Super-resolution microscopy of living bacterial cells

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Abstract. Currently several methods of super-resolution optical microscopy are known. One of them – super-resolution radial fluctuations (SRRF) microscopy – was successfully used in present work to study the structures, formed by FtsZ protein in living *E.coli* cells with a resolution well below the diffraction limit.

1. Introduction
The aim of this work was to visualize cell division process (in particular, structures formed by FtsZ protein) in living *Escherichia coli* cells using super-resolution microscopy. It is well-known that FtsZ protein plays one of the main roles in this process [1]. FtsZ forms so-called Z-ring at the site of future septum, which is considered to be a scaffold for several dozens of downstream proteins, thereby it directs the cytokinesis. FtsZ is a tubulin homologue and is able to polymerize *in vitro* into several high-order structures, including linear filaments, bundles and helices [2]. One of the most striking observations about FtsZ behavior *in vitro* is its ability to generate contractile force upon conditions close to *in vivo* situation [3], although the actual role and significance of this force *in vivo* seems to be doubtful [4]. To better understand the molecular mechanisms of FtsZ role in division, for instance, whether FtsZ actually generates the force, further study is needed.

In previous studies we successfully utilized the method of single-molecule localization microscopy (SMLM) in combination with immunofluorescence for visualization of FtsZ structures in fixed *E. coli* cells [5-7]. However for better understanding of mechanisms of bacterial division, it is essential to visualize the process of division in dynamics, i.e. in living cells. Thus, a significant interest for researchers is the dynamics of FtsZ structures in the course of fission, which is largely a result of the development of new methods of super-resolution microscopy. The study of dynamics testifies to the high dynamism of the processes of assembly and disassembly of the Z-ring. In particular, it has recently been possible to observe such a phenomenon as treadmilling [8]. Until recently the tools for FtsZ visualization in living cells were limited by the observation that all *E.coli* fluorescent fusions with FtsZ protein used for super-resolution visualization before were not fully-functional, thereby this fact could substantially distort the actual FtsZ action. Recently several fully-functional fluorescent FtsZ fusions were developed, including those incorporated into bacterial chromosome [9]. In current work we visualized FtsZ in living *E.coli* cells with super-resolution using aforementioned fully-

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functional proteins in combination with a novel technique of Super-resolution radial fluctuations (SRRF) [10].

2. Materials and methods
All experiments were conducted using E. coli strain harbouring fully-functional FtsZ fusion with mNeonGreen incorporated into genome [9]. Cells were grown overnight in LB medium at 37°C, then passaged to fresh medium and grown until OD₆₀₀≈0.5. Then 1 μl of cell suspension was placed into agarose pad slide, which preparation was described previously [11].

Visualization was carried out using custom-made set-up based on upright optical microscope which was previously described [12]. To excite fluorescence the set-up was equipped with a set of solid-state and diode lasers as well as Hg lamp. High-sensitive cooled EM-CCD camera (Andor Ixon 896) was used for registration of fluorescence. Series of frames (from 100 to 1000) were acquired with an exposition of 20 ms and maximum camera speed using MicroManager [13]. Consequently these series were processed using NanoJ-SRRF [10] software package a freely available open-source plugin for the popular ImageJ or Fiji image analysis software. Data processing was performed using ImageJ [14].

3. Results and discussion
SRRF method in combination with fully-functional fluorescent FtsZ fusion allowed us to successfully visualize the structures formed by FtsZ while bacteria were dividing (see Figure 1 and Figure 2). These structures are in strong agreement with data obtained previously using combination of SMLM and immunofluorescence [5, 12, 15]. It is important to note that dynamics of the process of division is extremely important because FtsZ forms various transient spatial structures, and their interpretation is indispensible for understanding of molecular mechanisms of bacterial division. Studying of FtsZ represents not only fundamental importance, since understanding of the mechanisms of bacterial cells division seems to be a crucial step for development of new antibiotics.
Figure 1. Comparison of image of FtsZ obtained using conventional fluorescence (left) and corresponding image obtained using SRRF technique. Charts correspond to intensity profiles along yellow lines on both images. Scale bar corresponds to 1 μm at all images.
Figure 2. Time-lapse imaging of cell division. Images of three individual cells during 100 minutes are presented. a, c, e – SRRF images of FtsZ, b, d, f – composite images of super-resolution image of FtsZ (green) and conventional transmitted light image (gray) in living E.coli cells. Scale bar corresponds to 1 μm at all images.

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