Localization microscopy study of FtsZ structures in *E. coli* cells during SOS-response

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Abstract. Localization microscopy allows visualization of biological structures with resolution well below the diffraction limit. This is achieved by temporal separation of single fluorophore molecules emission and subsequent localization of them with the precision of few tens of nanometers. This method was previously successfully used to obtain images of FtsZ structures in *Escherichia coli* cells using FtsZ fusion with fluorescent protein mEos2. In this work we obtained superresolution images of FtsZ structures in fixed *E. coli* cells using immunocytochemical labeling. Comparison of superresolution FtsZ structures in cells undergoing SOS-response and “healthy” cells shows that FtsZ structures are partially disassembled during SOS-response, but still retain some periodicity.

1. Introduction.
Localization microscopy is a novel fluorescence microscopy technique, surpassing the diffraction limit of resolution by an order of magnitude [1]. The increased resolution is extremely valuable in the study of biological structures. This method is based on creating conditions that render only small fraction of fluorescent molecules in the sample visible at the same time so that images of individual molecules are separated. This allows these molecules to be localized up to few tens of nanometers. A series of sample images (typically from few thousands to few tens of thousands) is collected and molecule positions are determined by computer algorithm. The principle of localization microscopy is illustrated by figure 1. One of the most convenient ways to control the number of fluorescent molecules in the sample is to use photoactivatable or photoconvertible dye to label the structure of interest and adjust the power of activating/converting laser to achieve desired fluorescent molecule density. One of the most promising areas of localization microscopy application is microbiology as most of the prokaryotic cells have the sizes comparable to the resolution limit of conventional fluorescent microscopy, which renders this method largely inapplicable to the study of inner structures of these tiny cells [2].

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Figure 1. Localization microscopy scheme. Time separated conventional fluorescent images of single molecules (a-e) are processed using software, this results in localized images (h-l). Next these localized images are combined into image with improved resolution: m – image reconstructed from 5 frames, n – from 1000 frames. There are conventional fluorescent images of these 5 and 1000 frames on figures f and g respectively. Scale bar – 1 um.

FtsZ is a prokaryotic tubulin homolog that plays a crucial role in cell division, participating in the formation of the septum between two dividing cells [3, 4]. Localization microscopy was previously used to visualize the structure of the Z-ring formed by FtsZ at the septum using FtsZ fusion with fluorescent protein [5]. Furthermore, well before FtsZ was shown to form spiral-like structures in mutant *Escherichia coli* cells [6]. Fluorescent labeling of proteins can introduce artifacts both when fluorescent proteins or antibodies are used. It is therefore important to compare structures obtained using different labeling techniques. It is also known that FtsZ polymerization is one of the main regulation points in cell-division arrest during SOS-response [7]. The aim of this work was to compare FtsZ superresolution structures in “healthy” *E. coli* cells and cells undergoing SOS-response and also to compare superresolution FtsZ structures obtained using fusion proteins and immunocytochemical labeling.

2. Materials and methods.

2.1. Bacterial strain and immunofluorescent staining.

For experiments with normally dividing cells *E. coli* Top10 strain was used. For SOS-response experiments *E. coli* strain KD 403 with CRISPR-system (genes casA,B,C,D,E,1 and 2 under the control of arabinose-inducable promoter), carrying a spacer, complimentary to the site in bacteria genome was used. Induction of casA-E,1,2 expression resulted in bacterial DNA double-stranded brakes and caused SOS-response.

Four hours after the induction cells were immobilized on poly-L-lysine coated coverslips with nonspecifically adsorbed polystyrene microspheres (2.35 μm, Bangs Laboratories). Then cells were fixed with methanol-water-acetic acid solution (3:4:1) for 10 minutes. Next bacteria were washed 3 times with PBS and permeabilized with lysozyme in GTE buffer (glucose 50 mM, Tris 32.5 mM pH=7.5, EDTA 10 mM). After blocking in 2% w/v BSA for 1 hour, cells were incubated with anti-FtsZ rabbit primary antibodies (Agrisera, 1 to 200) overnight. Then bacteria were washed 5x in 0.05 % Tween and stained with Cage 635 Goat anti-Rabbit secondary antibodies (Abberior, 1 to 100).

2.2. Experimental setup and data processing.

The observation of samples were performed using experimental setup based on Axioimager.Z1 (Carl Zeiss) upright microscope (see figure 3) with extended facilities (including quadrant back focal plane detection and multicolor fluorescent microscopy with laser excitation) [8].
Cage 635 was excited by 635 nm diode laser (power density about 10 kW/cm² in focal plane) and constantly activated by 405 nm diode laser (100 W/cm²). To reconstruct a superresolution image 1000 frames were collected and analyzed using QuickPALM [9] plug-in for ImageJ [10]. Due to increased resolution and long acquisition time localization microscopy is extremely sensitive to the sample drift. To overcome this problem we use an active sample stabilization system based on quadrant detection of 2.35 µm polystyrene microsphere position. It can achieve precision sufficient to render drift effect on localization microscopy resolution negligible in combination with high refresh rate (up to few hundred Hz) which allows it to compensate higher-frequency fluctuations of sample position compared to CCD-based stabilization. Low requirements for sample preparation allows this method to be easily incorporated into existing microscopy sample preparation protocols.

3. Results and discussion.
Localization microscopy of *E. coli* Top10 cells undergoing normal cell division (figure 3) showed that FtsZ forms various structures including Z-ring between two dividing cells (figure 3, column 4,5) and spiral-like structures along the cell membrane (figure 3, column 3). These observations are consistent with previous data obtained using fluorescent protein mEos2 [5].
Figure 3. Structures formed by FtsZ in “ordinary” cells. Transmitted light (a), “conventional” fluorescence (b), localization microscopy (c) and composite of a and c images (d). For clarity, the glow of the fluorophore on the composite image is shown in red. Scale bar – 1 μm.

Figure 4 shows a localization microscopy images of FtsZ in E. coli cell during SOS-response (b), corresponding transmitted light image (a) and “conventional” fluorescent image (c) obtained by averaging 2000 frames of the sample that were used for the reconstruction of a superresolution image. This images show evidence of the regular structure with the period of approximately 1 μm, but no ring-like structures are formed which could explain the division arrest in these cells. It was shown that SulA protein induced during SOS-response can partially inhibit FtsZ polymerization [7]. To test whether SulA is responsible for the observed change in FtsZ pattern either SulA inhibition or sulA gene deletion/knock-out mutation can be used. Three-dimensional localization microscopy should allow us to obtain a better view of the structures formed by FtsZ.

Fig. 4. FtsZ structure in E. coli during SOS-response. Transmitted light (a), “conventional” fluorescence (b), localization microscopy (c) and composite of a and c images (d) of a bacteria in SOS-response with fluorescently-labeled FtsZ. Bar – 1 um.

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