FAST TIRF-SIM SUPER-RESOLUTION MICROSCOPY OF THE BACTERIAL CELL WALL MACHINERY

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For a better observation of cytoskeletal dynamics on short timescales, fast imaging with high resolution is needed. We have developed a novel dual-color TIRF-SIM microscope using piezo scan mirrors and a Michelson-type beam duplexer to enable fast image acquisition with up to 8 Hz at 120 nm resolution.

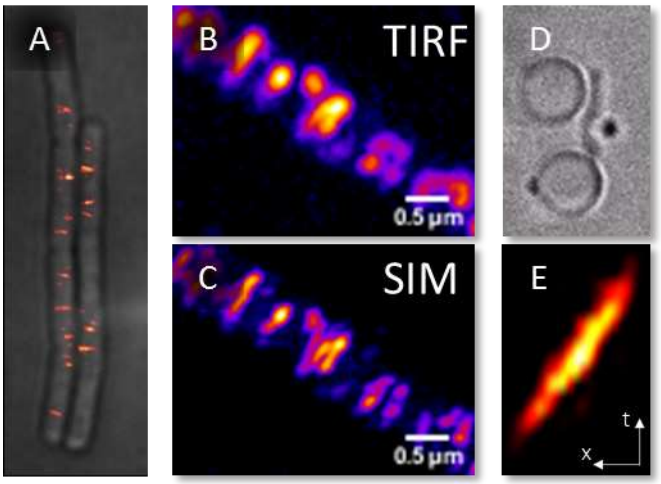

Figure 1: (A) GFP-labeled MreB-filaments in B. subtilis, taken with TIR-SIM microscopy. Overlay with wide-field standard microscopy. (B) Standard deviation analysis of dynamics sequence with TIRF microscopy and (C) TIRF-SIM. (D) Local micromanipulation of bacterium with optically trapped polystyrenel bead and (E) kymograph of moving MreB-filament.

We use these capabilities to image, analyze and manipulate the synthesis of the bacterial cell wall in B. subtilis. A key player in cell wall synthesis is MreB, which is essential for the cell shape of many rod-like bacteria like B. subtilis and E. Coli [1]. MreB filaments move underneath the cell membrane along its orientation with up to 80 nm/s. The filaments likely serve as mechanical coupling elements, which coordinate the parallel synthesis of PG-strands in the bacterial cell wall [2]. We use TIRF and TIRF-SIM to image the dynamics of MreB and other cell wall synthesis machinery proteins like RodA and PbpH. In addition, we probe the machinery's dynamics by manipulating the system chemically and mechanically with an optical trap and present a mechanistic model based on our results.

[1] C. Reimold, H. J. Defeu Soufo, F. Dempwolff, and P. L. Graumann, "Motion of variable-length MreB filaments at the bacterial cell membrane influences cell morphology.," *Mol. Biol. Cell*, vol. 24, no. 15, pp. 2340–9, 2013.

[2] P. V. Olshausen, H. J. Defeu Soufo, K. Wicker, R. Heintzmann, P. L. Graumann, and A. Rohrbach, "Superresolution imaging of dynamic MreB filaments in B. subtilis - A multiple-motor-driven transport?," *Biophys. J.*, vol. 105, no. 5, pp. 1171–1181, 2013.