PERSATLAB

ME480: Mechanobiology

Lecture #2

September 18th, 2024

By Laure Le Blanc, postdoc in the Persat lab

Outline

- Introduction 15min
- An example of a recent study in the field: my PhD work 45min
- Introduction to **project 1** 30min
- **Hands-on**: Image analysis tutorial 45min x2

I. Introduction

slido

Please download and install the Slido app on all computers you use

What is mechanobiology?

ⓘ Start presenting to display the poll results on this slide.

What is mechanobiology?

MECHANICS

Shear	**Friction**	**Tension**	**Compression**
Flow	*Substrate*	*Optical tweezers*	*AFM, Growth in a limited space*

Project 3

- How **physical / mechanical cues** shape the biology of living organisms
- How cells respond to mechanical forces

(MICRO)BIOLOGY

II. An example of a recent study

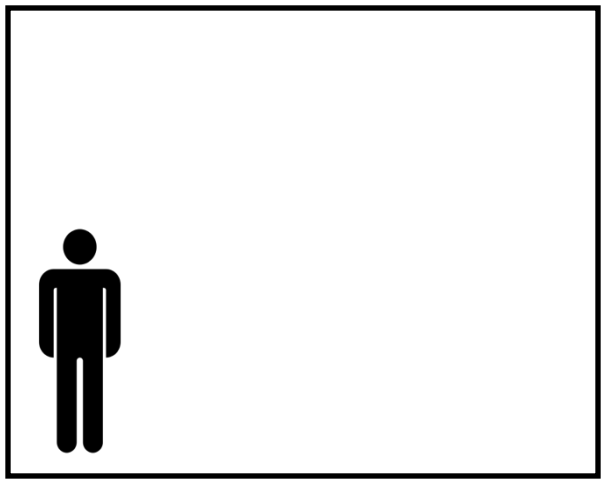
Bacterial growth under confinement

L. Le Blanc, …., M. Delarue*, G. Dumenil*, D. Bonazzi* *(under review)*

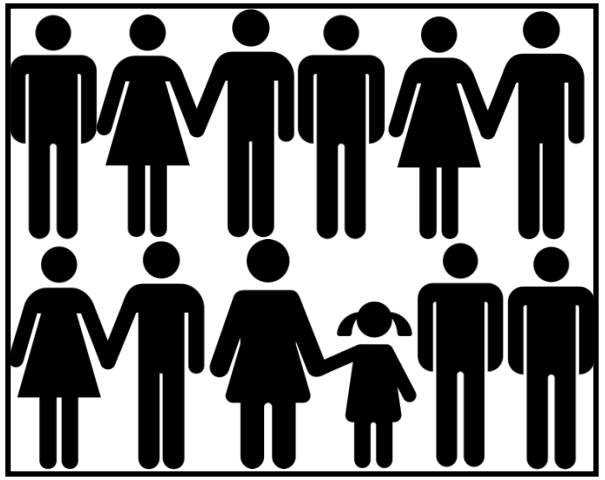
II.1. Introduction

II.1.1. Confinement: a visual definition

No confinement



Confinement



- **Limited space**
- **Accumulation** of people / cells
- **Close proximity to** neighbors
- **Change in behaviors**

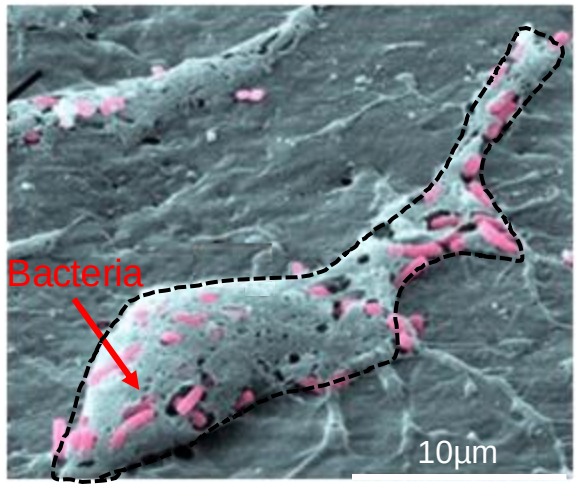
II.1.2. Prokaryotes also face confinement

Formation of dense bacterial colonies

Within biofilms

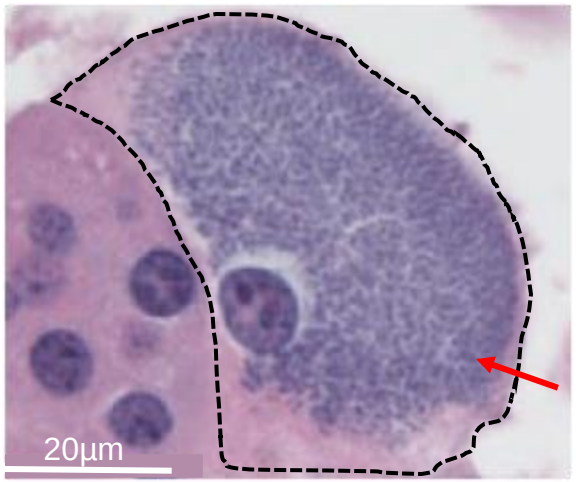
Within host cellular structures

Escherichia coli



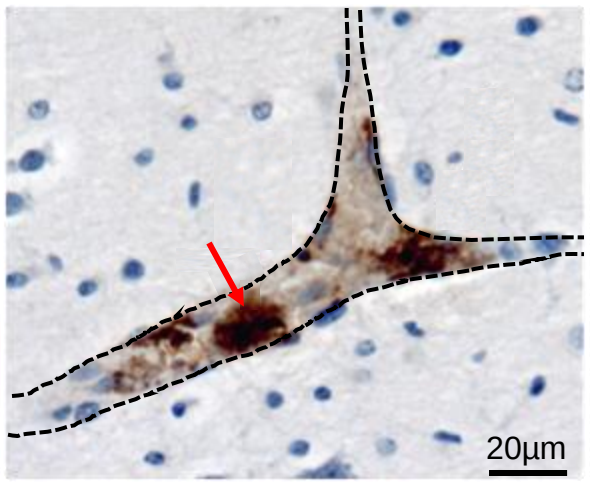
Khelissa et al., 2010

Escherichia coli



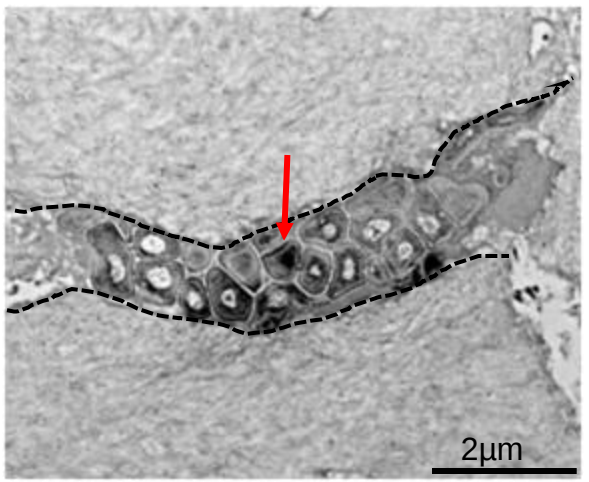
Anderson et al., 2003

Neisseria meningitidis



Melican & Duménil, 2012

Staphylococcus aureus



Masters et al. 2019

----- **Extracellular matrix**

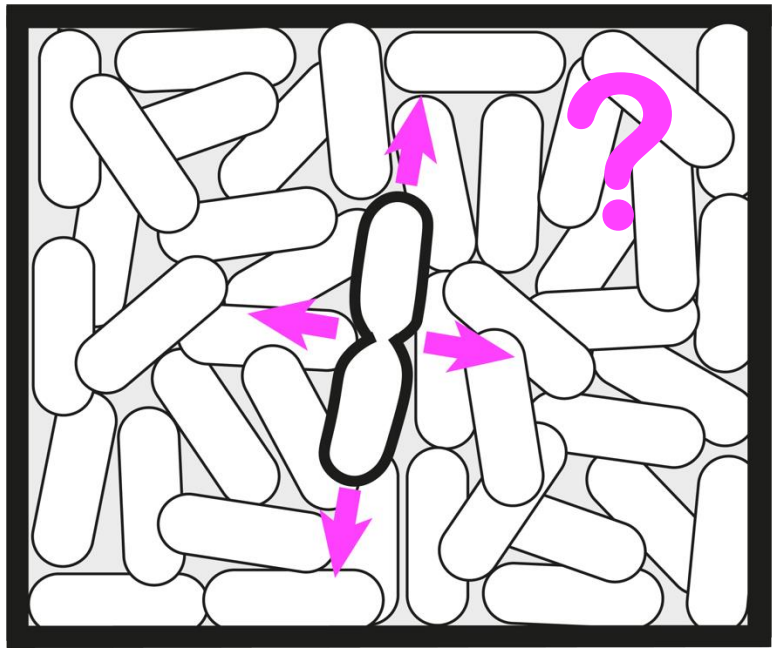
Host cell membrane

Endothelium

Bone fracture

Confinement is a general situation encountered by bacteria in the infectious context

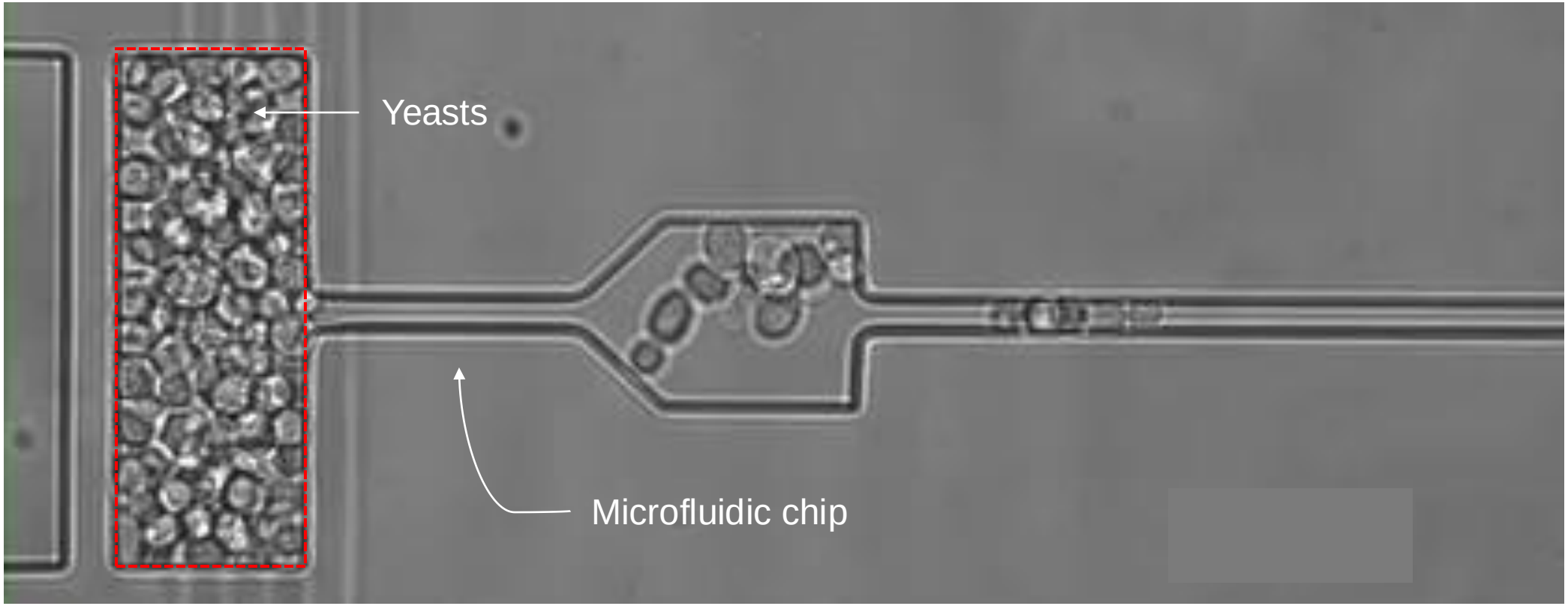
II.1.3. Main question



How **mechanical confinement** impacts **bacterial physiology?**

II.2. Methods

Yeast confinement

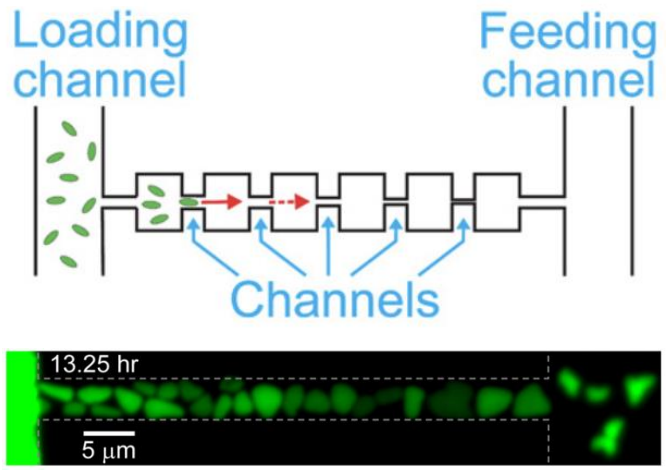


M. Delarue et al, Nat. Physics, 2016

II.2. Methods

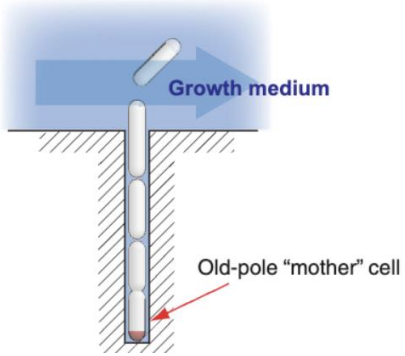
Microfluidics for microbiology

Design structured environments

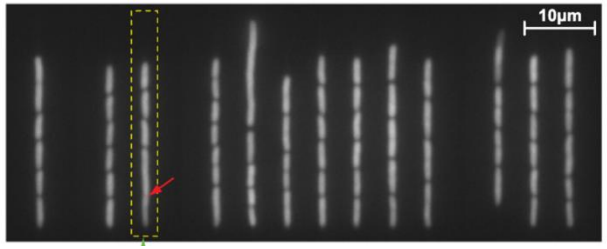


Männik et al. 2009

Study bacterial genealogy

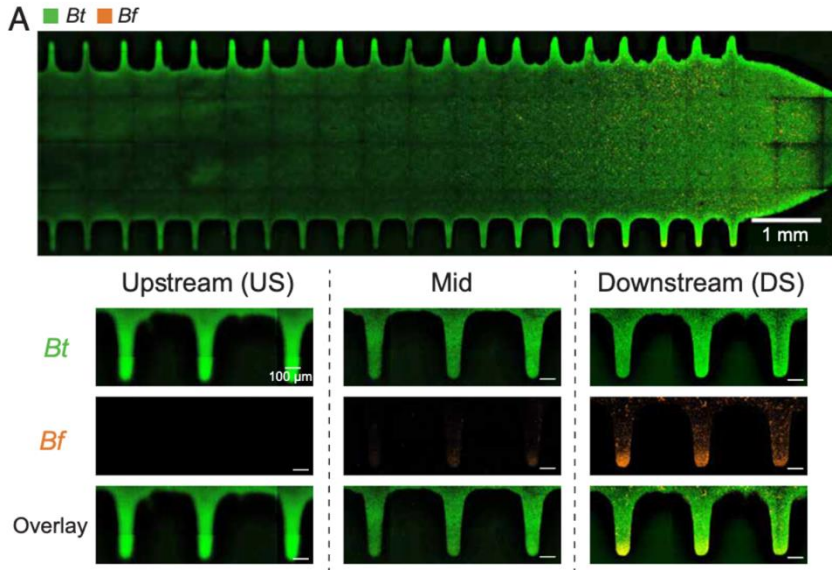


Mother machine



Wang et al. 2010

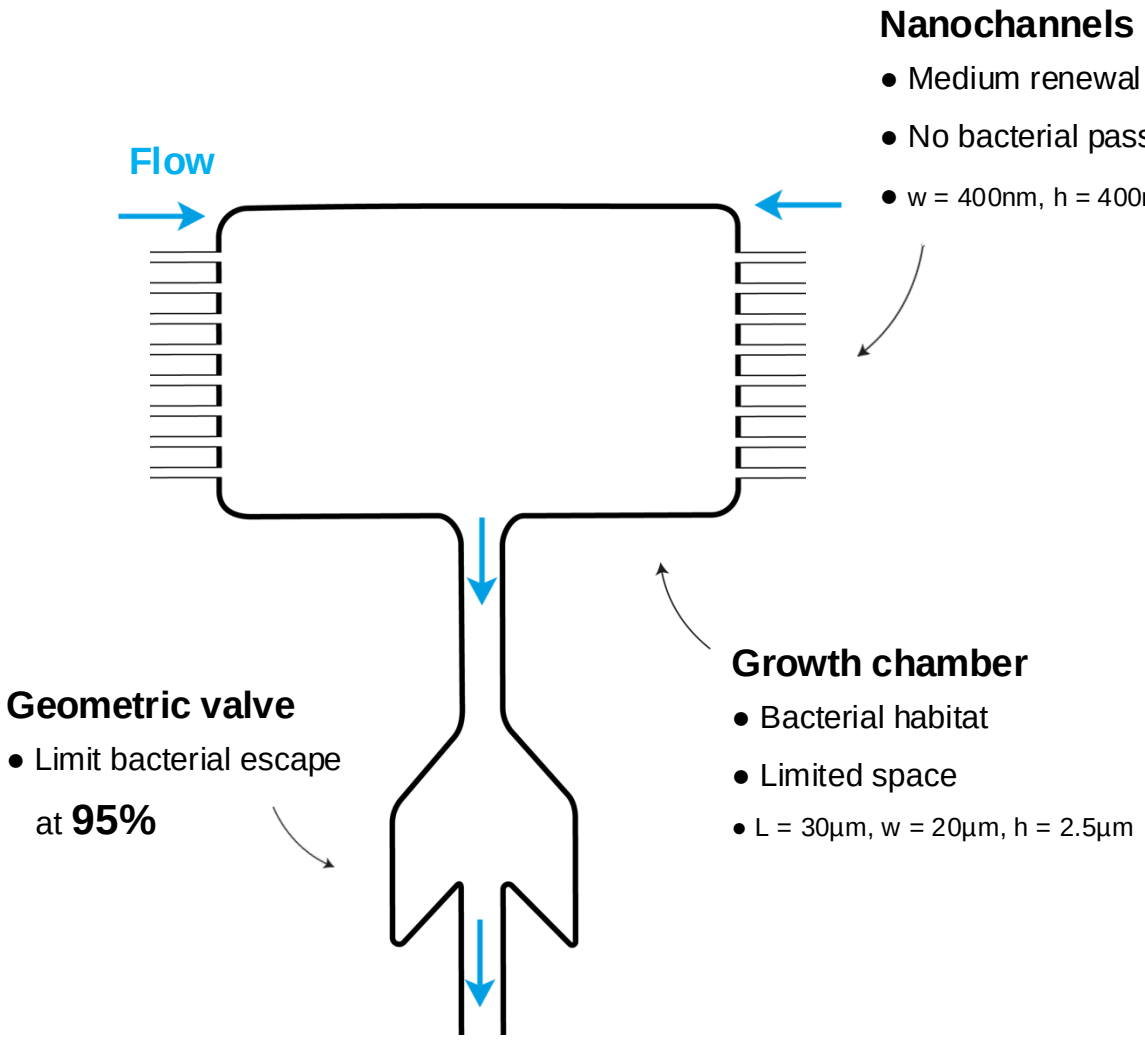
Study bacteria under flow



Wong et al. 2023

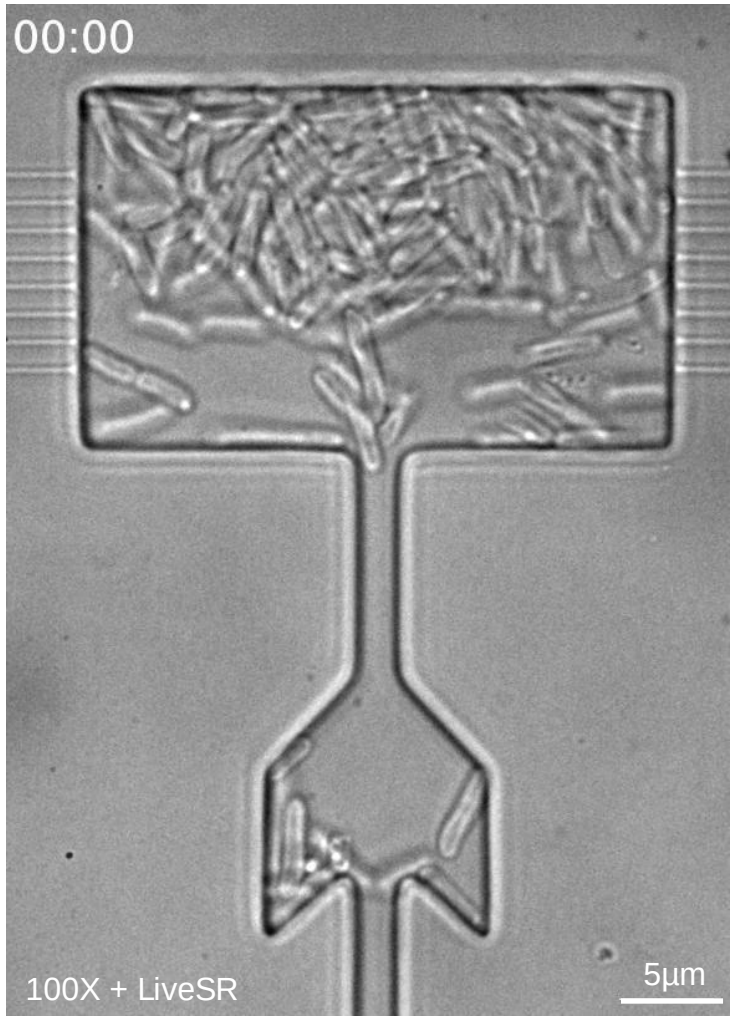
II.2.1. The bacterial confiner

Collaboration with:
Baptiste ALRIC, Laurent MAZENQ
and Morgan DELARUE
(LAAS-CNRS, Toulouse, France)



Inspired by *M. Delarue et al, 2016*

High-resolution live imaging



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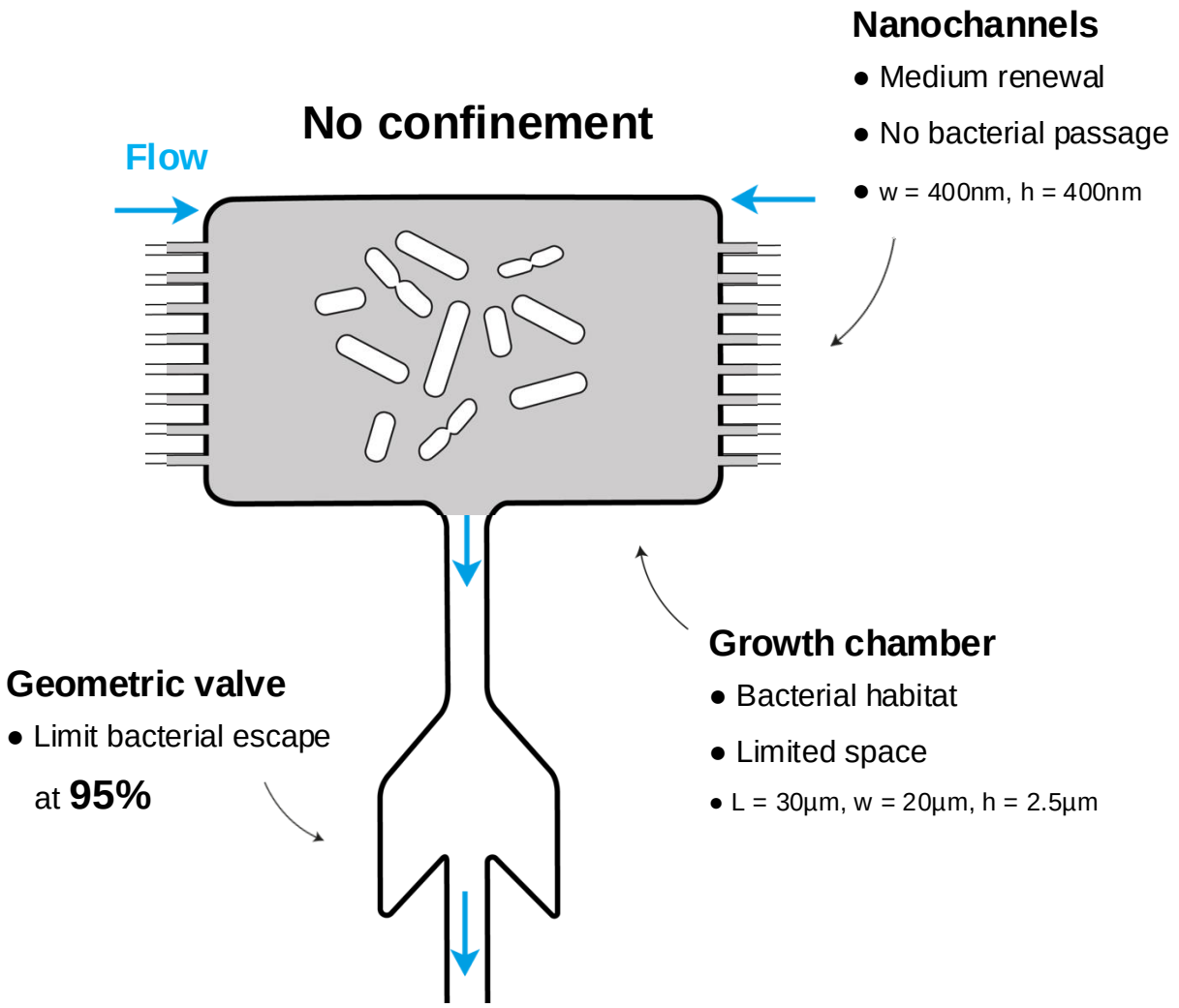

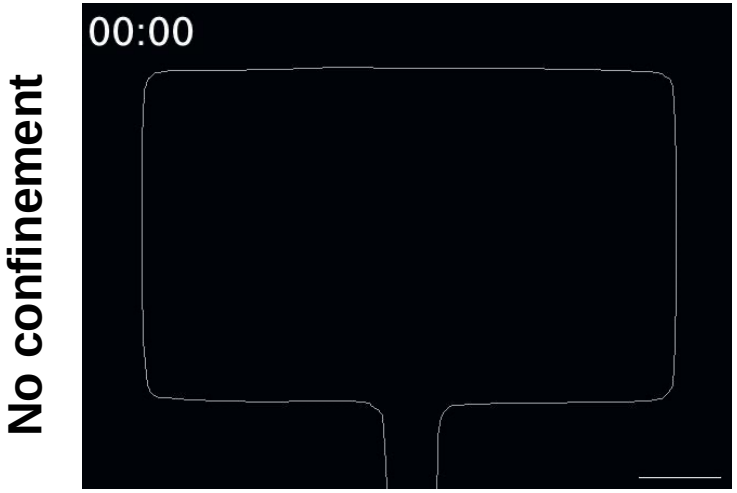

Inspired by *M. Delarue et al, 2016*

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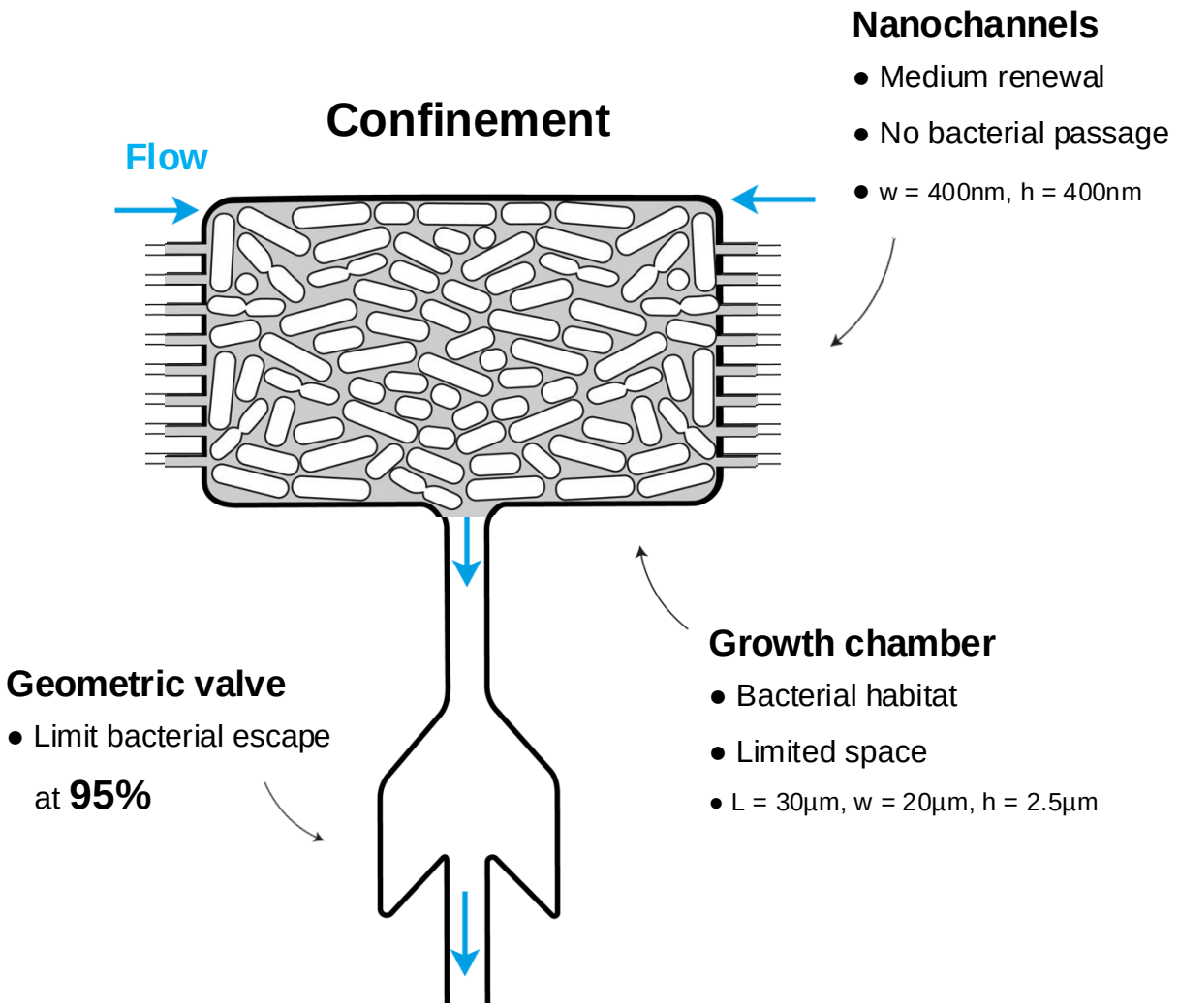
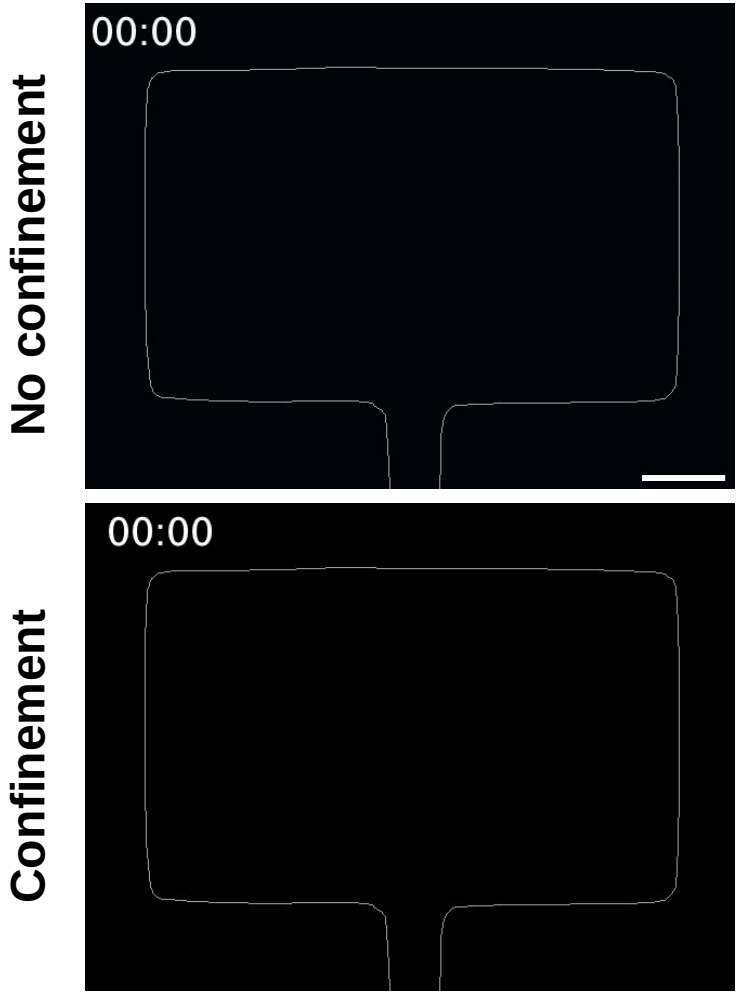




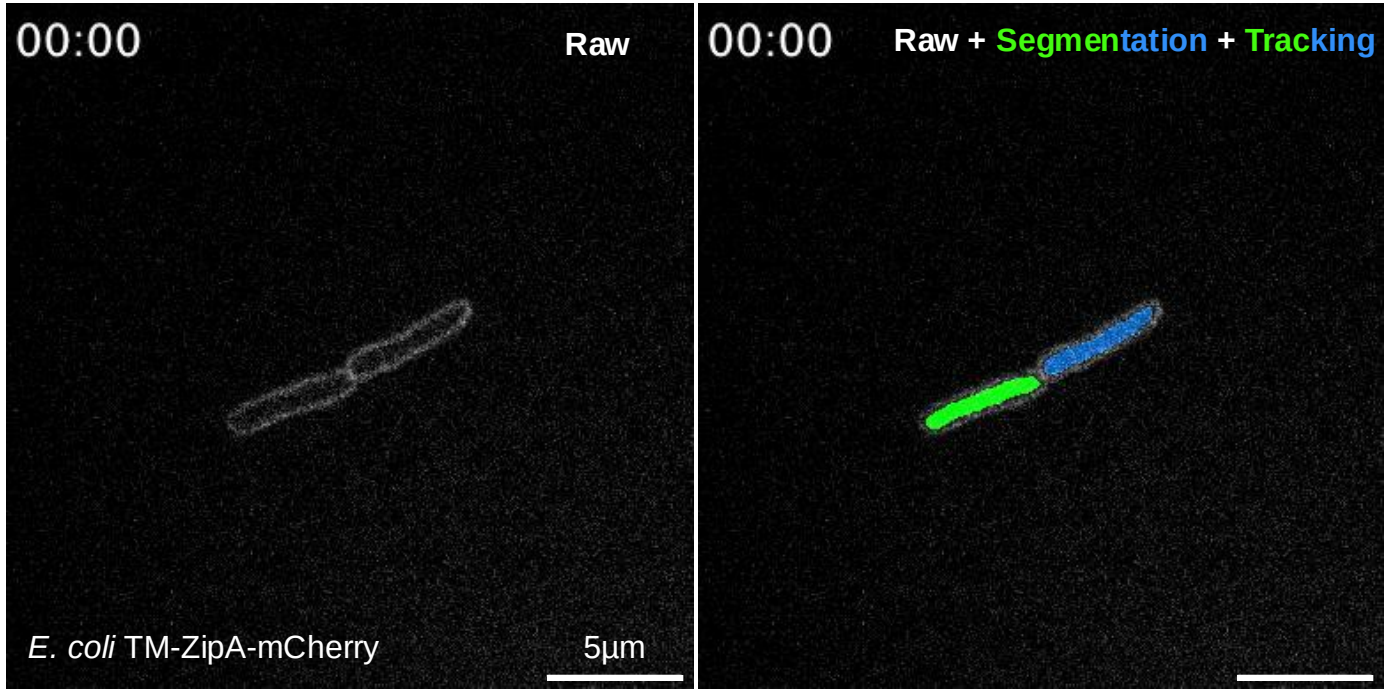
Inspired by *M. Delarue et al, 2016*

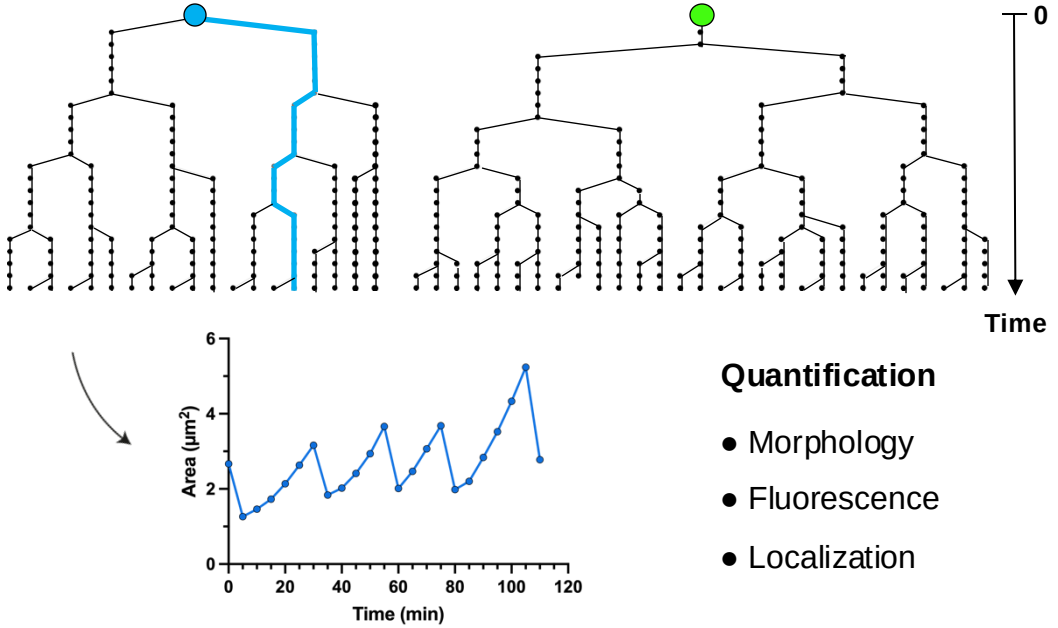
II.2.2. Image analysis

Collaboration with
Laura XÉNARD and Jean-Yves TINEVEZ
(Institut Pasteur, Paris, France)

Raw images → **Segmentation** Ilastik → **Tracking** TrackMate7 → **Post-processing** Python

Step 1: Segmentation of single cells in agar pad

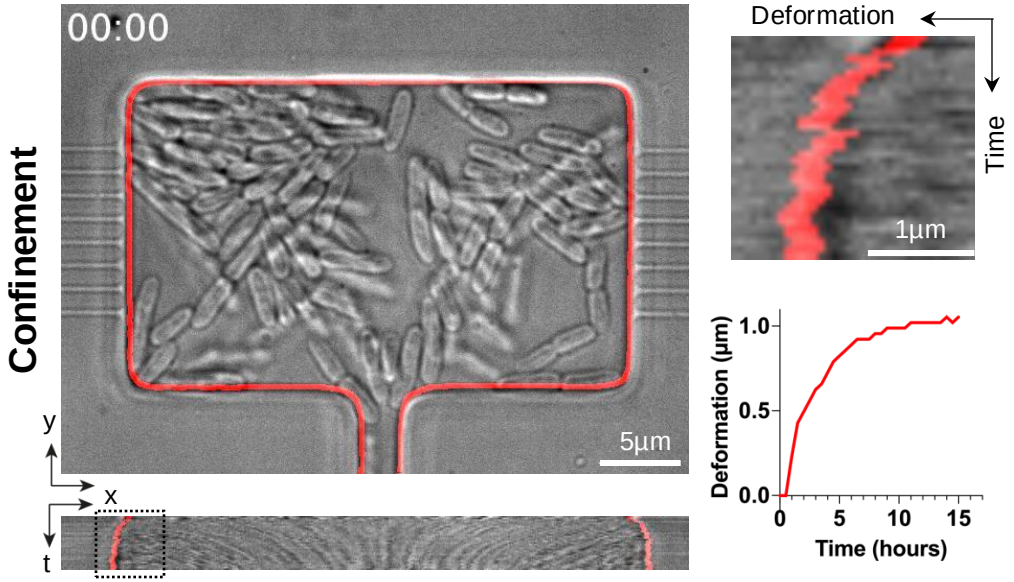


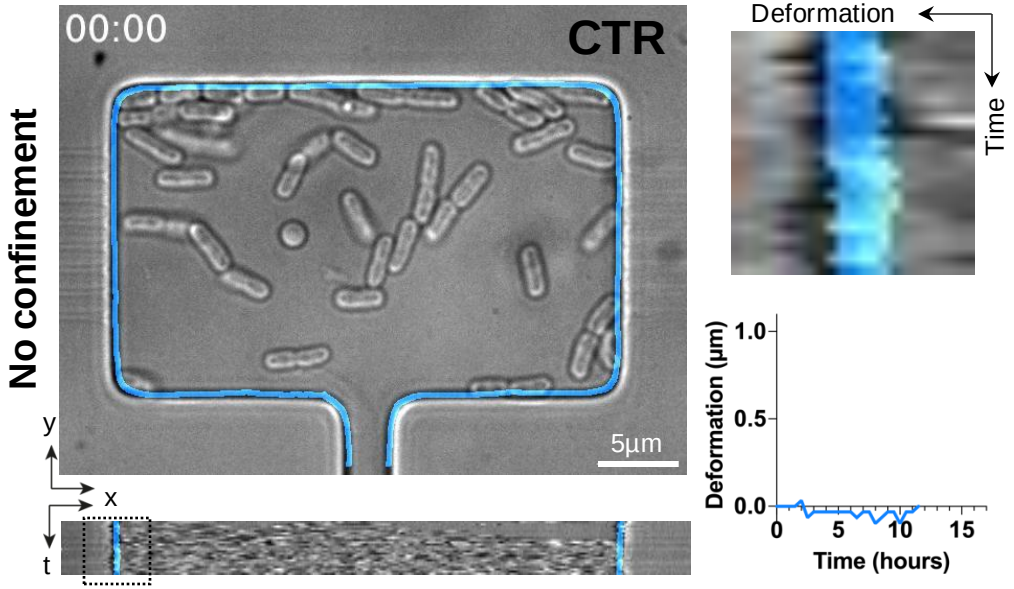


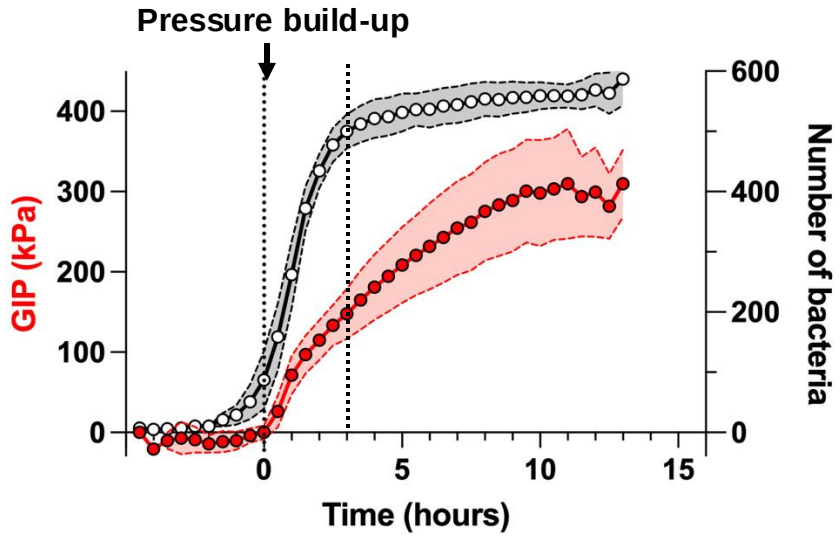
D. Ershov et al, Nat. Methods, 2022

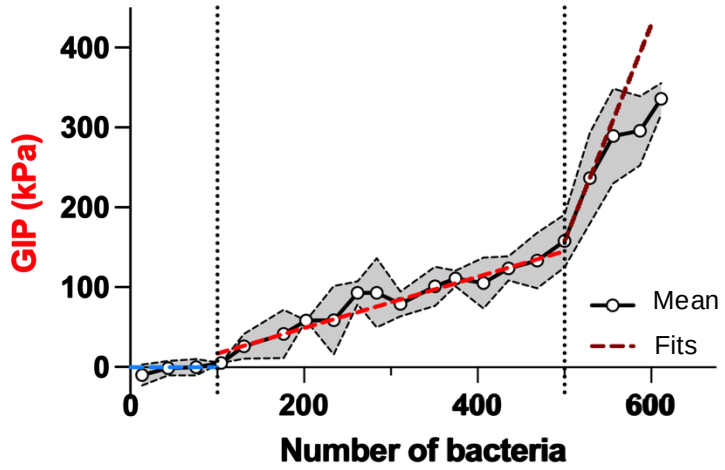
II.3. Results

II.3.1. Confined bacterial proliferation generates forces





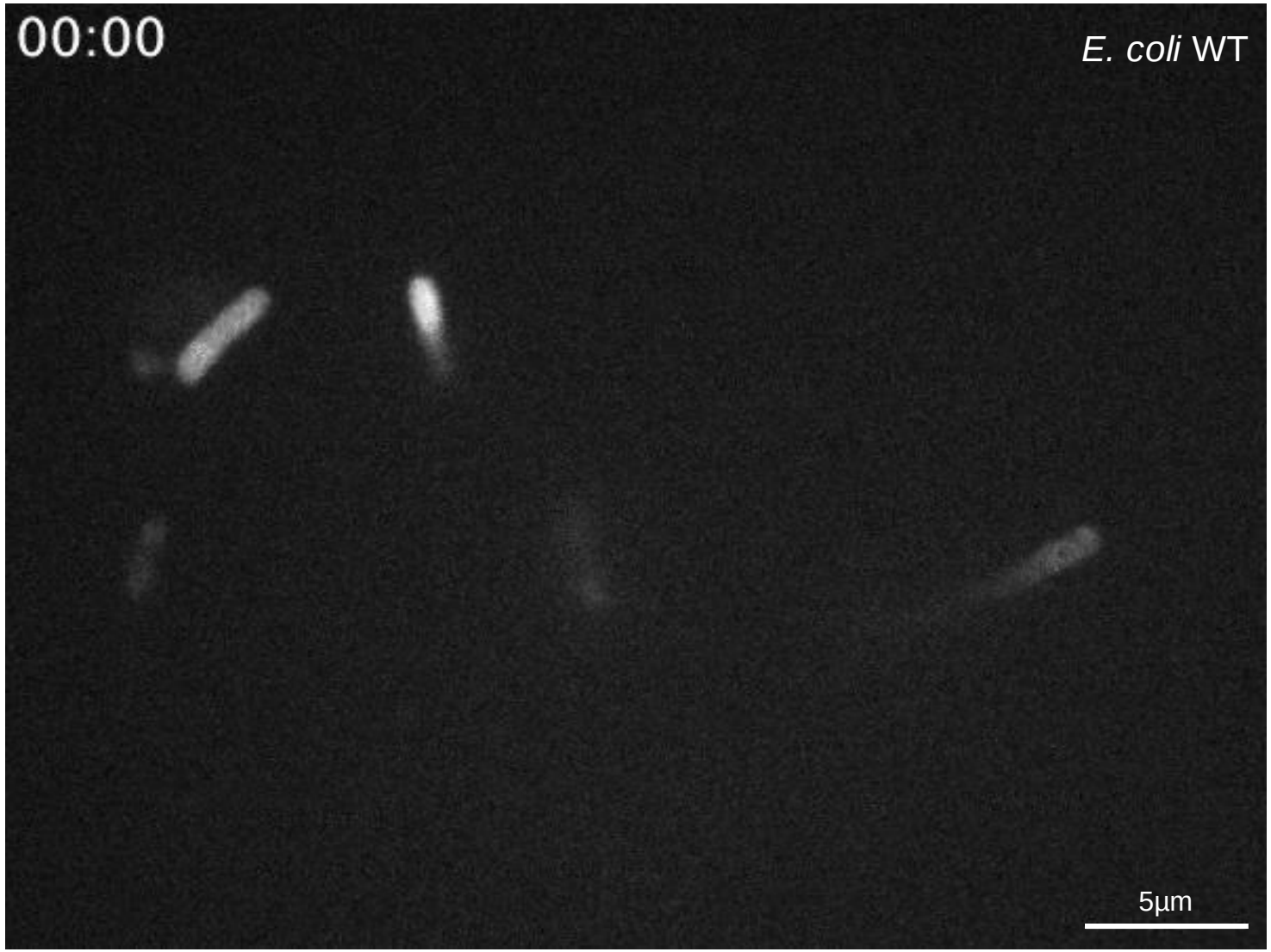




Bacterial proliferation exert large forces onto their microenvironment

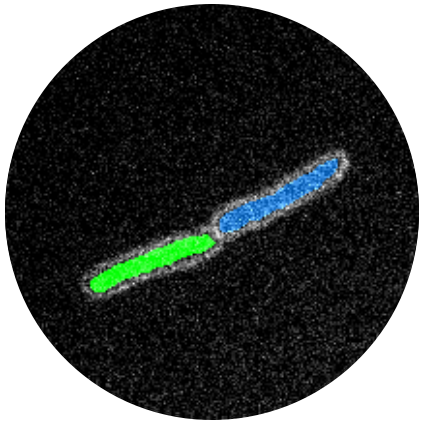
A. Cont et al. 2020

II.3.2. Confinement induces bacterial morphological changes



Min/Max rescaled by frame

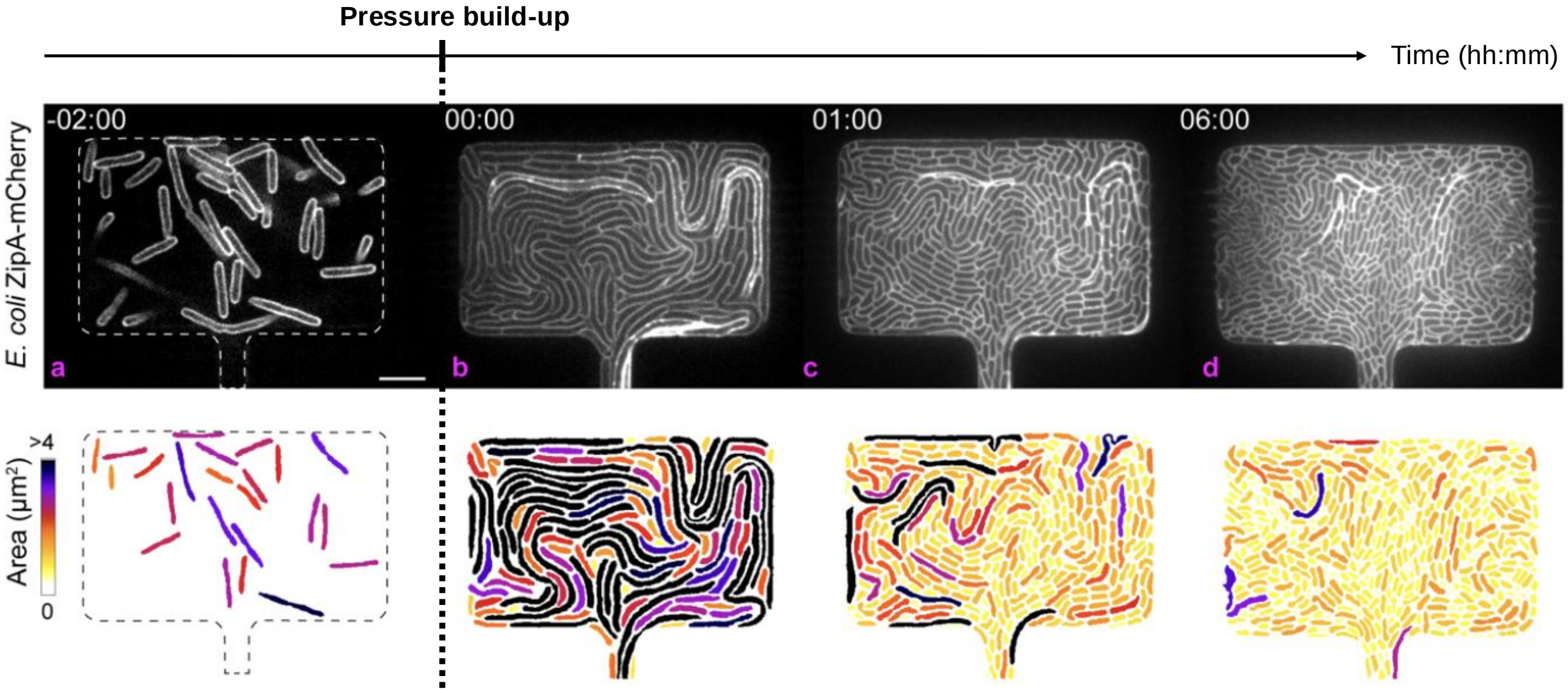
Quantification



requires membrane staining

(*E. coli* MG1655 TM-ZipA-mCherry)

II.3.2. Confinement induces bacterial morphological changes



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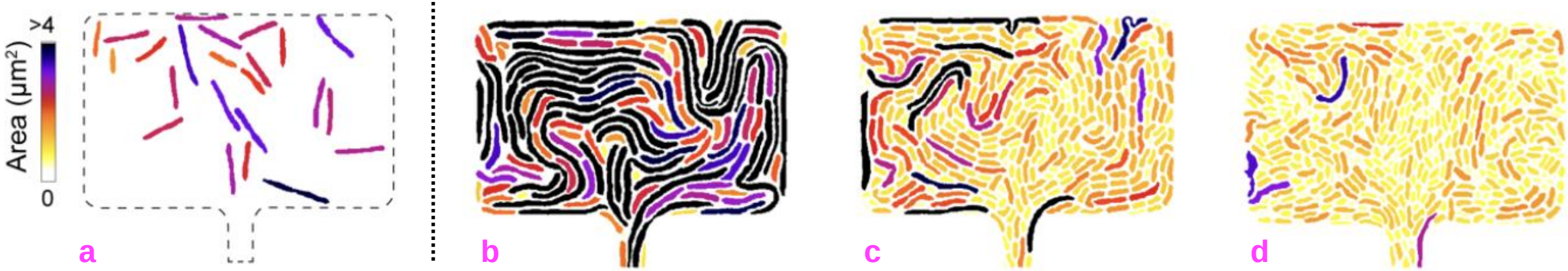
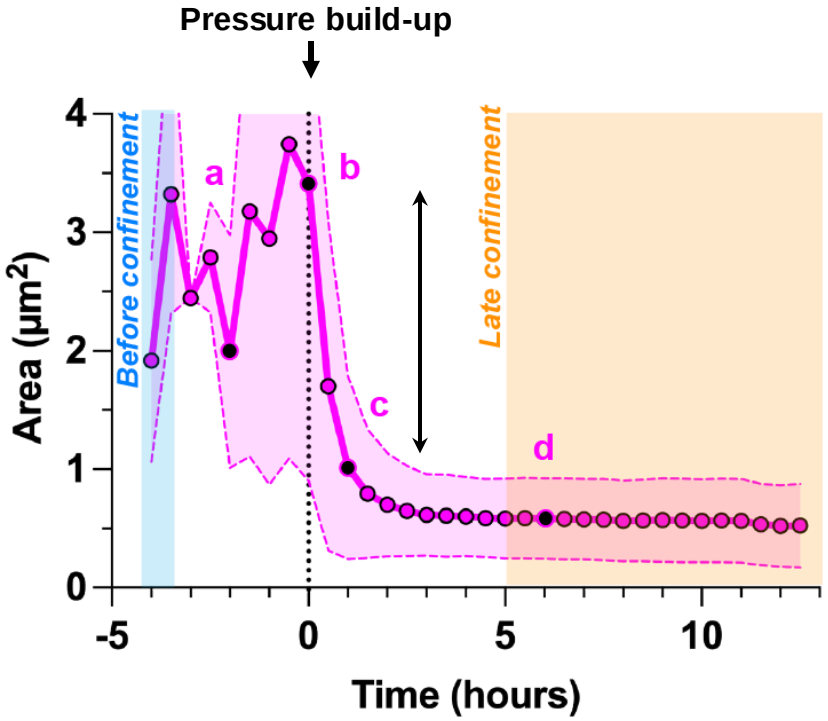
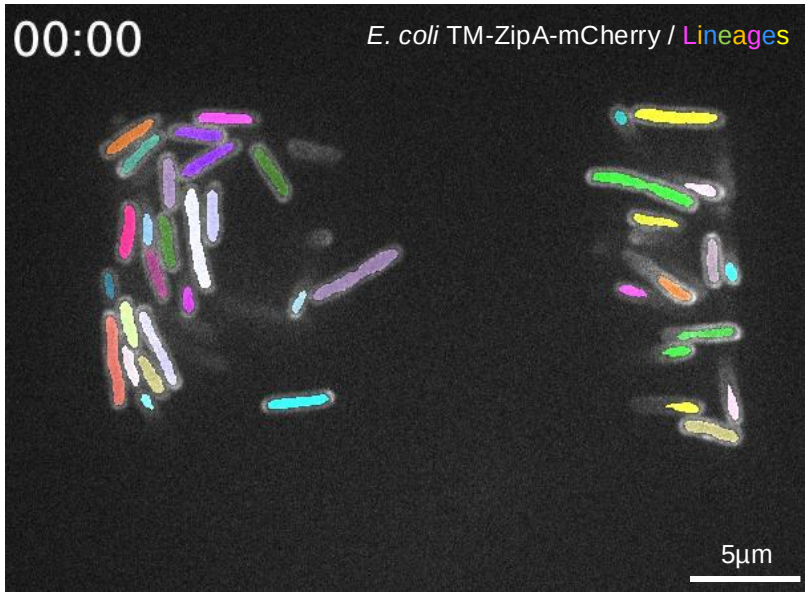




II.3.3. Confinement uncouples growth and division

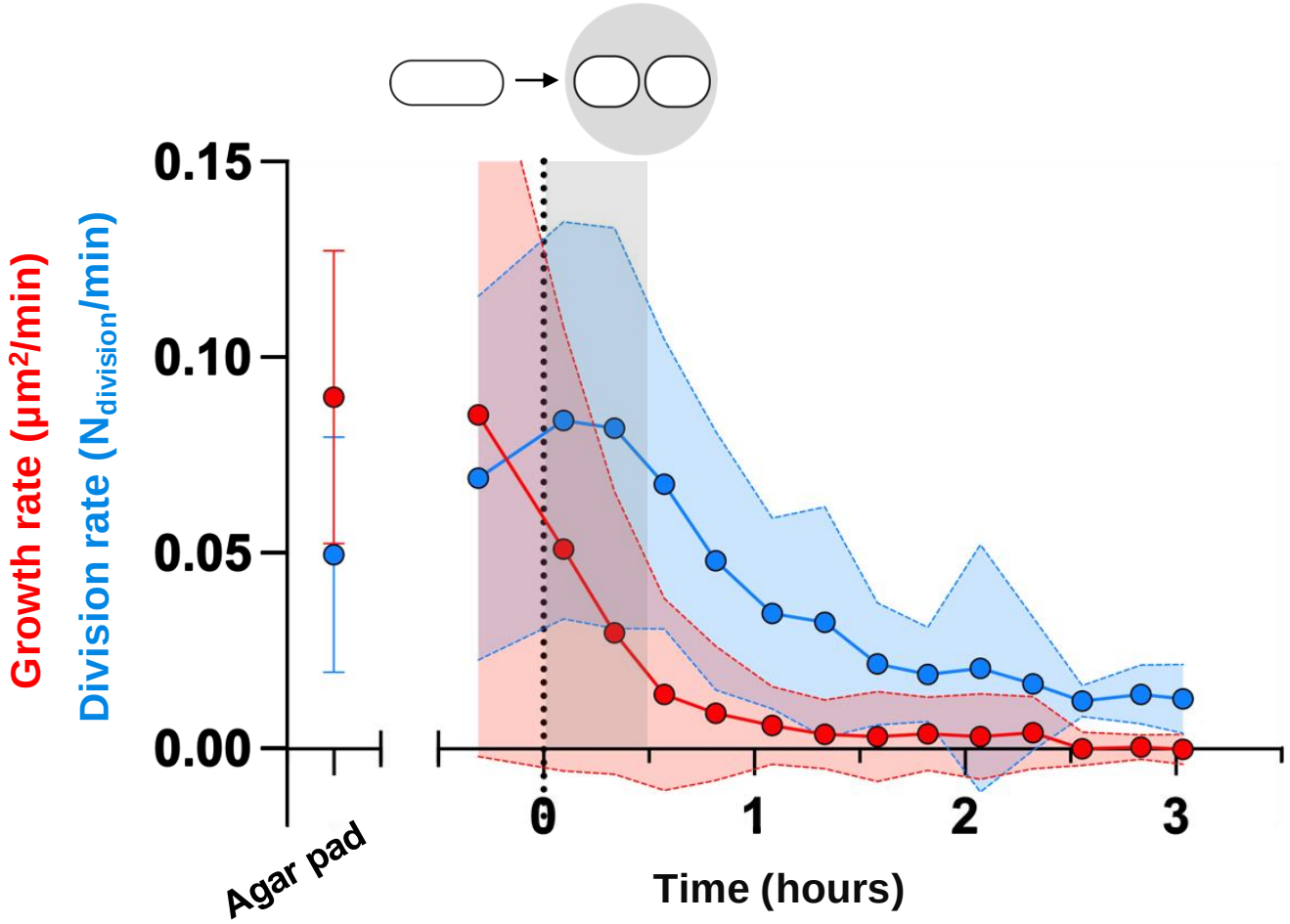


⇢ **GROWTH**

↳ Growth rate (µm²/min) = $\frac{\Delta \text{ Area}}{\Delta \text{ Time}}$

⇢ **DIVISION**

↳ Division rate ($N_{division}$/min) = $\frac{\text{Number of division}}{\Delta \text{ Time}}$



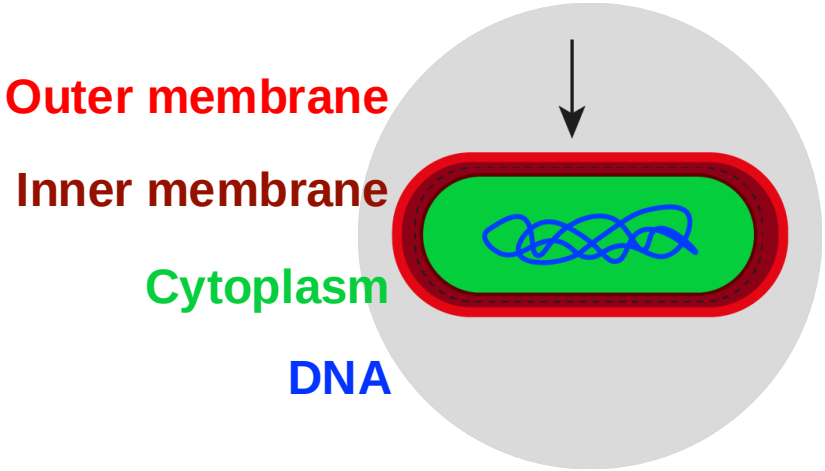
The uncoupling between growth and division explains why bacteria become smaller

II.3.4. Which mechanism(s) regulate(s) division?

Collaboration with:
Romain ROLLIN & Matthieu PIEL
(Institut Curie, Paris, France)

Hypothesis 1

Mechanical stresses



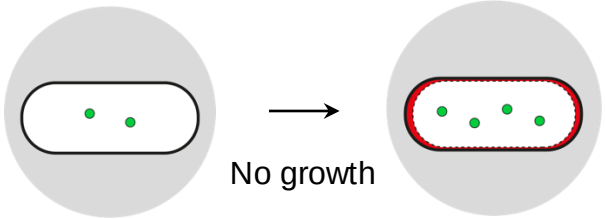
Activation of molecular pathways

Hypothesis 2

- Normal conditions

Protein synthesis
~ Growth rate

- Upon confinement



Protein synthesis
>> Growth rate

Increase in cytoplasmic crowding

Alric et al, Nat. Physics, 2022

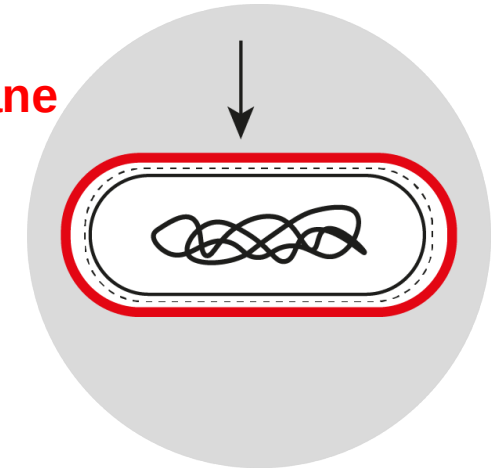
→ Increase in turgor pressure

II.3.5. Which mechanism(s) regulate(s) bact. adaptation?

Hypothesis 1

Mechanical stresses

Outer membrane



Activation of molecular pathways

Hypothesis 2

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Protein synthesis
~ Growth rate

- Upon confinement

Protein synthesis
>> Growth rate

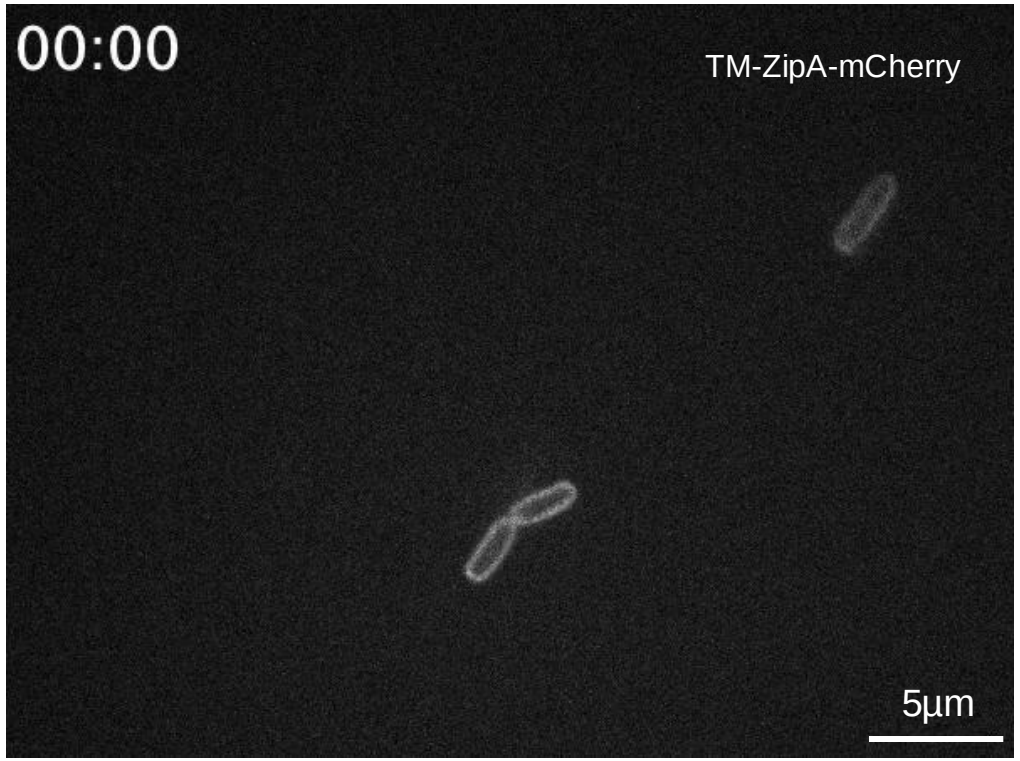
Increase in cytoplasmic crowding

Increase in turgor pressure

Alric et al, Nat. Physics, 2022

II.3.5.1. Mapping transcriptional adaptation at the envelope

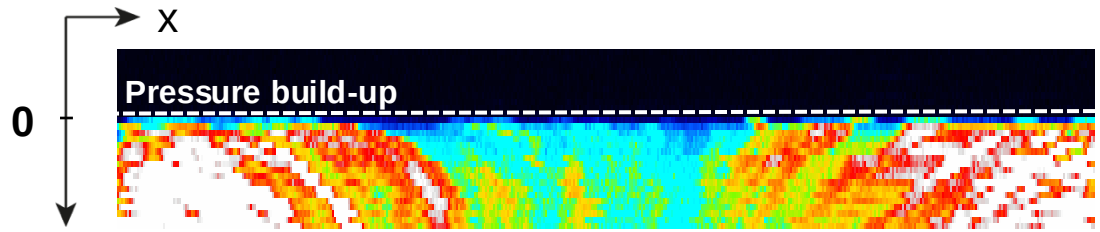
Fluorescent transcriptional reporter at the OM



Display only!

y
x

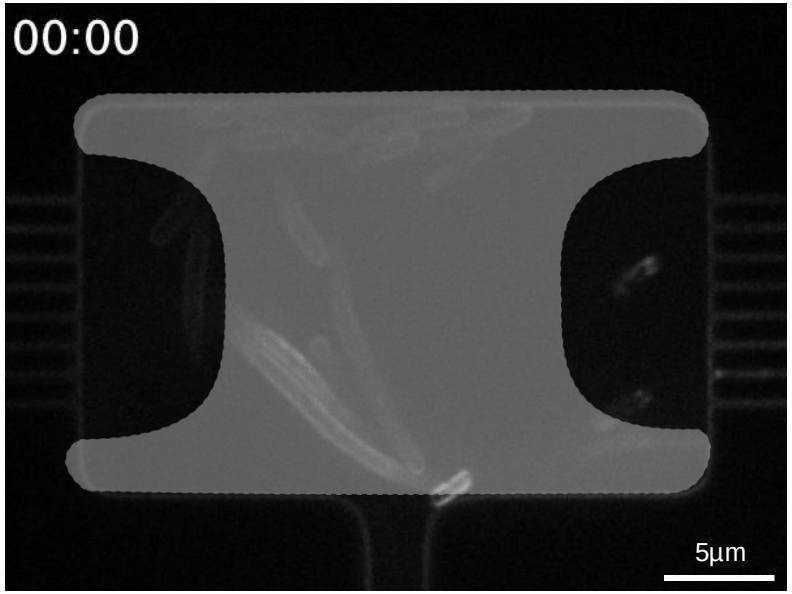
Kymograph

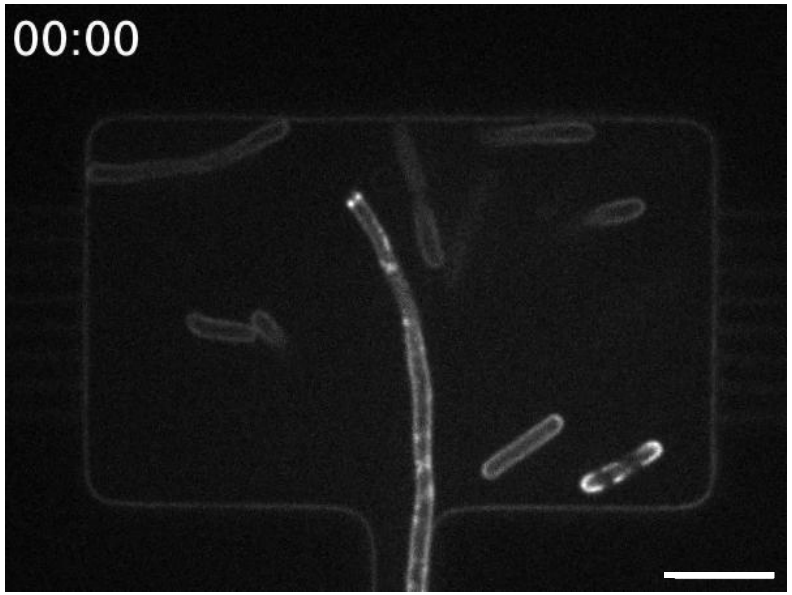


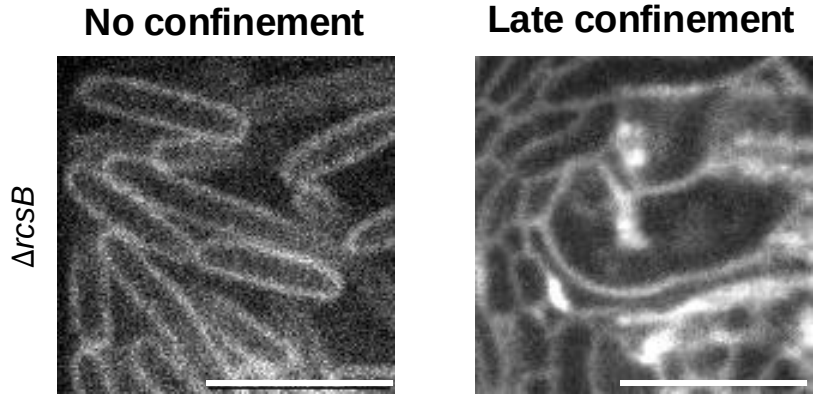
NO activation

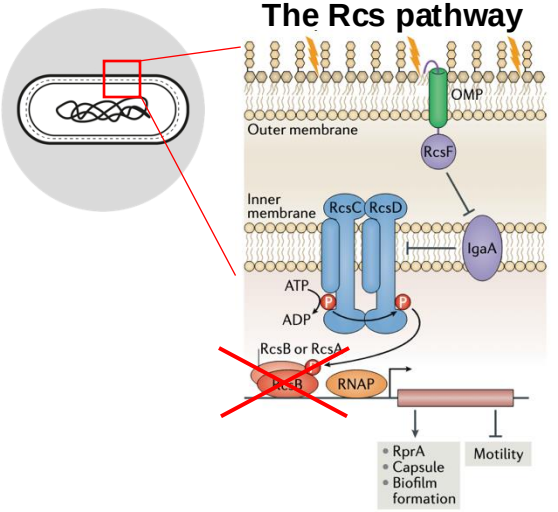
Activation

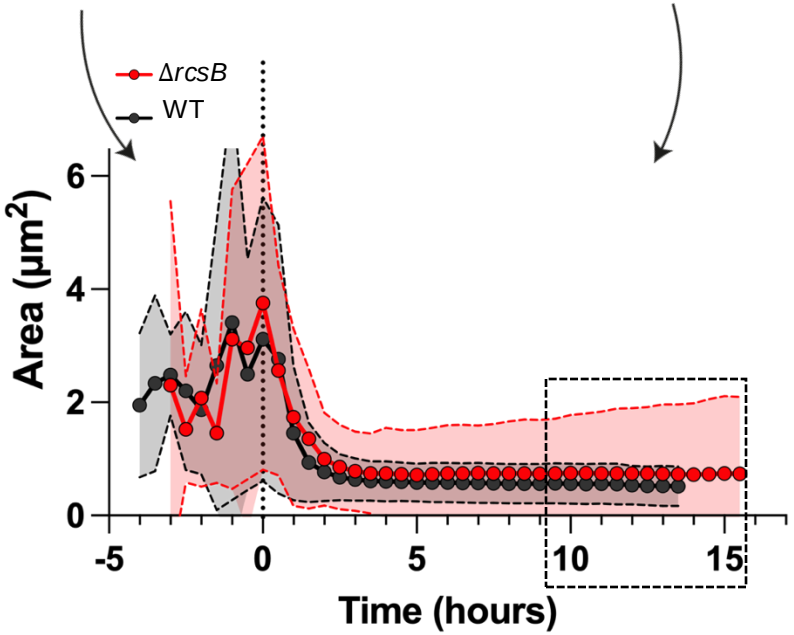
II.3.5.1. Envelope remodeling is essential to bact. adaptation

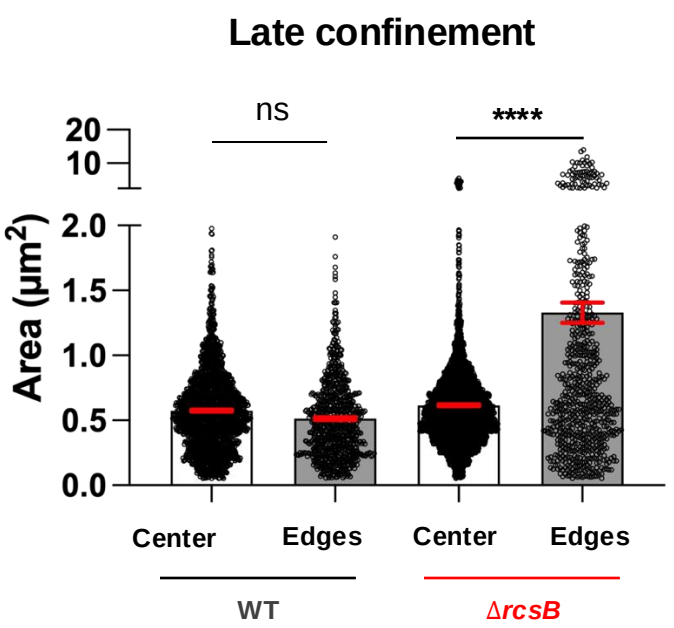








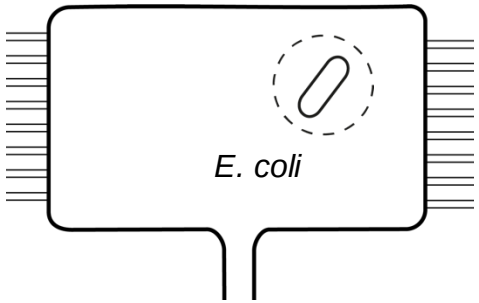


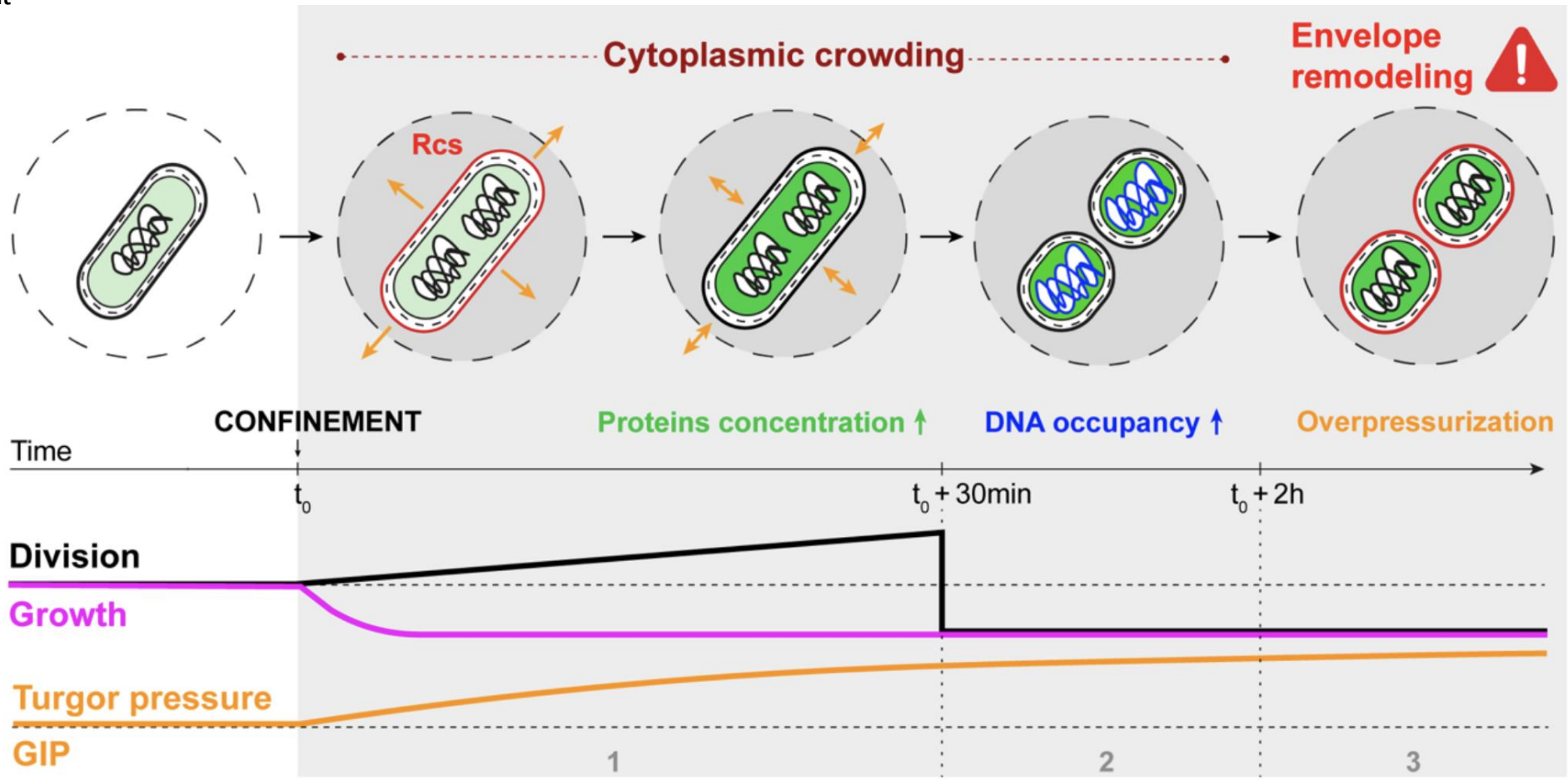


II.4. Summary: Impact of mechanical confinement on bacterial physiology

The bacterial confiner

Model of constrained environment



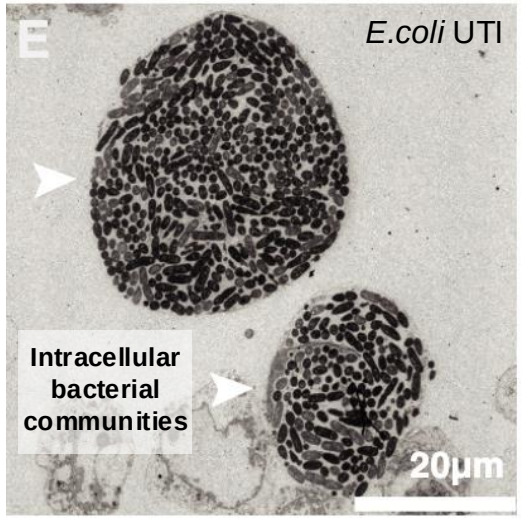


Video

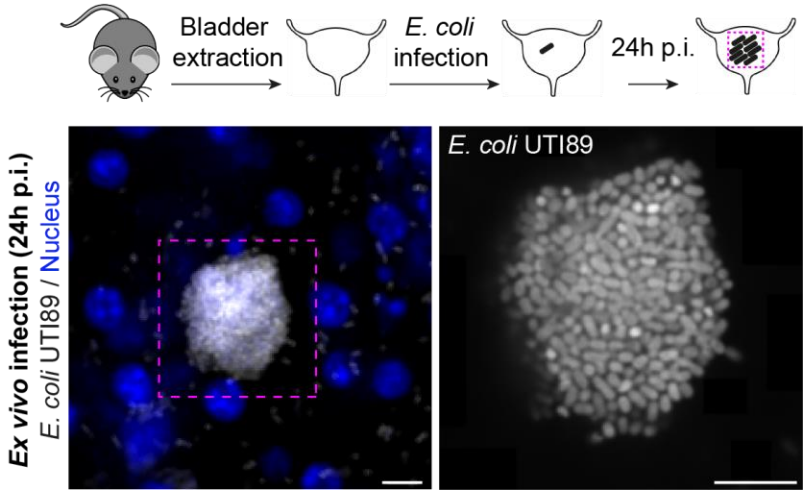
L. Le Blanc, …., M. Delarue*, G. Dumenil*, D. Bonazzi* *(under review)*

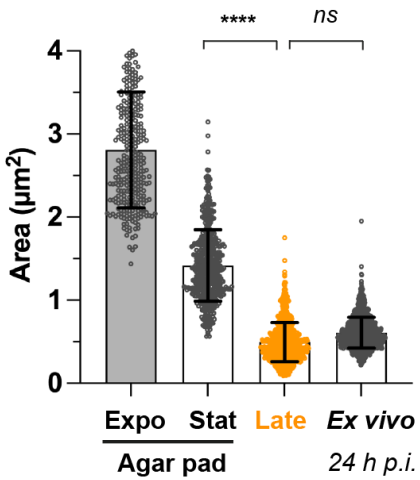
II.5. Relevance during urinary tract infections (UTIs)

- **Force generation & Morphological changes** ✅

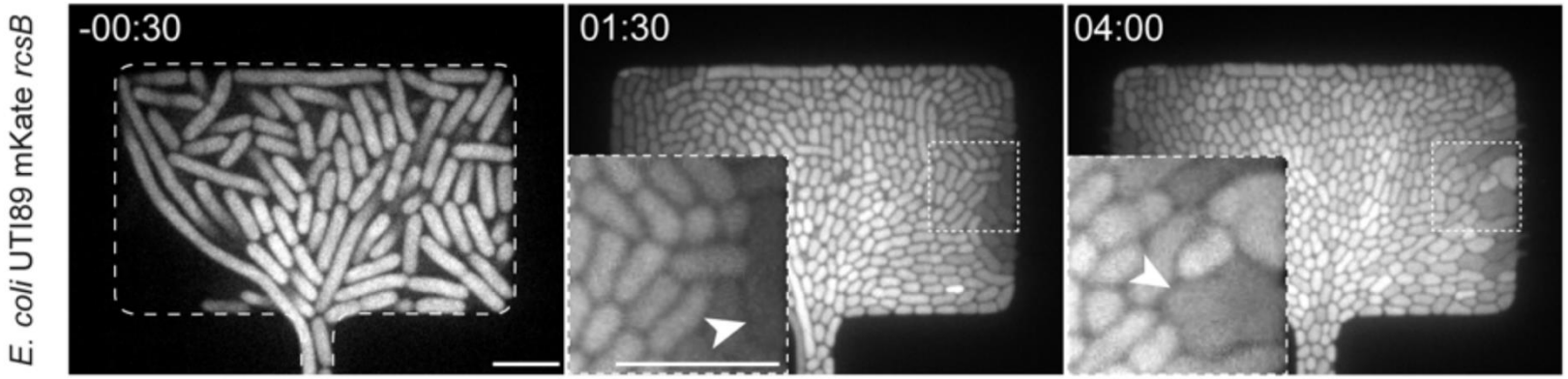


K. Sharma et al. 2021





- **Rcs-mediated envelope remodeling** ✅



Rcs: a potential target to UTIs?

II.6. Take-home messages

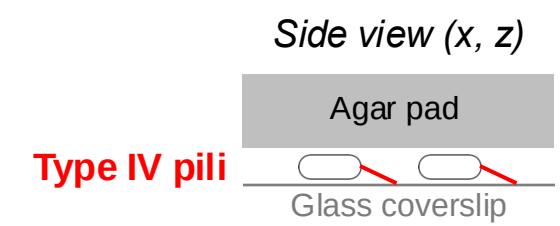
An example of a recent study, which highlights:

- The role of **mechanobiology** in understanding diseases
- **The biology of bacteria in groups is often different from isolated ones**

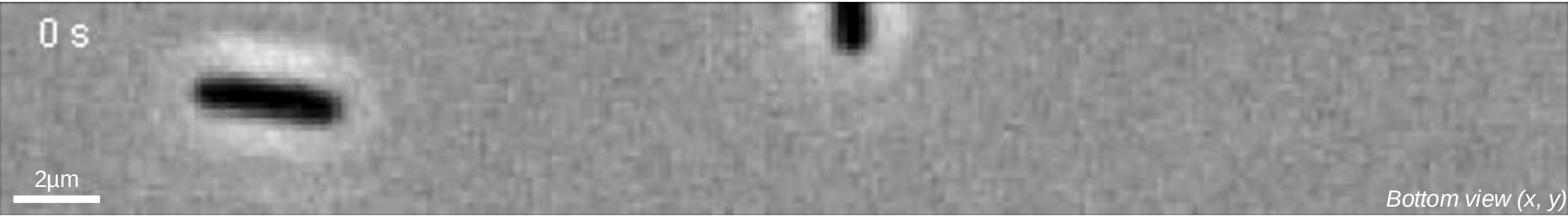
III. Introduction to project 1

Bacterial motility in crowded environments

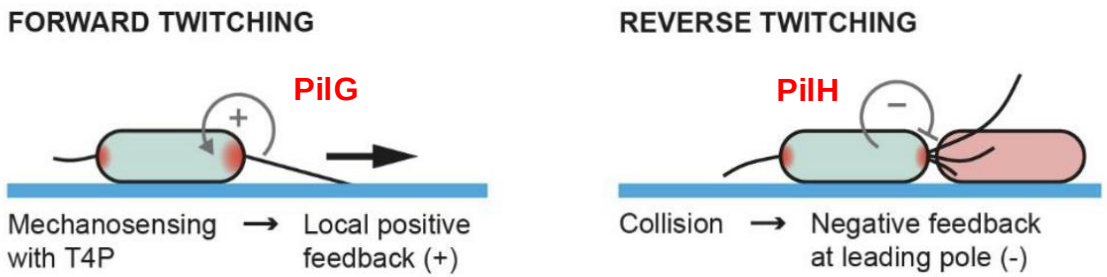
III.1. Background



P. aeruginosa twitching motility at the single-cell level



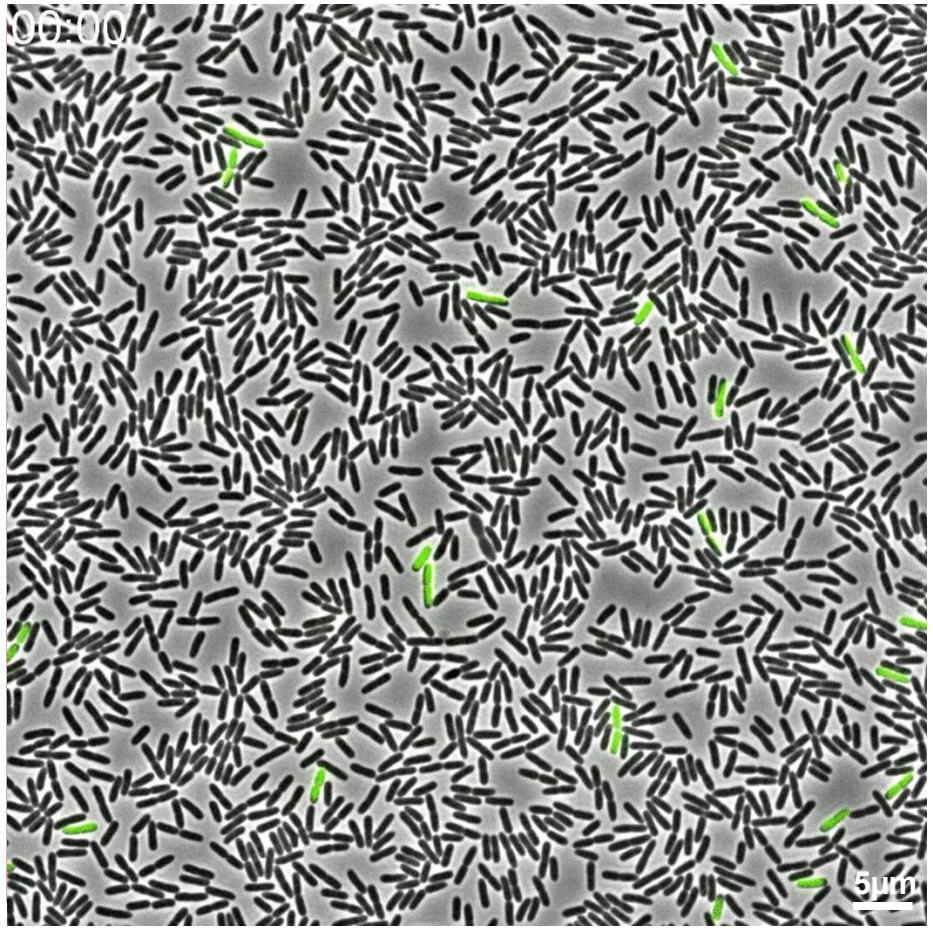
- **Bacteria move in the direction of the mechanical input**
- **Mechanotaxis is mediated by 2 response regulators: PilG and PilH**



M. Kühn et al, PNAS, 2021: Useful methodology !

III.2. In project 1, your goal is to…

Use image analysis to characterize bacterial motility at higher cell density



Pseudomonas aeruginosa WT

Time indicated as mm:ss

Step 0: Watch the movies

Step 1: Segmentation (Omnipose)

Select channel 1 (Phase contrast)

Step 2: Tracking (TrackMate)

Focus on fluorescent cells to make it easier

Step 3: Quantitative measurements

Bacterial density, Displacement, MSD, Speed, etc.

Step 4: Biological conclusions

Step 5: Discussion

IV. Image analysis tutorial

slido

Please download and install the
Slido app on all computers you use

Why is image analysis (very) useful?

ⓘ Start presenting to display the poll results on this slide.

Why is image analysis useful?

To extract **quantitative** data

Hands-on: let's get started !

1. **Install Fiji**
   https://imagej.net/software/fiji/downloads

2. **Open the dataset Training: *Training_Frame1_PC.tif, Training_Movie_MASK.tif***
   https://drive.switch.ch/index.php/s/GhAC3ZzOvAja28F

3. **Install Anaconda and create environment**
   https://www.anaconda.com/download/
   https://pypi.org/project/omnipose/

4. **Live tutorial: How to analyze single image (45min) and stacks (45min) ?**

☞ Overview of Fiji main functions: image processing, segmentation and tracking
☞ Towards batch analysis (macro, Python)
☞ Tracking: Fiji > Plugin > TrackMate
→ **If you don't find it**: update Fiji, otherwise go to Fiji > Help > Update… > Manage Update Sites, tick TrackMate, click « Apply changes », then restart Fiji

Omnipose code (The basics in 2D):
https://omnipose.readthedocs.io/examples/mono_channel_bact.html

Useful references

Publications

☞ **Bacterial motility:** Kühn et al. 2021 https://pubmed.ncbi.nlm.nih.gov/34301869/

☞ **Omnipose:** Cutler et al. 2022 https://pubmed.ncbi.nlm.nih.gov/36253643/

☞ **TrackMate:** Ershov et al. 2022 https://pubmed.ncbi.nlm.nih.gov/35654950/

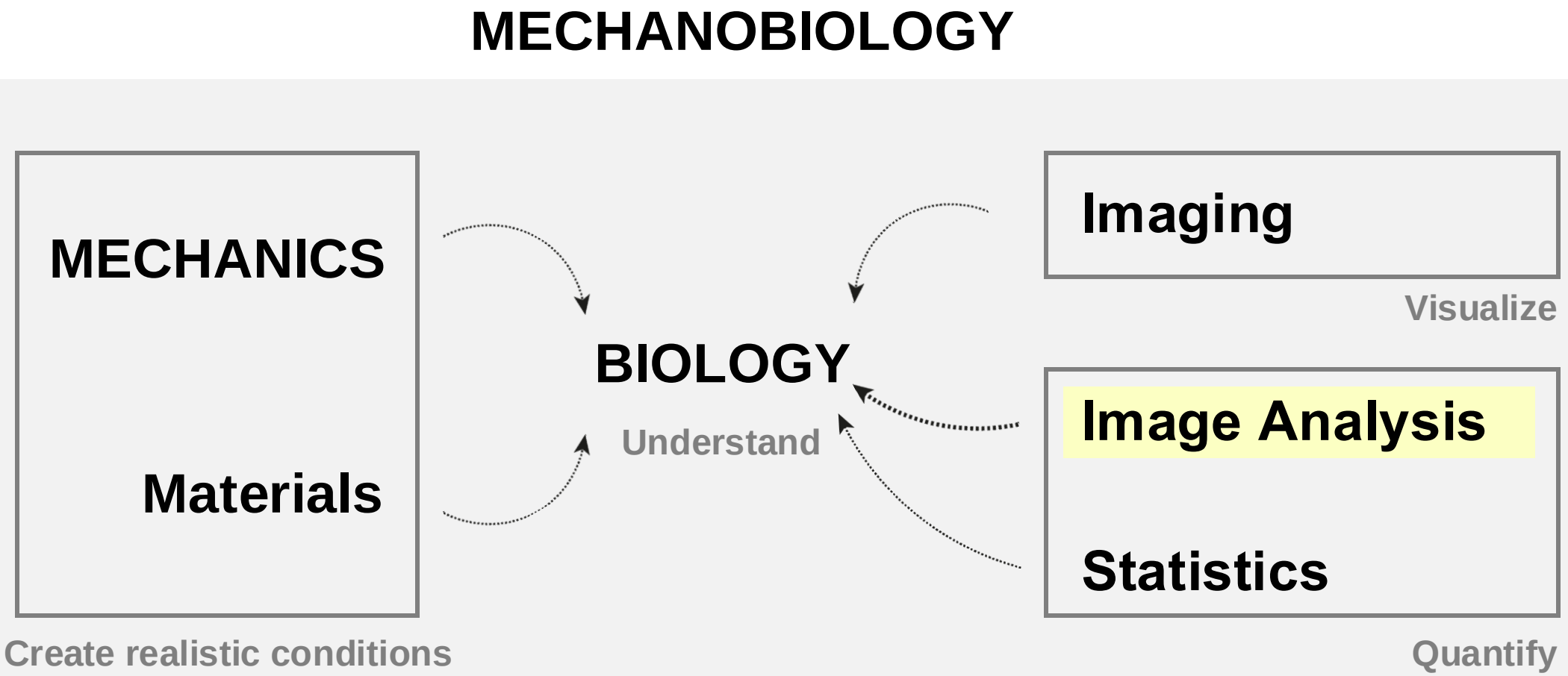
Softwares: Fiji / Omnipose / TrackMate

☞ **Fiji tutorial** for beginners: https://www.youtube.com/watch?v=y567dfZ_reI

☞ Fiji plugin's **TrackMate tutorial**: https://imagej.net/plugins/trackmate/

☞ **Omnipose documentation** (« The basics in 2D »):
https://omnipose.readthedocs.io/examples/mono_channel_bact.html

Conclusion


MECHANOBIOLOGY
MECHANICS
Materials
Create realistic conditions
BIOLOGY
Understand
Imaging
Visualize
Image Analysis
Statistics
Quantify