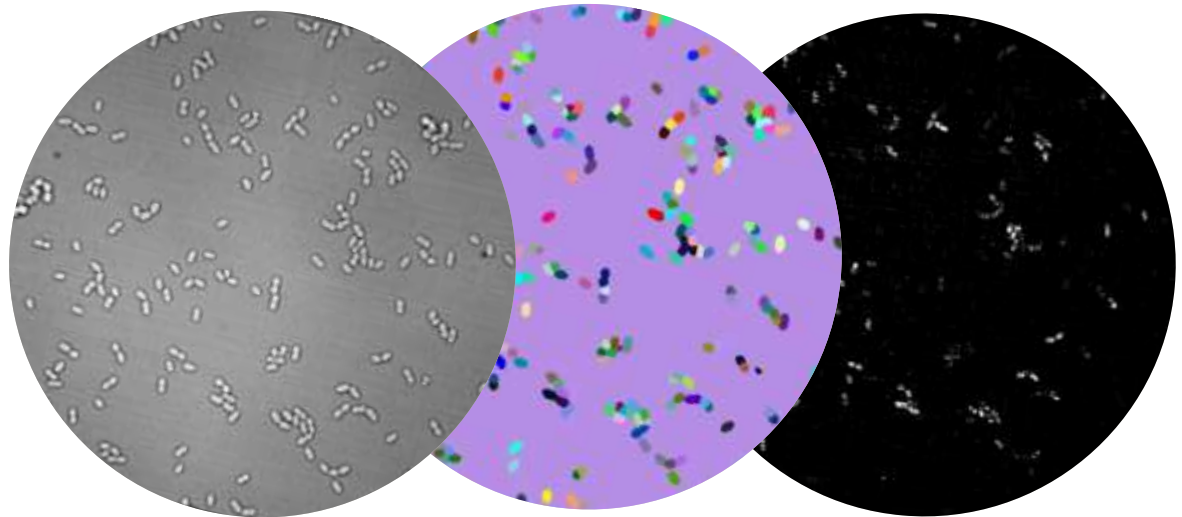
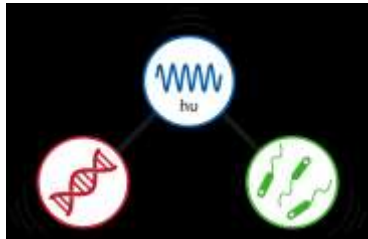


# Towards applications of fluorescence super-resolution microscopy in food research

Laboratory of Biophysics, Wageningen University & Research

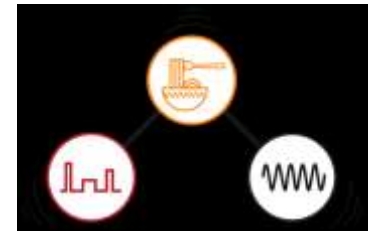
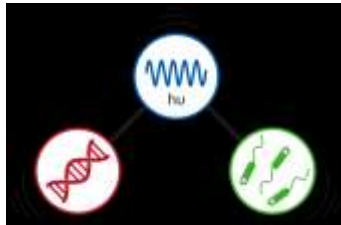
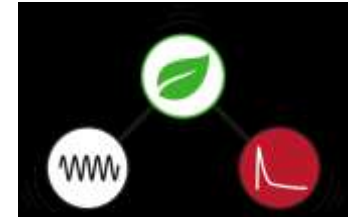
10.09.2019, Johannes Hohlbein  @HohlbeinLab



# Laboratory of Biophysics at WUR

## ■ Mission statement:

- Develop and apply advanced methods to study functional dynamics of biological systems and food
- Teach the fundamentals of (bio)physics and its application in the life sciences.



# Motivation for using super-resolution microscopy

Bacterial starter cultures in the dairy industry?



Anisotropy in "vegetable steaks"?

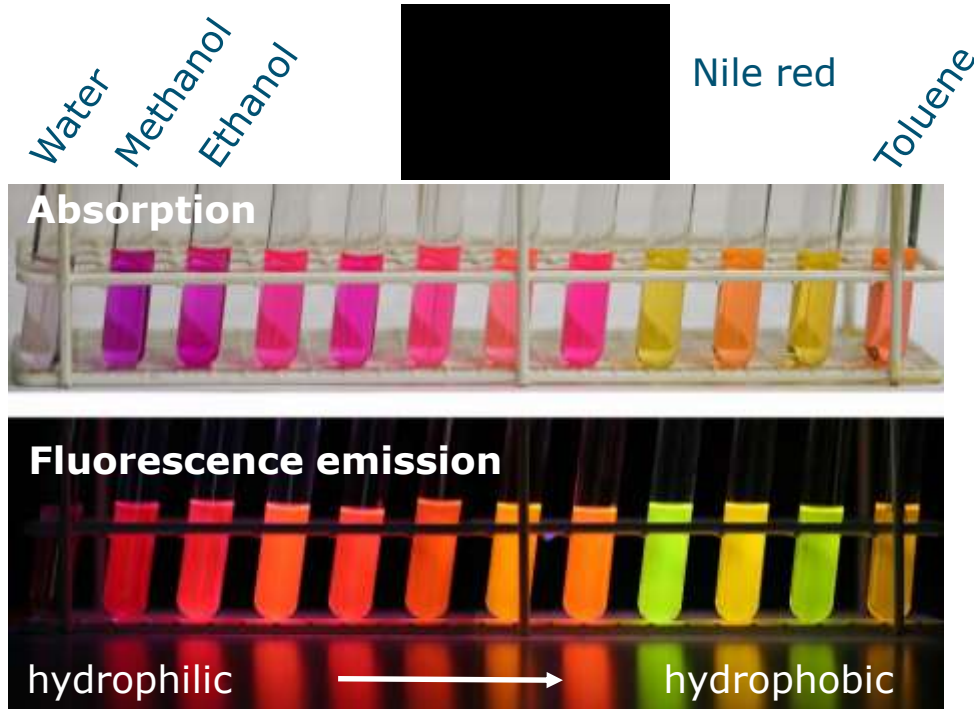


Localising lipid oxidation? Where and when?



van der Groot @ WUR

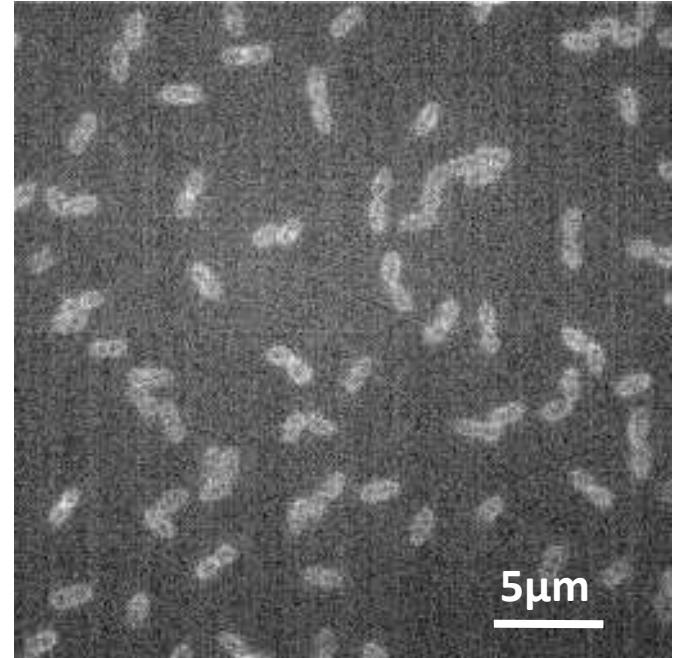
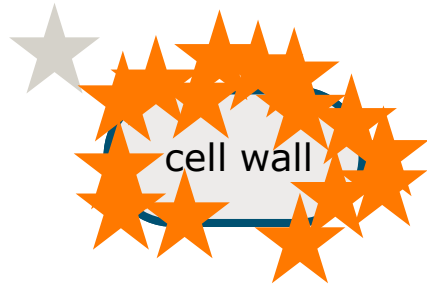
# Fluorescence imaging with Nile red



# Fluorescence imaging with Nile red

- Conventional staining with Nile red (*Lactococcus lactis*)

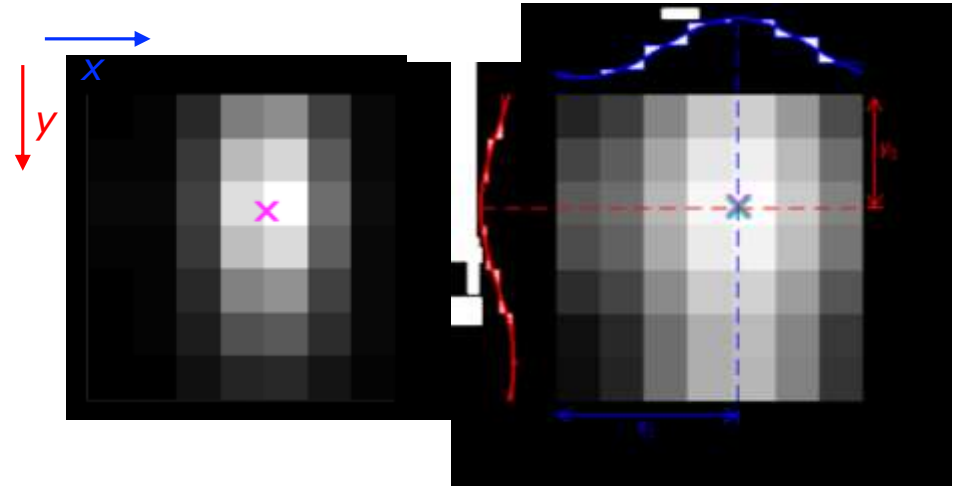
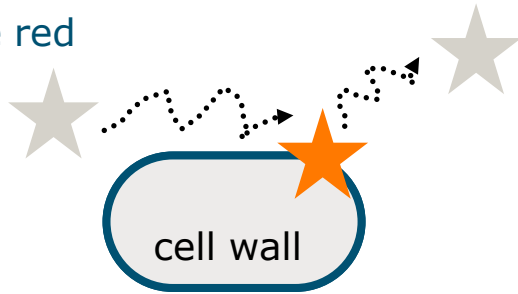
Nile red



# Fluorescence super-resolution imaging

- PAINTing with Nile red (*Lactococcus lactis*)

Nile red



- Single-molecule detection requires sophisticated microscope
- Our contribution: the miCube

PAINT: Sharanov & Hochstrasser, PNAS, 2006  
iPAINT: Aloï et al, Nanoscale, 2016

# miCube: modular fluorescence microscopy

- Dev
- Lice

See also POSTER by Koen Martens!



# miCube: modular fluorescence microscopy

← → 🏠 📧 🔒 https://hohlbeinlab.github.io/miCube/index.html 📄 ⋮ 📧 ☆ ↻ 🔍 Search 📄 📄 📄 📄 📄 📄 📄 📄 📄 📄

## #miCube: Modular fluorescence microscopy

- [Main page](#)
- [miCube manuscript](#)
- [Component overview](#)
- [Phasor-based SMLM](#)
- [Related projects](#)
- [People involved](#)

📄 [View On GitHub](#) 🐦 [Twitter](#)  
📄 [Hohlbein lab](#)  
📄 [Wageningen University: Biophysics](#)



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Theme adapted from [Andrew York theme](#).

## The miCube

Fluorescence microscopy is an extremely powerful and versatile technique contributing to many areas of the life sciences. Especially variants featuring the ability to monitor single-molecule fluorescence, however, require sophisticated instrumentation that is either very expensive when bought commercially (>> 100 kEuro) or demands extensive expertise in optics and engineering.

Here we present an open and modular hardware framework aiming for

- cost effectiveness: build your own starting at 20k Euro (~100k Euro for state of the art capabilities)
- modularity: all parts can be accessed and replaced by the user
- simplicity: set up the microscope in a few hours without prior knowledge
- customizability: confocal or widefield/TIRF microscopy,...
- openness: part lists and drawings will be made available
- stability and throughput: minimizing drift and utilise well plate scanners



(change this slider to rotate the miCube)

Rotating view of miCube: An interactive figure

For a detailed description of the complete microscope, go to [the related manuscript](#) or to the [miCube component list](#).





# miCube: modular fluorescence microscopy

Conventional microscope

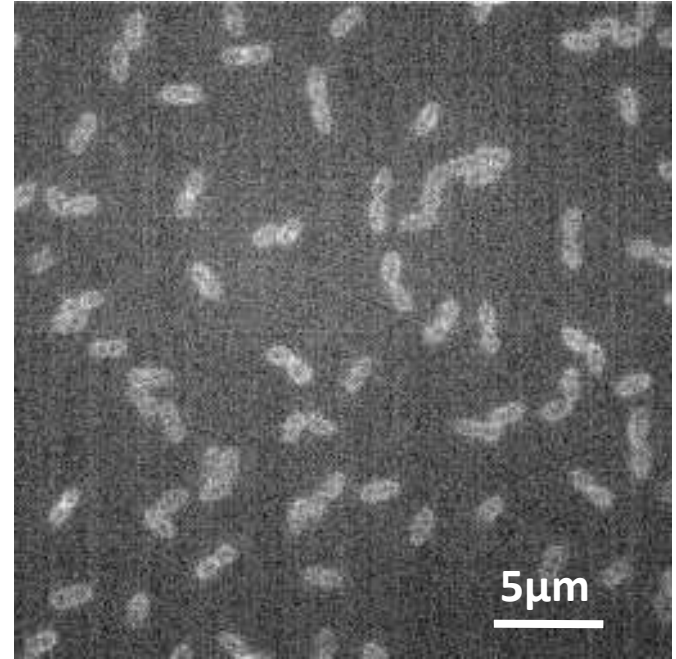
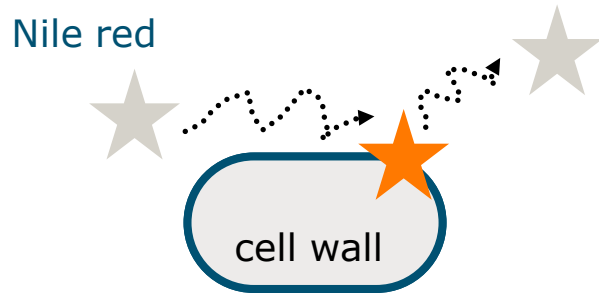


**miCube**



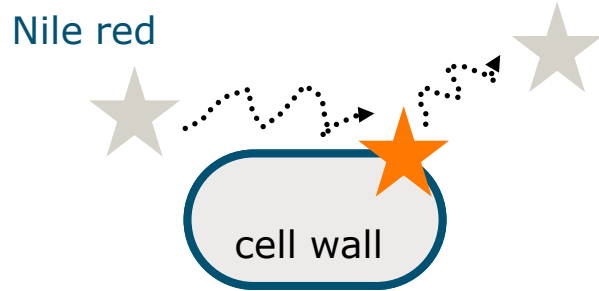
# Fluorescence super-resolution imaging

- PAINTing with Nile red (*Lactococcus lactis*)

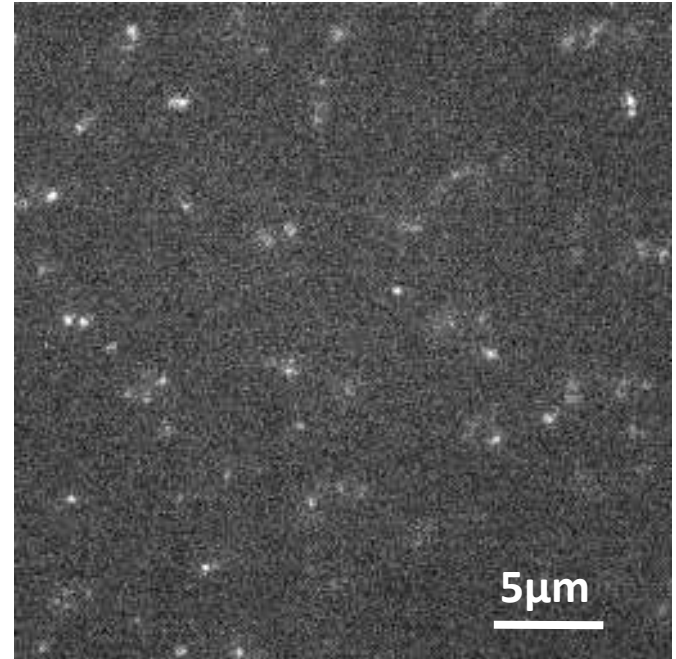


# Fluorescence super-resolution imaging

- PAINTing with Nile red (*Lactococcus lactis*)

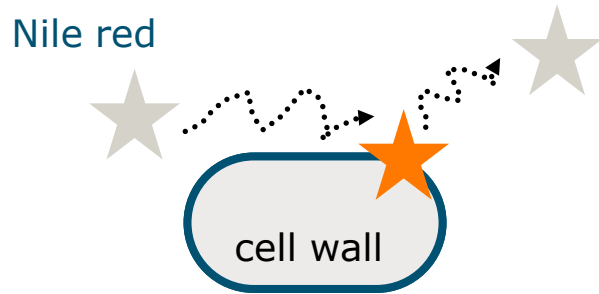


- Video (real time, raw data)

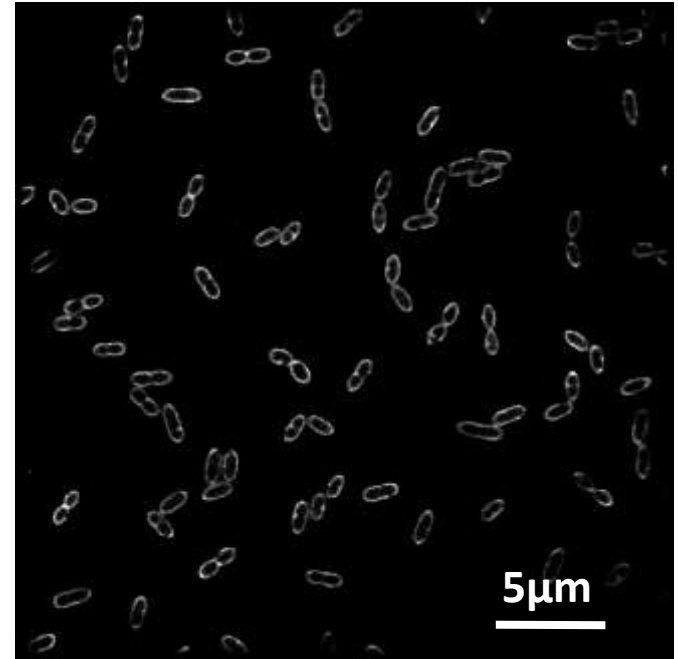


# Fluorescence super-resolution imaging

- PAINTing with Nile red (*Lactococcus lactis*)

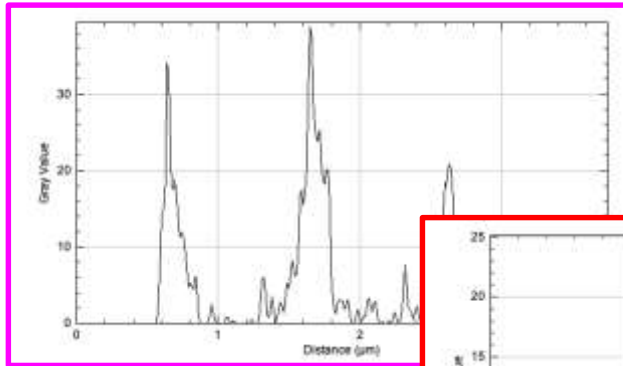


- Super-resolved image

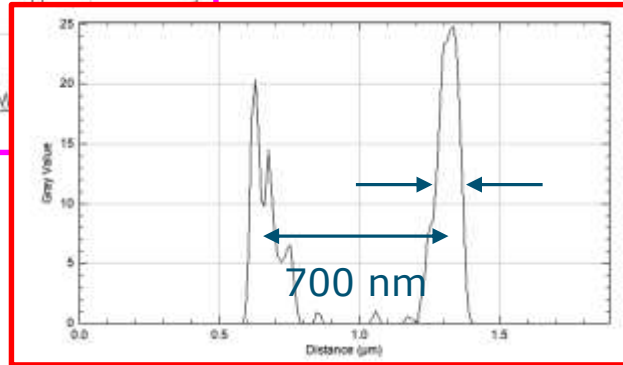


# Fluorescence super-resolution imaging

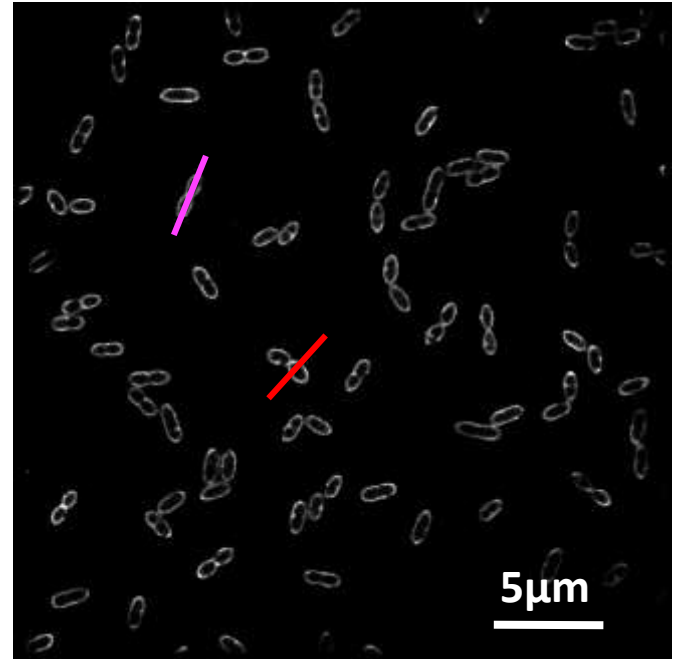
- PAINTing with Nile red (*Lactococcus lactis*)



FWHM: 100 nm



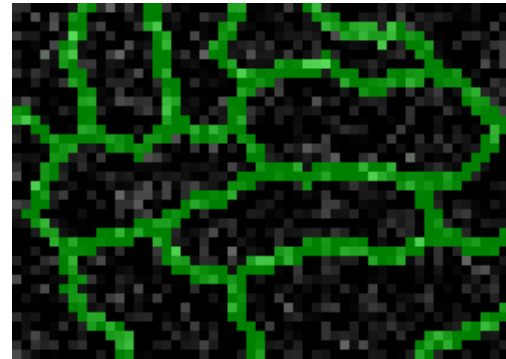
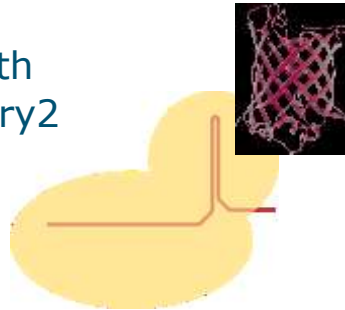
- Super-resolved image



# Single-particle tracking

- Fluorescent proteins tagged to genes in bacterial cells or (later) probes in “vegetable steak” to probe anisotropy

dCas9 with  
PAmCherry2

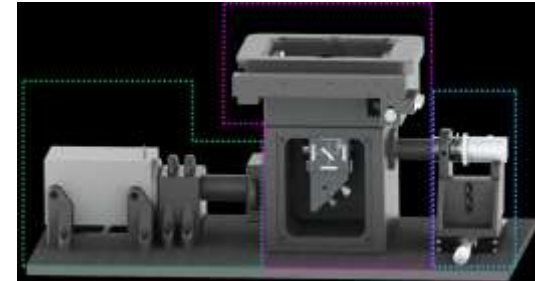
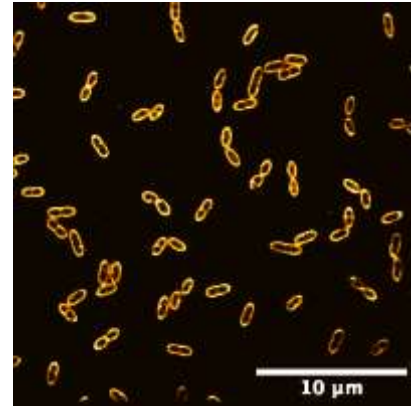


See also POSTER by Koen Martens!

# Summary

- New technologies for food-related research
- Super-resolution techniques allow to overcome the diffraction limit of light ( $\sim 250$  nm  $\rightarrow$   $< 50$  nm resolution)
- miCube provides “easy” access to super-resolution imaging

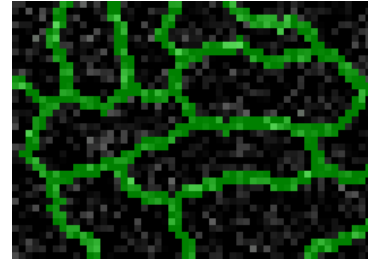
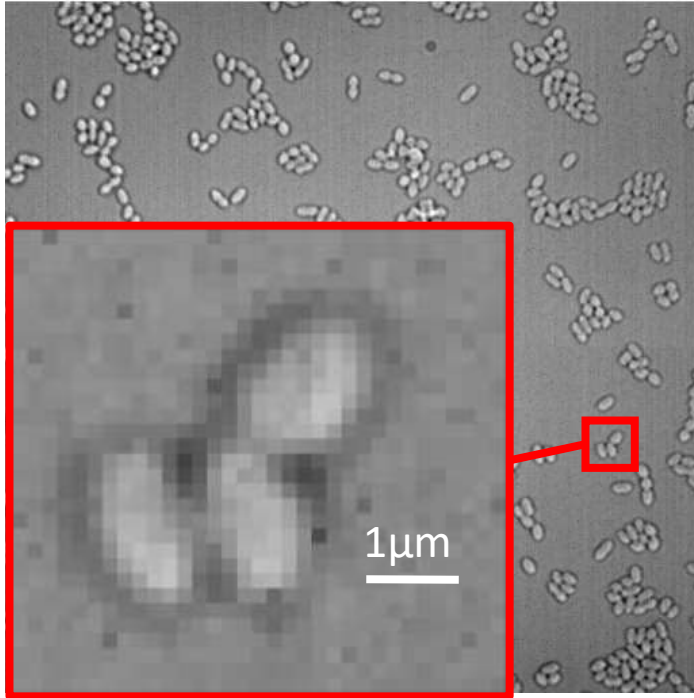
People (Hohlbein lab & BIP): **Koen Martens**, **Sam van Beljouw**, **Ben Tumulero**, **Sander Baas**, Mattia Fontana, Arjen Bader, Suyeon Yang, Abbas Jabermoradi, Ge Vogelaar, Sven Koens, Vincent Boerkamp



# Blank



# miCube: super-resolution microscopy data



Single particle tracking  
PALM of dCas9

Monitoring cell division with <math>< 50\text{nm}</math> resolution

