FAST TIRF-SIM SUPER-RESOLUTION MICROSCOPY OF BACTERIAL AND EUKARYOTIC CYTOSKELETON DYNAMICS

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We have developed a novel TIRF-SIM microscope using piezo scan mirrors and a Michelson interferometer to enable fast image acquisition with up to 8 Hz at 120 nm. This allows us to better understand the fast reorganization of the cellular cytoskeleton, which is strongly involved in the response behavior of cells. We show actin dynamics in mouse macrophages as well as the dynamics of the bacterial cytoskeletal protein MreB, which is a homolog to the eukaryotic actin.

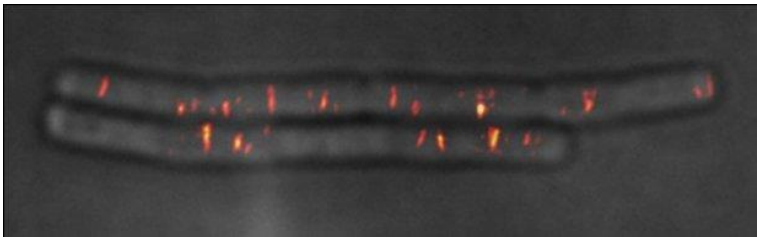

Figure 1: GFP-labeled MreB-filaments in B. subtilis, taken with TIR-SIM microscopy. Overlay with wide-field standard microscopy.

MreB 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 50 nm/s. The filaments likely serve as mechanical coupling elements, which coordinate the parallel synthesis of PG-strands in the bacterial cell wall.[2]. However, some open questions remain about the precise role of MreB in the cell wall construction and its "stop, go and return" movements. On the other side, macrophages exhibit fast reorganization of their actin cytoskeleton, which is driven by a "stop and go" dynamics of myosin motors.

For a better observation of cytoskeletal dynamics on short timescales, fast imaging with high resolution is needed. Dual-color imaging allows co-localization experiments with other membrane proteins. We present first imaging results of cytoskeleton dynamics.

[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.