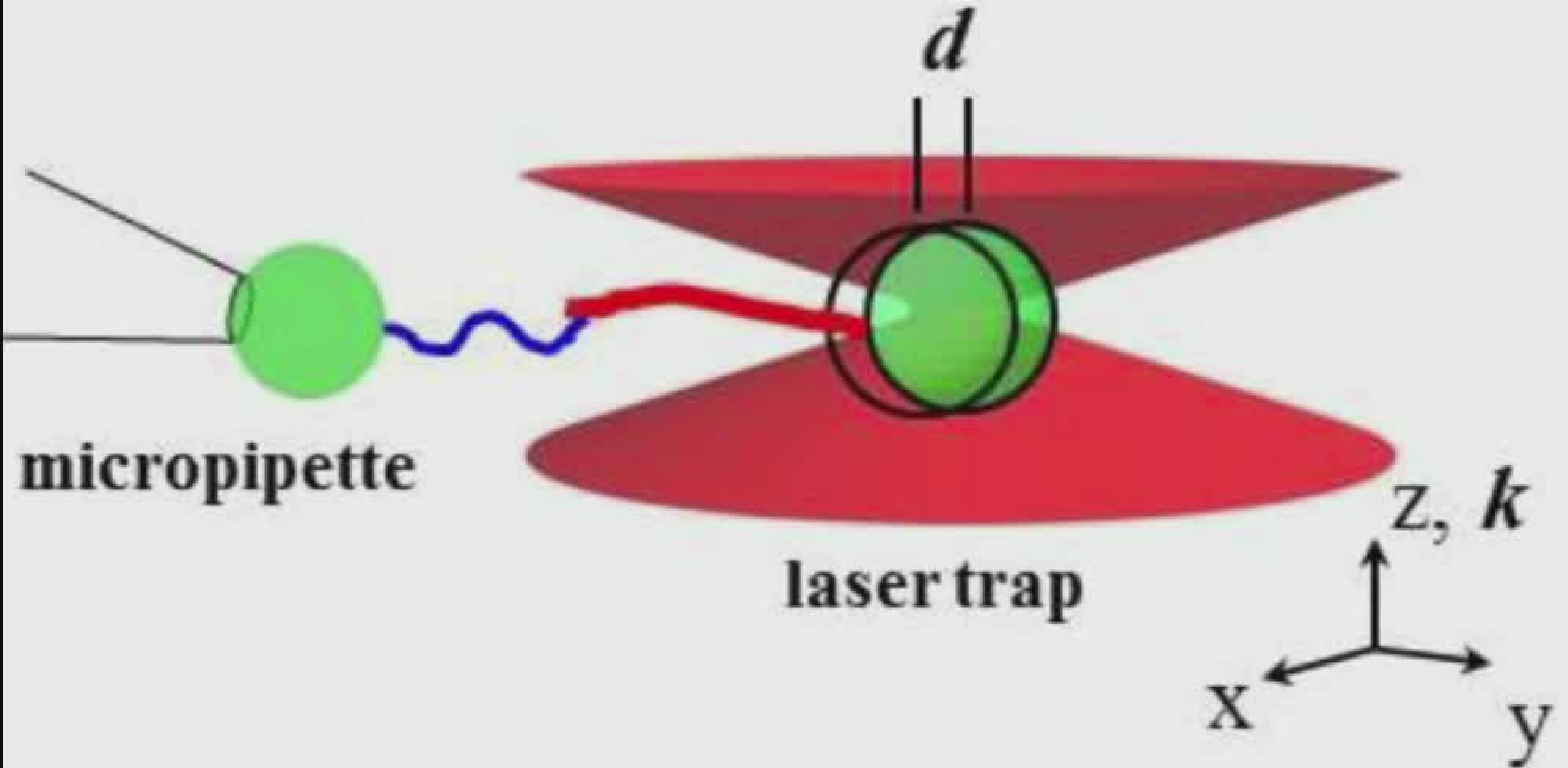


Spreading

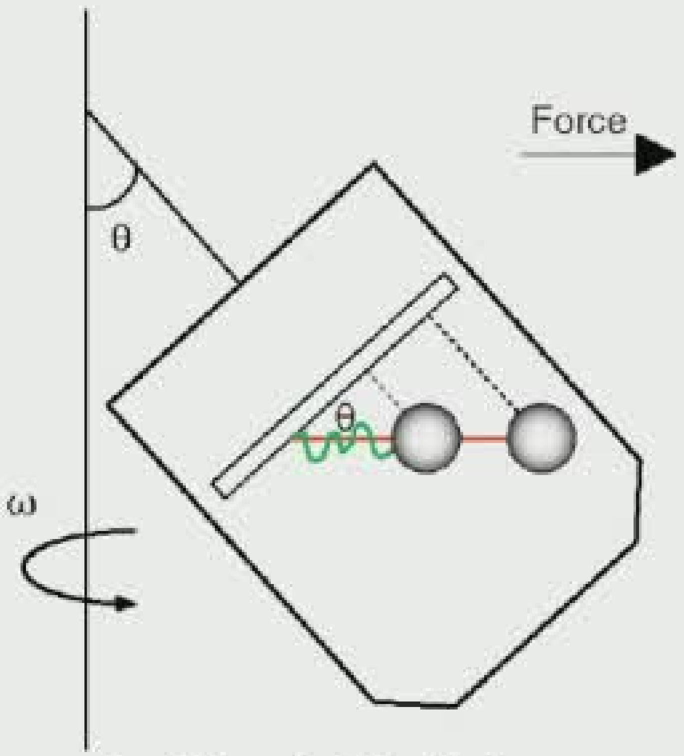


Also, for <u>biofilm removal</u> and developing <u>new materials</u>.

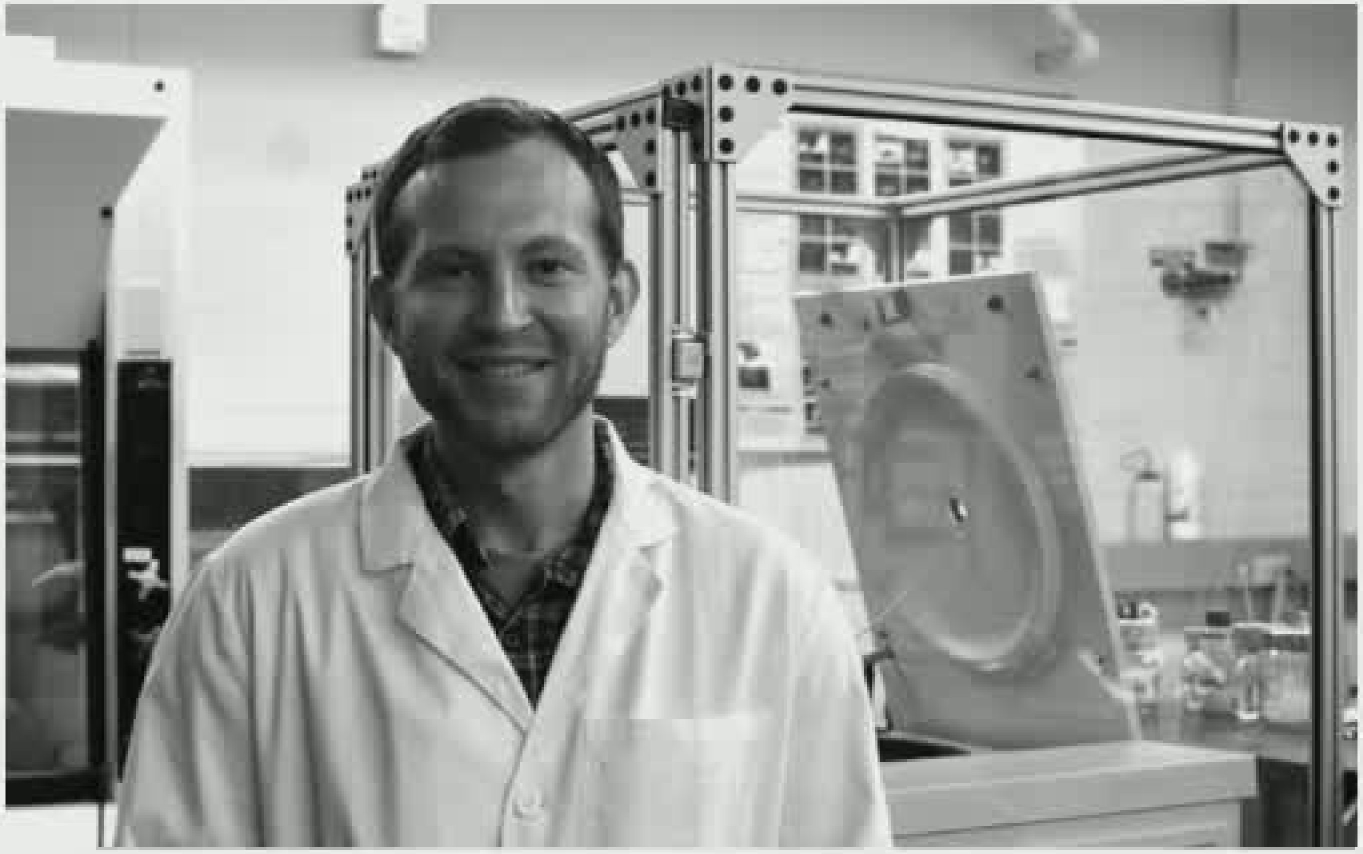
Measure interactions between matrix components



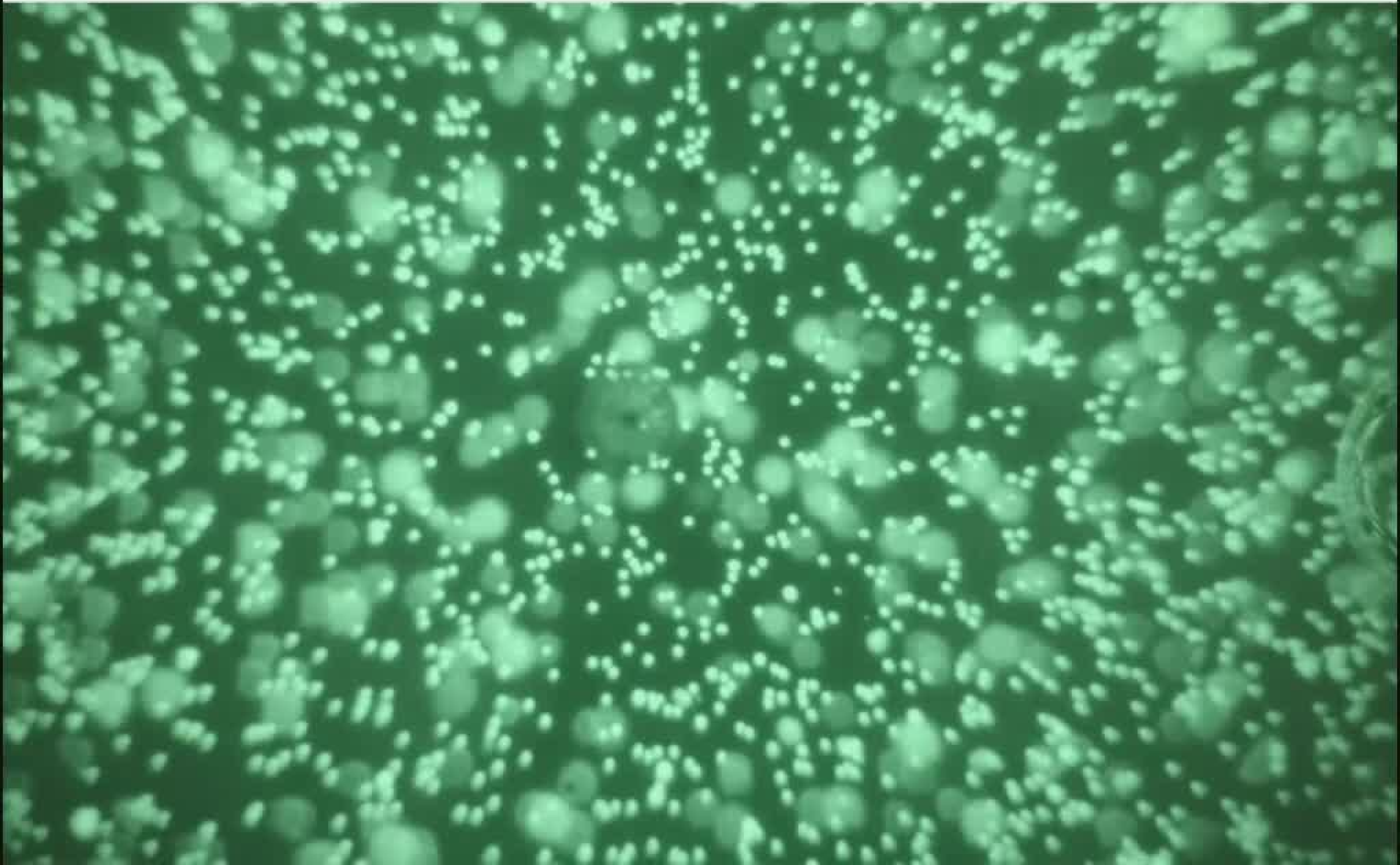
Measuring matrix molecule interaction forces with a microscope-in-a-centrifuge



D. Yang, A. Ward, K. Halvorsen, W. P. Wong, Nature Communications, 2016.



Measuring matrix molecule interaction forces with a fluorescence microscope-in-a-centrifuge



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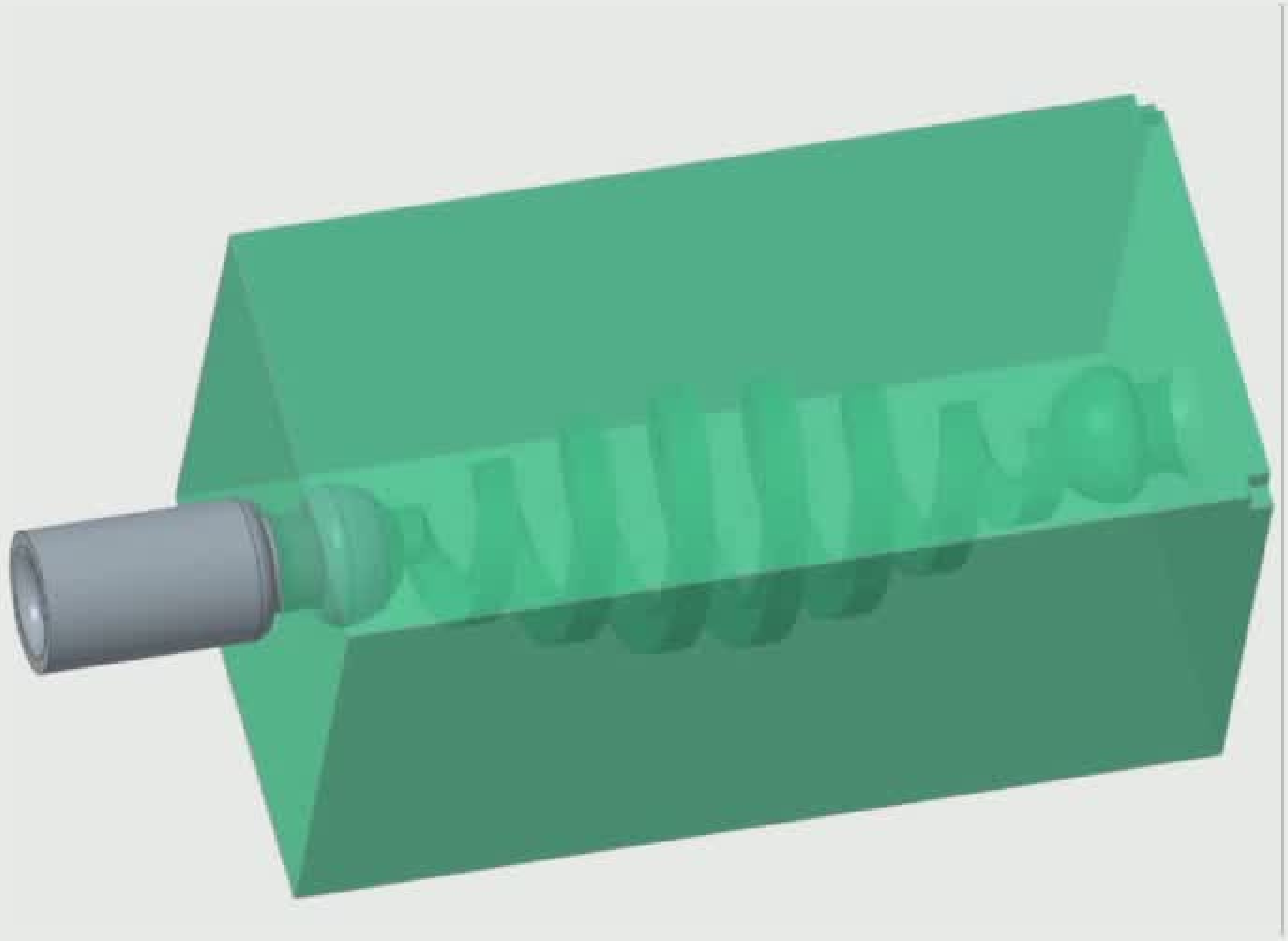
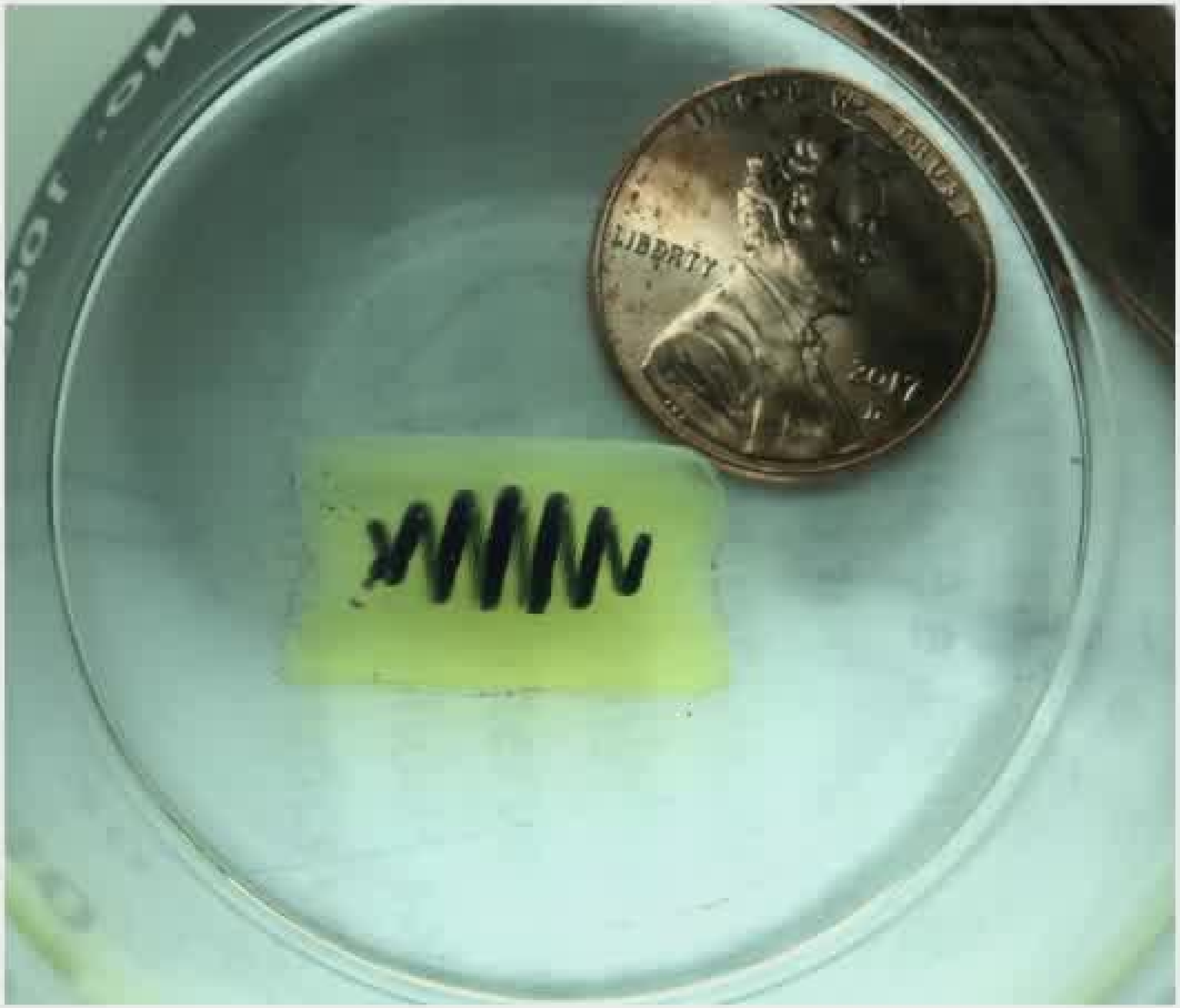
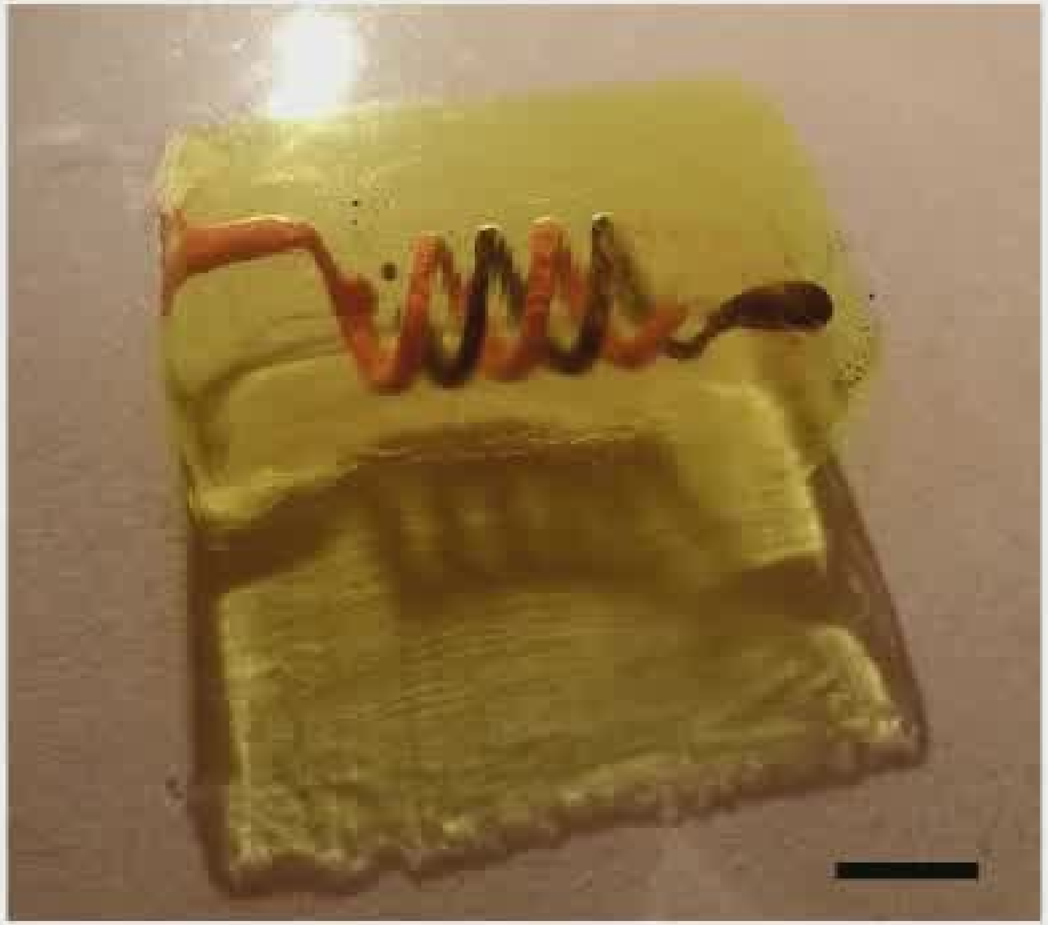
Demonstration of liquid flow through submillifluidic hydrogel channels



Can repeat this optimization process with any photoblocker.





A. D. Benjamin, R. Abbasi, M. Owens, R. J. Olsen, T. B. LeFevre, D. J. Walsh, J. N. Wilking, Light-Based 3D Printing of Hydrogels with High Resolution Channels, *Biomedical Physics & Engineering Express* (2018).

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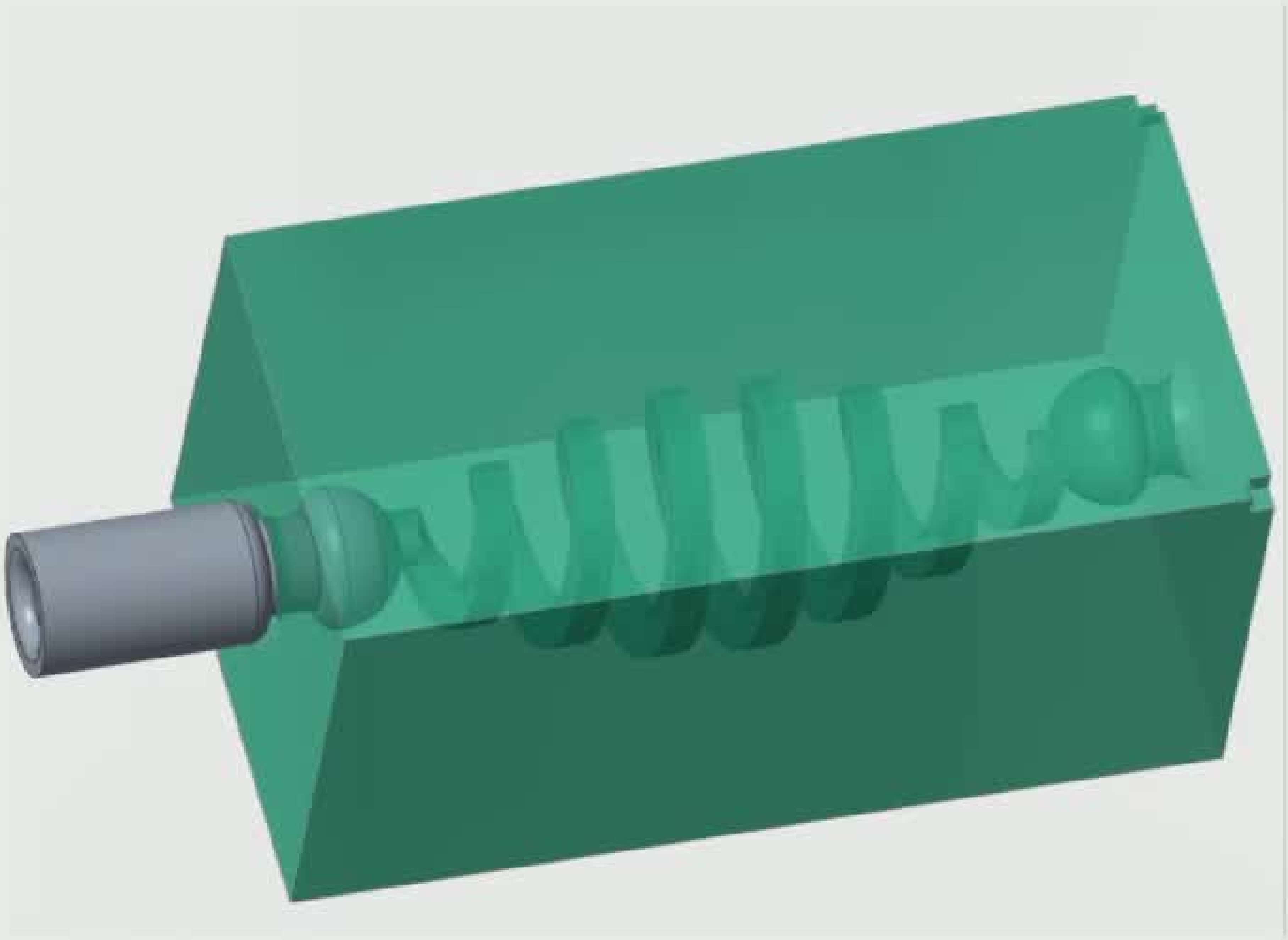

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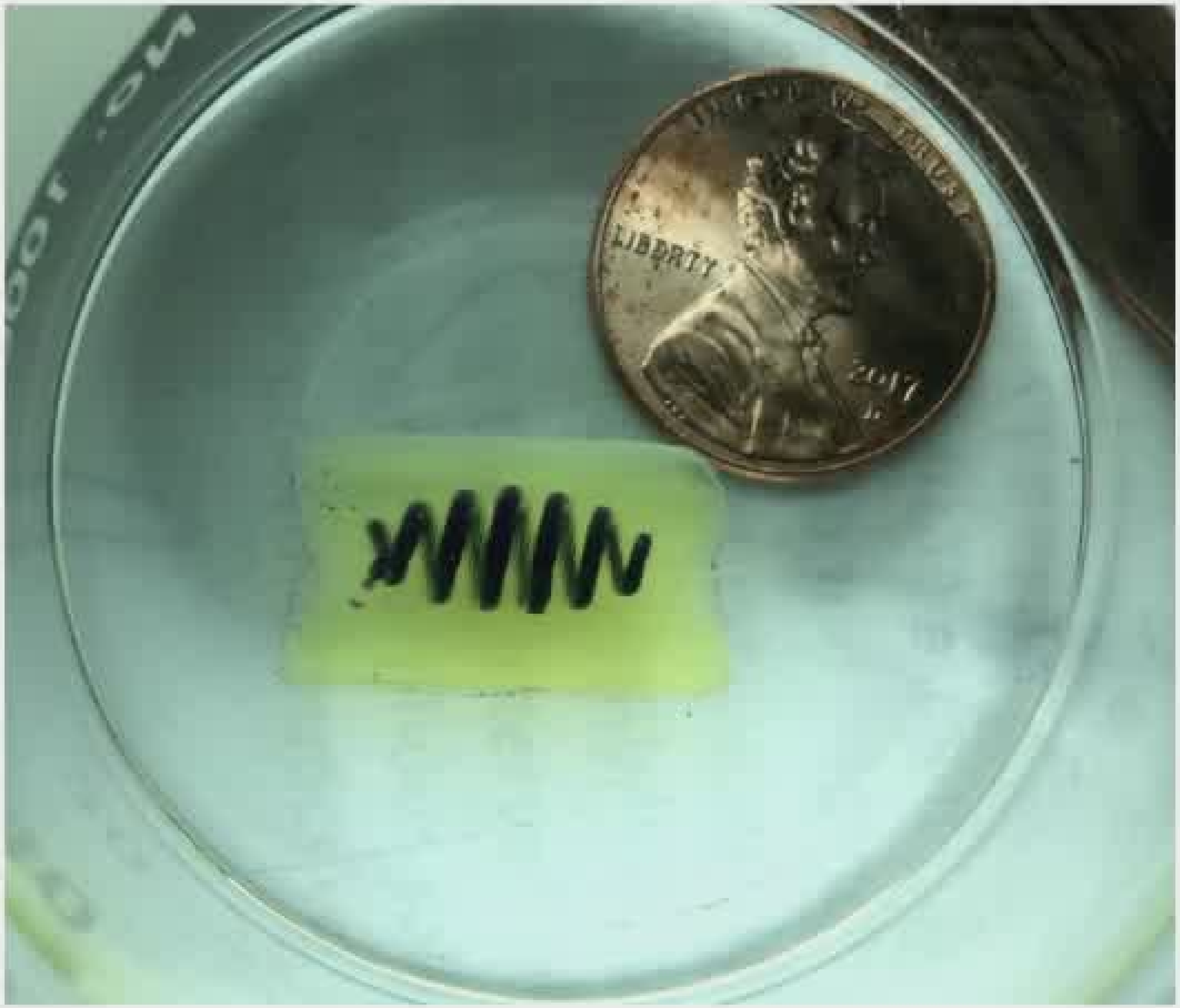

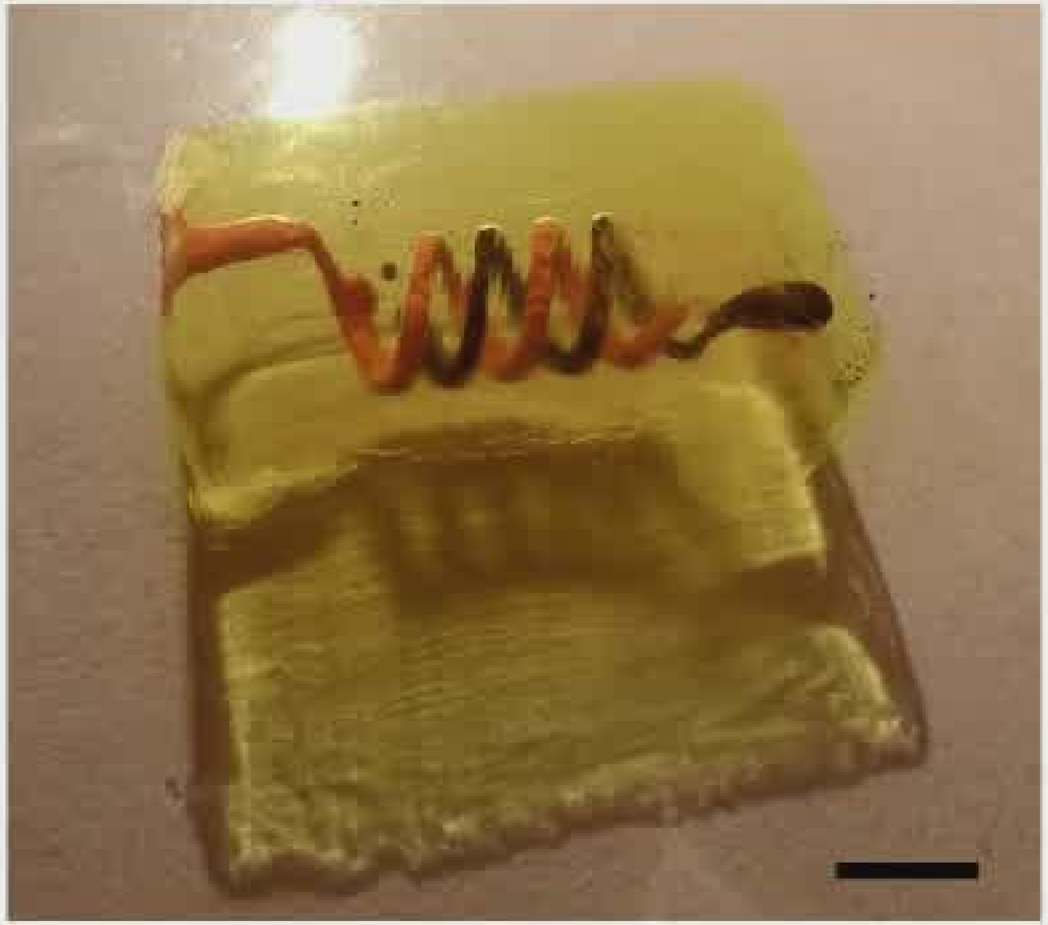

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Summary

- Goal: to understand the structure, mechanics, and dynamics of a model biofilm.
- Osmotic spreading in *B. subtilis* biofilms is driven by matrix production.
- Liquid-filled channels exist in *B. subtilis* biofilms with flow driven by differential evaporation.
- *B. subtilis* biofilms are viscoelastic and the interaction between amyloid fibers and polysaccharides requires further study → single molecule measurements.
- We have developed an approach for creating well-defined channels with any directional orientation in hydrogels using light-based 3D printing → printing biofilms.