Superresolution Microscopy of Live Cells – Imaging MreB Dynamics in *Bacillus Subtilis*

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High resolution fluorescence microscopy is of special interest in all life sciences as it allows investigating living matter like e.g. cells. In combination with structured illumination microscopy (SIM) one can increase the lateral resolution of fluorescence microscopes beyond the classical Rayleigh resolution limit (≈ 250 nm) to about 100 nm [1]. Applying this to biological samples enables the revelation of otherwise undetectable details, even in living cells [2].

We combine an objective launched total internal reflection fluorescence (TIRF) set-up with structured illumination. This brings together the advantages of high contrast and a superior resolution. The evanescent sinusoidal light grid is created with the help of a spatial light modulator (SLM) [3]. In combination with a fast camera for image acquisition this enables high frame rates of up to 1 Hz, sufficient to image many dynamic processes in living cells.
We show that the combination of TIRF-microscopy with structured illumination yields images of high contrast with superresolution of living bacteria. We imaged GFP-labeled MreB in *bacillus subtilis* with about two-fold increased resolution (Fig1) and in timesteps of a few seconds. Thus we could proof that MreB forms filaments that move on tracks perpendicular to the cells long axis. Furthermore we could resolve details in the protein dynamics that usually remain unobservable, like e.g. the slight deviation from straight tracks.

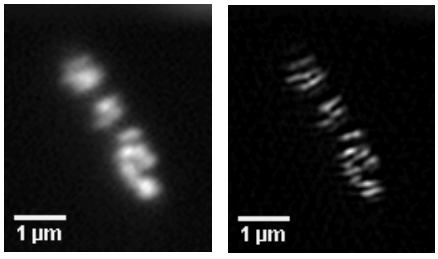

Figure 1:
(*left*) Usual TIRF-image of MreB in *bacillus subtilis*.
(*right*) Reconstructed TIRF-SIM image with superresolution. Much finer details are visible.

[1] M.G.L. Gustafsson, "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy", *Journal of Microscopy*, **198**, 82-87 (2000)
[2] P. Kner, B.B. Chhun and M.G.L. Gustafsson, "Super-resolution video microscopy of live cells by structured illumination", *Nature Methods*, **6**, 339-344 (2009)
[3] R. Fiolka, M. Beck and A. Stemmer, "Structured illumination in total internal reflection fluorescence microscopy using a spatial light modulator", *Optics Letter*, **33**, 1629-1631 (2008)