

Light up your research

# Imaging microbes at the nanoscale

Because the size of bacterial cells is particularly small (~1-3  $\mu\text{m}$ ), single molecule microscopy is particularly well-adapted for revealing subcellular structures and dynamics that cannot be resolved with classical light microscopy. Recent advances in single molecule microscopy now open-up a whole new range of applications in bacteriology. However, practical aspects need to be considered to design imaging experiments: from sample preparation to image acquisition and data analysis.

## What can I do with single molecule imaging in bacteria?

Depending on the goal of the study, single molecule microscopy allows to (i) probe the subcellular structure of bacterial cells and (ii) follow the dynamics of single proteins within bacterial membranes, poles or cytoplasm for example. Among the most popular applications: bacterial replication, bacterial cell morphology, antibacterial molecules localization within bacteria, protein subcellular localization,...etc.

### In practice

What do I need to do single molecule microscopy on bacteria?

- Fluorophores compatible with STORM, PALM or PAINT
- A specific imaging buffer for STORM imaging.
- A software dedicated for acquisition, processing and analysis

## PREPARATION

The design of single molecule microscopy experiments relies on the type of structure or dynamics you want to image.

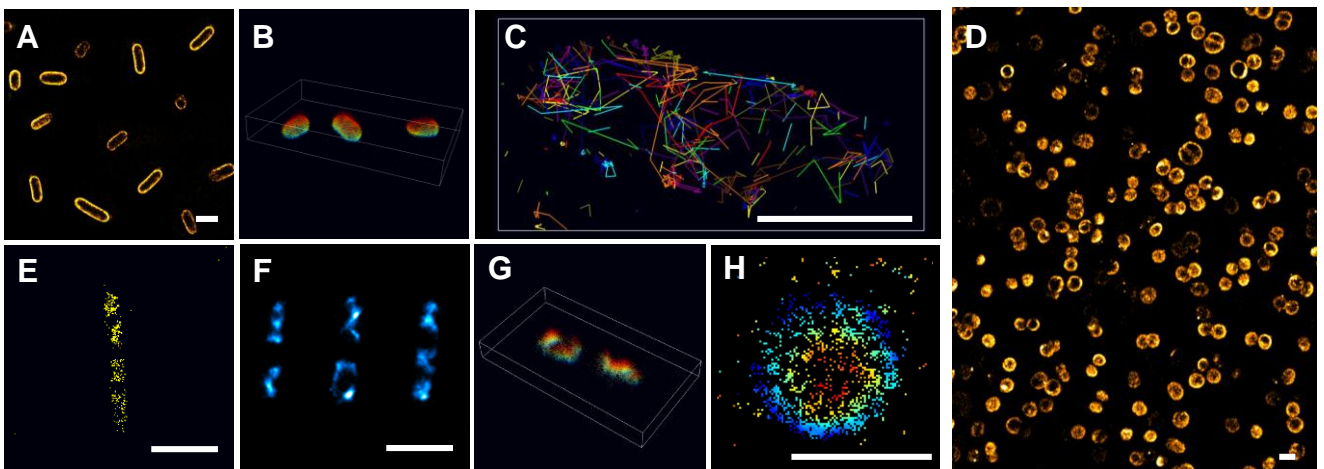
- STORM imaging will be adapted to image fixed cells, stained with organic fluorophores like cyanines or rhodamines.
- PALM or SPT-PALM will be used for living cells whose proteins are fused to photo-activatable or convertible proteins
- PAINT will be chosen to stain the membranes of living bacteria.

## IMAGING

Abbelight SAFE modules (SAFE180 and SAFE360) can both be used for single molecule microscopy in bacteria. These modules can adapt to any inverted microscope. In both cases, an astigmatic lens is recommended for 3D imaging to visualize the whole shape of the single cells. In the case of simultaneous multicolor imaging, SAFE360 dual-view system is recommended

## ANALYSIS

The data obtained with single molecule experiments include not only high-resolution images but also 3D coordinates for each molecule detected. To acquire, process and analyze such data, Abbelight offers a dedicated software. Designed to handle single molecule data, NEO software give real-time reconstruction of the images, fast processing and various analysis tools (clustering, single particle tracking, spectral demixing,...).



**Examples of applications using single molecule microscopy in bacteria with abbelight setup. (A)** STORM imaging of *E. coli* stained with WGA-AF647, (B) 3D visualization of *E. coli* cells, (C) Single particle tracking of proteins (confidential) within a single bacterial cell, (D) PAINT imaging of *S. aureus* membranes stained with Nile Red, (E) PALM imaging of RNA polymerase fused to PA-mCherry in *E. coli*, (F) Replication sites in dividing *E. coli* cells stained with EdU-AF647, (G) 3D visualization of the replication sites, (H) Daptomycin antibiotic localization in *S. aureus*. The scale bar represents 1  $\mu\text{m}$ .