# EXTENSIVE CHARACTERIZATION OF PATHOGENIC BACTERIAL SPECIES BY STED MICROSCOPY

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*The cell morphology of eleven bacterial species has been characterized using STED microscopy. The KK114 dye stains both Gram-positive and Gram-negative species, and peculiar nanostructures of bacterial cells have been highlighted.*
**Keywords**: Bacteria, STED.

## 1. Introduction

During the past two decades, Stimulated Emission Depletion (STED) microscopy has been demonstrated a reliable nanoscopic approach to overcome the Abbe's diffraction limit, achieving a resolution less than 30 nm. The optical resolution of a laser scanning confocal microscope (LCSM) is increased by switching off the fluorescence in the outer regions in the excitation area, using an intense doughnut-shaped laser beam [1]. We have stained eleven different bacterial species using KK114 dye, which has been previously utilized for eukaryotic cells [2]. We demonstrate that KK114 labels the membranes of both Gram-positive and Gram-negative bacteria, allowing also the visualization of peculiar bacterial cell features.

## 2. Materials and Methods

All the bacterial species were grown in Tryptic Soy broth at 37°C for 24 h, washed twice and diluted in distilled water before staining. Only *S. pyogenes* was cultured in Todd-Hewitt broth. The bacterial suspensions were stained with KK114 (10 µg/ml), air-dried on a glass coverslip and imaged with the laser scanning LCSM/STED microscope (Aberrior), using a 100× immersion oil objective, 640 nm excitation and 750 nm STED lasers. The obtained images were processed using Huygens software.

## 3. Results

The Gram-positive (*B. subtilis*, *E. faecium*, *S. aureus* and *S. pyogenes*,) and the Gram-negative (*A. xylosoxidans*, *A. baumannii*, *Enterobacter aerogenes*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. maltophilia*) bacteria are stained by the KK114 in the cell periphery (Fig.1). In some cases, KK114 enters and homogeneously stains the whole cell. We observe a large resolution improvement using STED, compared to LSCM, which allows us to distinguish division septa in *S. aureus*. Similarly, division structures can be visualized in *B. subtilis*, which usually generates membrane invaginations around the partially formed cross wall, during cell division. In addition stained *B. subitilis* cells revealed also a typical pole-to-pole helix structure resembling the bacterial tubulin homolog FtsZ involved in the Z-ring formation, which is essential for daughter cell separation [3].
These results demonstrate that STED microscopy can be an effective approach to characterize the morphology and functionality of bacterial cells, and to investigate their antimicrobial resistance.

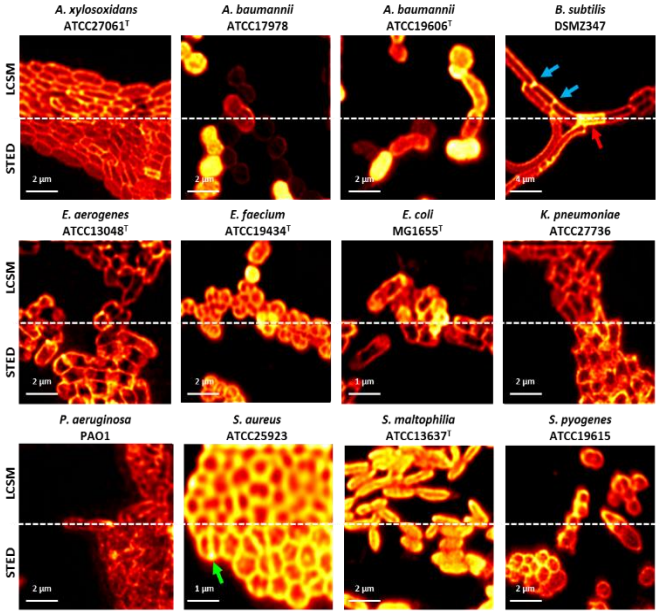


Fig. 1 LSCM and STED images. Blue arrows mark the membrane invagination during the *B. subtilis* cell division; the red arrow evidences its intracellular helix-pattern (bacterial cytoskeleton). The green arrow indicates a division septum of *S. aureus*.

## References

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