Imaging of bacterial chromosome organization by 3D super-resolution microscopy

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Abstract

The bacterial nucleoid is highly organized, yet it is dynamically remodeled by cellular processes such as transcription, replication or segregation. Many principles of nucleoid organisation have remained obscure due to the inability of conventional microscopy methods to retrieve structural information beyond the diffraction limit of light. Structured illumination microscopy has recently been shown to provide new levels of spatial details on bacterial chromosome organisation by surpassing the diffraction limit. Its ease of use and fast 3D multicolor capabilities make it a method of choice for imaging fluorescently labeled specimens at the nanoscale. We describe a simple high-throughput method for imaging bacterial chromosomes using this technique.

1. Introduction

How chromosomes are organised within the cell is a fundamental problem in biology. Most bacteria possess a single circular chromosome, that must be tightly compacted to fit within a micrometer sized volume, called the nucleoid. Sophisticated regulatory mechanisms ensure the packaging and dynamic organisation of the DNA content within the nucleoid at different hierarchical levels, from the nanometer to the micron scale. Traditional ensemble approaches in biochemistry, genetics or molecular biology have provided invaluable insights into this organisation. Of particular interest, bacterial chromosomes have been shown to be structured by several hierarchical levels of organization, including topological domains (10-20kb) (*1*, *2*), chromosome interaction domains (50-300kb) *(3–5)* and macrodomains (*6*). In particular, it is not yet understood how these different levels of structural organisation are orchestrated at the single cell level, coordinated with the cell cycle, or coupled to DNA management processes such as replication.

Fluorescence microscopy enables the *in vivo* observation of DNA while permitting the localization of proteins at the single cell level with high specificity. However, the maximum resolution attainable by conventional microscopy approaches is intrinsically restricted to the detection of spatial scales above the limit imposed by the diffraction of light (typically 200–300 nm and 700–900 nm in the lateral and axial directions, respectively). As most hierarchical organisation levels of the bacterial nucleoid lie in the 10-500 nm scale, they cannot be accessed by conventional microscopies. In recent years, a plethora of techniques using various strategies to overcome the intrinsic microscope optical resolution have been developed (*7–10*). Among these techniques, three dimensional structured illumination microscopy (3D-SIM) enables a two-fold increase in both lateral and axial resolution as compared to diffraction-limited microscopies (*11*). 3D-SIM relies on the modulation of the frequency content of the specimen using an excitation grid pattern that is shifted or rotated during image acquisition. Reconstruction procedures permits high-frequency information to be extracted to obtain a 3D super-resolved image. Using this technique on the *Bacillus subtilis* and *Escherichia coli* chromosomes, we revealed the existence of a new higher-order level of organization of the nucleoid that was not visible by diffraction-limited fluorescence microscopy methods (*4*, *12*). Nucleoids in those species appear structured by High-density DNA Regions (HDRs). Besides circumventing the diffraction limit of light, 3D-SIM offers an ease of use and fast 3D multicolor capabilities that will likely ensure it to become a method of choice for imaging fluorescently labeled specimens at the nanoscale. However, like any super-resolution technique, its use, especially on live biological specimens, requires careful data acquisition and processing procedures to avoid reconstruction artifacts.

Here, we describe a method to image the bacterial nucleoid and DNA-binding proteins simultaneously at super resolution and at high-throughput. We provide a step-by-step protocol describing the cell culture, sample preparation, and acquisition and reconstruction of multicolor super-resolution images on a commercially available 3D-SIM platform (GE Healthcare OMX).

Specifically, we demonstrate our method by imaging the nucleoids of *Bacillus subtilis* (*B.subtilis*) or *Escherichia coli* (*E.coli*) using protein fusions or chemical labeling, and simultaneously detect the nuclear distribution of ParB, a protein that assembles at centromeric sequences to form a mitotic-like apparatus assuring the inheritance of duplicated genetic material before cell division.

2. Materials

All solutions are prepared using ultrapure water and analytical grade reagents when possible. Prepare and store all stock reagents at 4°C. Solutions percentage are weight/volume (w/v %) unless otherwise indicated.

2.1. Bacterial strains

1. *Bacillus subtilis* strains : wild type *B. subtilis* (PY79 background), chromosomal ParB-GFP strain (HM671, PY79 background*),* with resistance to kanamycin.
2. *Escherichia coli* strain: HU-mCherry labelled nucleoids and plasmidic ParB-mVenus (DLT3053/pJYB234), with resistance to chloramphenicol.

2.2. Growing media

1. Luria Bertani (LB) medium: 4 g NaCl, 4 g Tryptone, 2 g Yeast Extract, bring volume to 400 mL with water. For *E.coli* culture add 4 mg of Thymine.
2. Minimal media (M9, 10X) : 60 g of Na_2HPO_4, 30 g of KH_2PO_4, 5 g of NaCl, 10 g of NH_4Cl. Bring to 1L with H_2O.
3. *B. subtilis* minimal media (M9bs): 50 µL of 2 mg/mL $FeNH_4$ citrate, 0.6 mL of 1 M $MgSO_4$, 0.1 mL of 100 mM $CaCl_2$, 0.2 mL of 65 mM $MnSO_4$, 0.1 mL of 1 mM $ZnCl_2$, 0.1 mL of Thiamine 2 mM, 2 mL of 5% Glutamate, 7.5 mL of 20% Succinate. Bring volume to 100 mL with M9 (1X) solution. Store at 4°C.
4. *E. coli* minimal media (M9ec): 2 mL of 20% Glucose, 100 µL of 1 mM $MgSO_4$, 100 µL of 0.1 M $CaCl_2$, 100 µL of 1 mg/ml Vitamin B1, 2 ml of 2 mg/ml Thymine, 200 µl of 10 mg/ml leucine. Bring volume to 100 mL with M9 (1X) solution. Store at 4°C.
5. Casamino Acids (CAA) supplement: 20%. Store at 4°C.
6. Antibiotics final concentrations: Chloramphenicol 10 $\mu g.ml^{-1}$, Kanamycin 2 $\mu g.ml^{-1}$.

2.3. Sample preparation

1. Rectangular coverslip (#1.5H, Deckgläser, 24 x 60 mm).
2. 1 mm thick glass slide (SuperFrost, Ultra Plus, 25 x 75 mm)
3. Acetone
4. Bunsen burner
5. Home-made Teflon rack
6. Double-side adhesive tape
7. Razor blade
8. Fluorescent beads (40 nm, Trans FluoSpheres and 100 nm Tetraspeck, Invitrogen).
9. Agarose (A4804, SigmaAldrich).
10. Minimal medium (M9bs/M9ec).
11. Dry bath heater
12. Optional : plasma cleaner Femto (Diener Electronic)

2.4. Structured illumination microscope and software

Instrumental setup (GE Healthcare OMX version 3)

- 405/488/561 nm excitation lasers
- DAPI filter : 419-465 nm
- A488 filter : 500-550 nm
- A561 filter : 581-618 nm
- Objective 100X, NA 1.4,
- Nanometric piezo-stage
- 4 electron-multiplying charge-coupled device (EM-CCD) cameras (Photometrics Evolve)

Software

- OMX acquisition software
- SoftWoRx version 5
- ImageJ (Bioformat, SIMcheck plugins)
- Icy/Imaris/Matlab.

3. Methods

3.1. Growing cells

Bacterial strains are stored in a mix of LB and glycerol (50%) at -80 °C. All cell culture steps should be carried under flame or in a hood.

1. Streak bacteria from -80°C glycerol stock onto solid (plate) Luria Bertani (LB) medium (i.e. agar plate) complemented with appropriate antibiotics.
2. Culture agar plates 24 hours at 37°C.
3. Select a single colony from plate and perform at least 5 serial dilutions with M9bs/M9ec supplemented with CAA 20 µg/mL in sterile 50 ml falcon tubes (1/10 each in 5 ml final volume) and grow overnight at 30°C with agitation at 200 rpm.
4. The next morning measure the optical density (OD) at 600 nm and select dilution of cells exponentially growing (OD ~0.3-0.5). Further dilute to OD ~0.05 in M9 (M9bs or M9ec) supplemented with CAA 20 µg/mL (25 mL final volume in 250 mL flasks for optimal aeration) and incubate at 30°C with agitation at 200 rpm.
5. Measure OD at 1h intervals and when reaching OD ~0.3-0.4, take 1 mL of bacterial suspension and place it into a 1.5 mL Eppendorf tube.
6. If necessary, add 7.5 µl of a 0.3 µM solution of DNA dye (DAPI, Invitrogen) to 1 ml of bacterial suspension and incubate for 5 minutes.
7. Spin down cells in a bench centrifuge at 1500 g for 4 min for *B. subtilis* or 7000 g for 1.5 minutes for *E. coli*.
8. Resuspend in 1 ml of M9bs or M9ec.
9. Repeat steps 7 and 8 two more times.
10. Discard supernatant and resuspend pellet in 20 µl M9bs or M9ec.
11. Add 1 µL of 1/10 dilution of 40 nm fluorescent beads to the suspension and gently mix (see Note 1).

3.2. Mounting agar pads

1. Rinse microscope coverslips and glass slides with acetone and dry them over an open flame to eliminate any remaining fluorescent contamination (see Note 2).
2. Place microscope coverslips and glass slides on a Teflon rack (home made). Plasma clean it for 15 minutes with O_2 gas at 0.4 mbar at 70% power (see Note 3).
3. Deposit a piece of double-side adhesive tape on a glass slide and extrude a ~5 mm V-shaped channel from its center using a razor blade (Figure 1, see Note 4).

4. Spread 15 µl of 2 % melted agarose (diluted in M9bs or M9ec media, melted at 90°C) on the center of the glass slide and cover right away with a second glass slide to ensure a flat agarose surface (Figure 1).
5. Let agarose cool down to room temperature for ~2 minutes at a horizontal position with external pressure to ensure a flat agarose surface.
6. Carefully remove the top glass slide by sliding it down towards the bottom of the V-shape channel to ensure the agarose pad remains on the bottom glass slide.
7. Deposit ~4 µl of vegetatively growing bacterial resuspension (see section **3.1**) onto the agarose (see Note 5) and let settle for 2 minutes.
8. While carrying step 7, remove the second face of the double side tape then gently seal the pad with a clean coverslip by pressing its top surface (see Note 6).
9. Store your agar pad in a sealed box with water to prevent sample from drying.

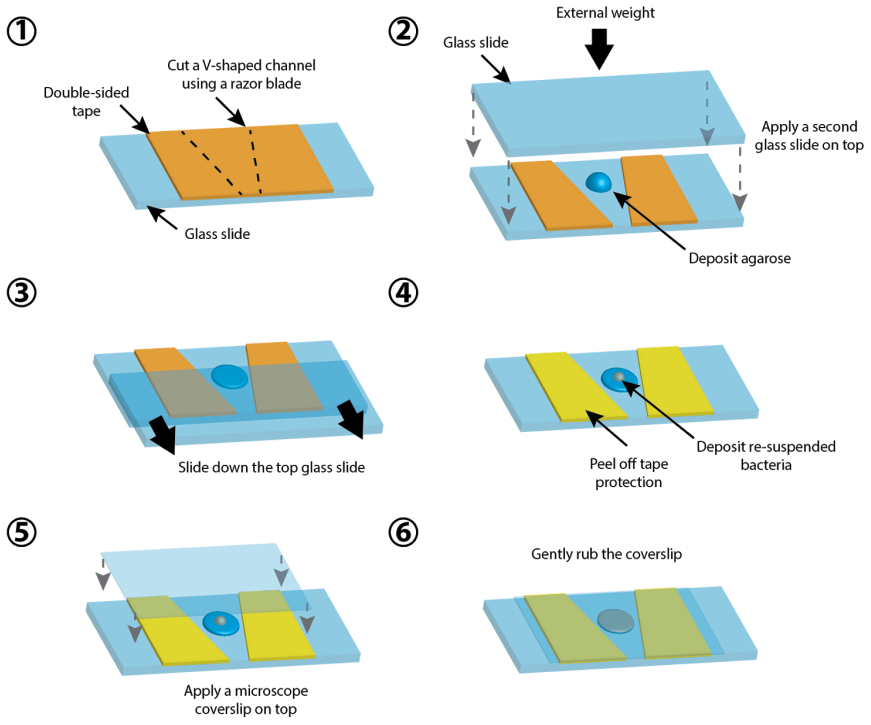

Figure 1 : Agar pads mounting for live-cell 3D-SIM. 15 µl of 2 % melted agarose are sandwiched into a V-shaped channel between two 1 mm thick glass slides. Once cooled to room temperature under external pressure (see main text), 4 µl of re-suspended bacteria are deposited onto the agarose before sealing the sample with a microscope coverslip.

3.3. Imaging

This section describes initial acquisition conditions and optimisations for imaging bacterial chromosomes in 3D-SIM. Before starting high-throughput acquisitions, additional controls described in section 3.5 should be performed.

1. Set software to the 100X objective settings and Z scan acquisition mode (center of the stack).
2. Mount slide with agarose pad onto the microscope and find the focus on the agar pad - coverslip interface using brightfield illumination.
3. Under brightfield illumination, explore sample and while verifying its integrity find a region of the pad with a dense monolayer of bacteria (~300-500 bacteria per field of view can be found in optimal conditions)(see Note 7).

4. Assign the appropriate fluorescent filters to the cameras for ParB (GFP or mVenus labelled) and DNA (DAPI-stained or Hbs-mCherry) emission and excitation wavelengths (see Note 8) and switch to structured illumination mode (see Note 9).
5. Re-adjust the focus to find the optimum axial position between the two fluorescence emission channels for ParB and DNA imaging (see Note 10).
6. Adjust laser excitation intensities and camera integration times (see Note 11) and gains to exploit as much as possible the dynamic range of the camera (>10^4 levels of grey for a 16 bits camera) while minimizing photobleaching and phototoxicity. Anticipate a few hundreds images using your current imaging settings for volumetric SIM acquisition, see Note 12-13)
7. Set the Z step between each slice to 125 nm (see note 14) and the number of slices to 15-17 to anticipate the chromatic shift between emission channels and any slight tilt of the sample plane by including information above and below signal's plane of focus.
8. Move to a nearby non-illuminated region of the pad (~100 µm), let the sample stabilize for 10-15 seconds and launch the acquisition of a 3D-SIM stack.
9. Open the acquired 3D-SIM stack in a data inspection software (e.g. ImageJ/Icy/Matlab) for a rapid visual inspection of your data.
10. Inspect your data for minimum fluorescence intensity variations during acquisition (Figure 2, see Note 15), good modulation contrast, and absence of artifactual signatures (Figure 2). Check for presence of first and second modulation peaks and absence of atypical structures in the fourier transform of the raw data. A more thorough assessment of image acquisition will be performed in section **3.5**.
11. Optimize imaging conditions by repeating steps 5-10 until you get satisfying imaging conditions.

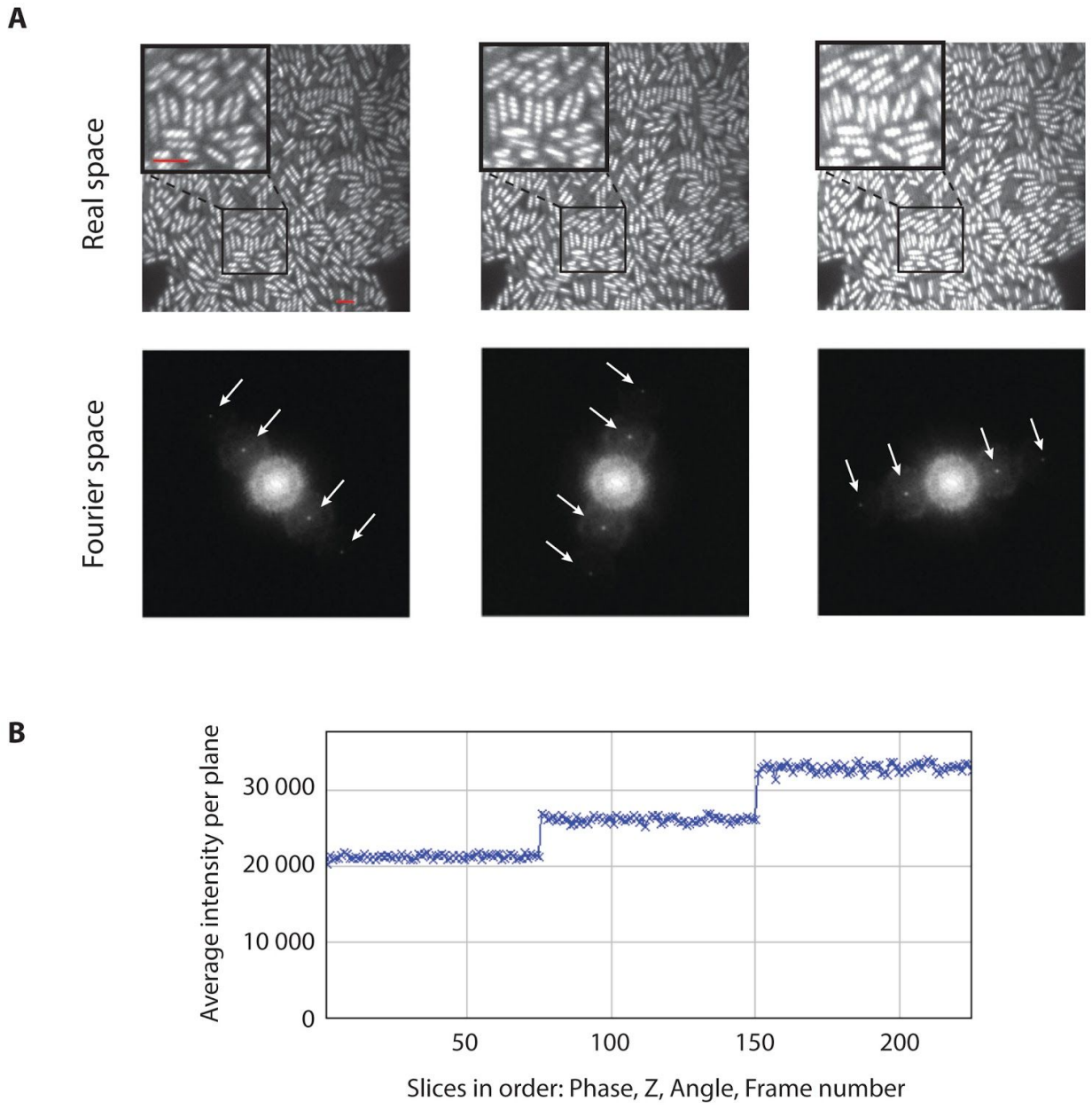

Figure 2 : Visual inspection and optimization of 3D-SIM acquisition. A. Single plane images of a 3D-SIM raw acquisition of *B.subtilis* nucleoids stained with DAPI, showing the illuminated sample with three excitation pattern orientations. Top panels show the illuminated sample in the real space with insets showing a higher magnification in which the illumination pattern is clearly visible, while bottom panels display their respective Fourier transform. White arrows in bottom panels point toward the first and second diffraction order of the structured illumination ensuring optimal modulation of the frequency content of the sample with the structured illumination pattern. **B.** Average intensity for each imaging plane (in the order: Phase, Z, Angle, Time). This allows to ensure minimum fluorescence intensity variations during acquisition due to photobleaching, intensity differences between angles (as illustrated with the increasing fluorescence signal on the above figure), and illumination flickering amongst other measures.

3.4. Data reconstruction and alignment

Reconstruction of 3D-SIM raw data is performed using softWoRx v5.0 (Applied Precision, see Note 16). Channel specific Optical Transfer Functions (OTFs) are used for reconstruction and are computed from an experimental point spread function (PSF). This experimental PSF is obtained from 3D stack of images of 100 nm multi-color labelled FluoSpheres (LifeTechnologies) provided as input to softWoRx. For all emission channels, set the reconstruction filter (Wiener filter) settings and background subtraction value to optimal values (0.002 and 65, respectively, under the imaging conditions of this report, See Note 17). These settings will depend on your image background and signal to noise ratio and may be adjusted depending on your imaging conditions.

For multi-color acquisitions, images from different emission channels are aligned to each other to get the most accurate overlay of fluorescent structures (**Figure 3**). This step is critical, particularly on the GE Healthcare OMX platform since emission on each channel is recorded on a distinct camera. Multichannel alignment calibration is typically performed using fluorescent beads bound to a coverslip as alignment markers, but other strategies such as nanostructures consisting of arrays of pinholes can be used. The 3D coordinates of each alignment marker then provide references to correct for X-Y-Z translation, distortion, magnification and rotation of each fluorescent channel. Alignment markers must be immersed in the same media used for experiments, as optical aberrations depend on the refractive index of the sample media. OMX Editor performs automated extraction of the coordinates of alignment references and efficiently applies the obtained correction parameters to align multichannel 3D-SIM acquisitions.

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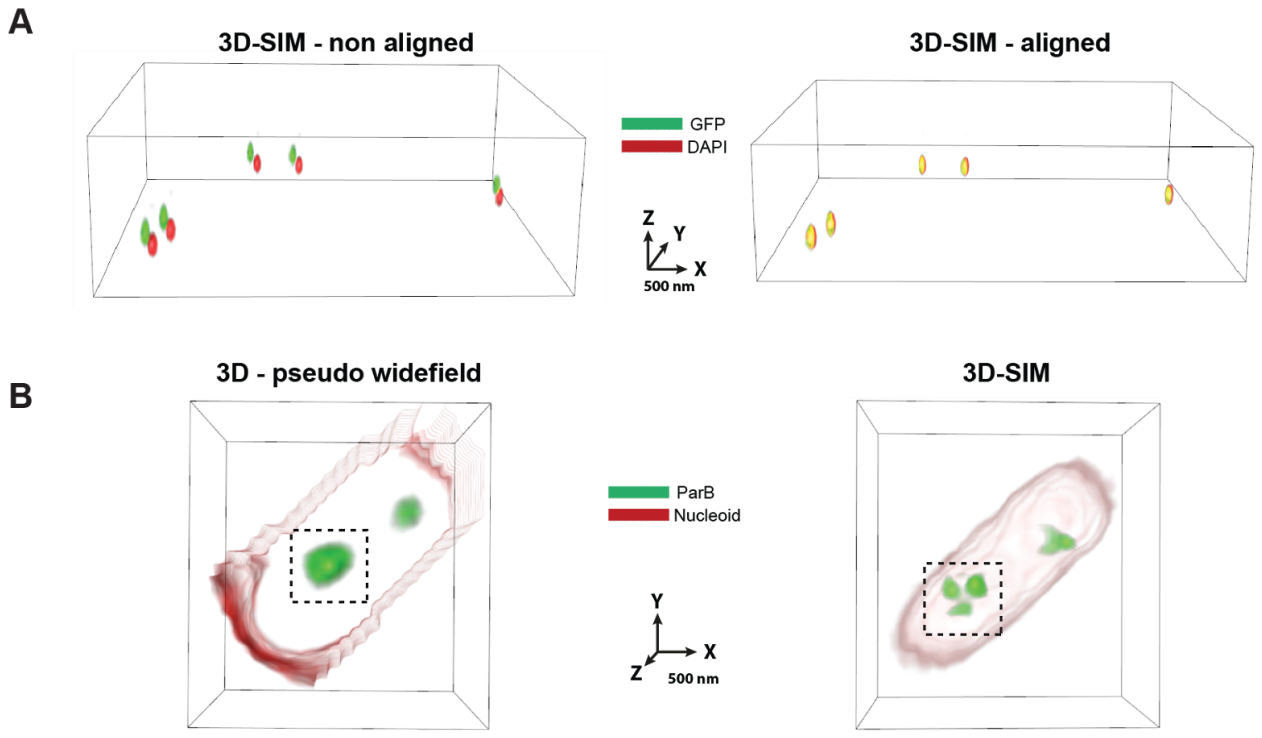

Figure 3 : 3D-SIM alignment and reconstruction. A. 3D-SIM images of beads imaged in GFP (green) and DAPI (red) channels before and after alignment (left and right panels, respectively). **B.** Top view of the same nucleoid (red) and ParB foci (green) from *B. subtilis* in 3D epifluorescence (pseudo-widefield reconstruction from 3D-SIM raw data without channel alignment, see Note 18) and reconstructed from 3D SIM raw data (with channels alignment). Dotted square in left and right images highlights the same ParB focus composed of three ParB sub-clusters only resolvable when using 3D-SIM.

3.5. Data acquisition and reconstruction validation

Inaccurate instrument settings and suboptimal imaging conditions can cause artifacts that are often difficult to distinguish from relevant structural features (See Note 19). Absence of artifacts in 3D-SIM reconstructions can be verified by employing different strategies (**Figure 4**). These include the recently developed SIM-Check software (*13*), a plugin application of ImageJ, which allows to verify:

- degree of photobleaching during acquisition (See Note 20),
- absence of spherical aberrations (See Note 21),
- absence of movement or drift of the sample,
- proper modulation contrast (see Note 22),
- absence of artifactual signatures in the fourier transform of the reconstructed data,
- presence of the first and second orders of the excitation modulation in the Fourier transform of the raw data

We highly recommend users to employ SIM-Check as it additionally provides tools for assessment of the resolution, image quality and identification of sources of errors and artifacts in SIM imaging.

Besides SIM-Check software, additional experimental controls can be performed to ensure the absence of artifacts (**Figure 4**):

- Imaging cytosolic GFP (or other fluorescent markers with similar spectral properties) on anucleated bacterial cells (obtained by treatment with mitomycin at 50 ng/ml during 30 min) using the same imaging conditions as those for whole labeled chromosomes. High-frequency signal should be exclusively distributed at the frontier between the cytosol and membrane where the fluorescence intensity signal drops to zero.
- 3D-SIM time-lapse acquisition of labeled nucleoids. The overall nucleoid morphology should be conserved between the images and only small dynamical changes between acquisitions should be apparent. To minimize dynamical changes during sequential acquisitions as well as photobleaching, the total number of Z slices can be reduced to ~12.
- Two-colors 3D-SIM imaging of nucleoids simultaneously labeled with spectrally distinct probes (for example Hbs-GFP and DAPI). Nucleoid structures should appear similar in both colors and only small dynamical changes between acquisitions should be apparent.

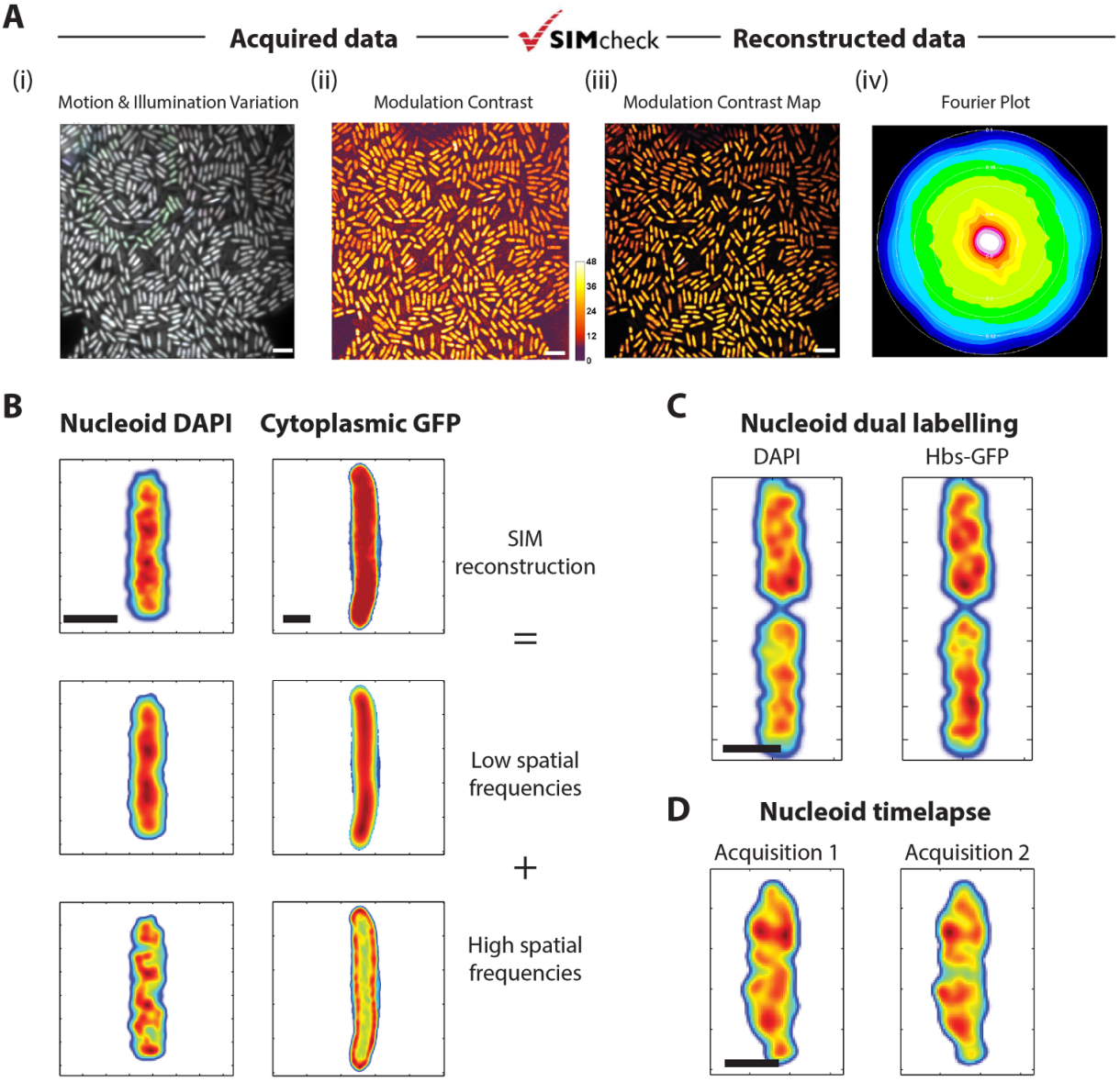

Figure 4 : 3D-SIM acquisition and reconstruction checkings. A. Examples of data acquisition and reconstruction checkings using SIMCheck on *B.subtilis* chromosomes stained with DAPI. Image (i) assesses motion or illumination variations of the sample during the acquisition by averaging and then assigning false colors to images corresponding to the same illumination angle (see Note 23). Image (ii) maps the adequacy of the modulation contrast in the image for reconstruction (see Note 24). Color-code for structures reconstruction should read as inadequate, adequate, good, very good and excellent for purple, red, orange, yellow and white respectively. Image (iii) maps the reliability of the structures in the reconstructed data using the same color code of ii (see Note 25). Image (iv) represents the lateral Fourier transform of the full reconstructed 3D-SIM dataset to assess the resolution of the reconstructed data as well as potential reconstruction artifacts. Scale bar is 5 µm. **B-C-D.** Experimental controls to assess the absence of artifacts in 3D-SIM nucleoids images of *B. subtilis*. (A) Maximum intensity projections of spatial frequencies decomposition of bacterial nucleoids (chromosomes stained with DAPI, left panels) and cytosolic GFP (right panels, anucleated cells, see section 3.5) imaged in 3D-SIM using similar imaging conditions. High spatial frequencies corresponding to high DNA density regions should solely be observed in fluorescently labeled nucleoids images and be absent in cytosolic GFP images. (C-D) Comparison of 3D-SIM maximum intensity projections of chromosomes imaged in two colors (panel C, DAPI and Hbs-GFP labeling) or in timelapse (panel D, DAPI labeling). Overall nucleoid morphology and high DNA density regions should be

conserved between images and only small dynamical changes between acquisitions should be apparent. Color-codes represent DAPI/GFP fluorescence intensity increasing from blue to red. Scale bar is 1µm.

3.6. Reconstruction visualisation

Volumetric visualisation and inspection of structures of interest can be challenging due to mixture of both low and high spatial frequencies in three dimensions. Exploration of 3D-SIM datasets can be performed using freely available software packages such as Icy, which permits fast and handy representations of your data, or using more sophisticated or automated solutions such as Imaris or Matlab (**Figure 5**). We here give a step-by-step guide to represent your reconstructed nucleoids in 3D using Icy:

1. Open your reconstructed and aligned 3D-SIM data in Icy.
2. Define a Region Of Interest (ROI) containing your selected nucleoids of choice (see Note 26)
3. Re-scale your ROI by doubling its dimensions (number of rows, columns and slices) in order to attenuate aliasing effects of the voxel size.
4. In the drop-down menu of your active sequence, select 3D representation
5. If your computer is equipped with a compatible graphic card, tick the OpenGl option on the right-side panel of the software.
6. Still on the right-side panel of the software, select the fluorescent channel corresponding to your nucleoid data and pick the colormap of your choice.
7. Adjust the minimum and maximum intensity values for each color of the selected colormap to display your features of interest (Figure 5, panels A-B)

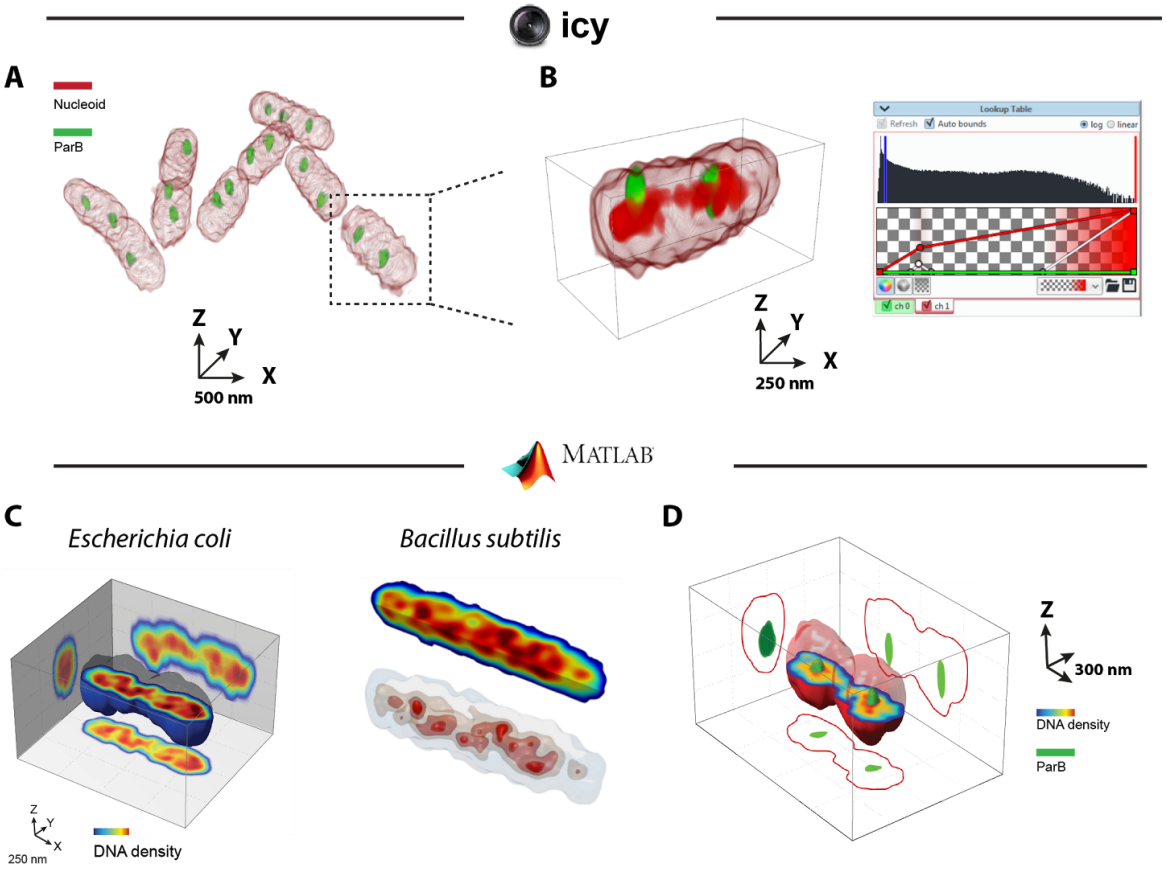

Figure 5 : 3D-SIM data visualisation. A-B. 3D-SIM visualisation with Icy. (A) Multicolour 3D-SIM images of *E. coli* HU-mCherry labelled nucleoids (red) and plasmidic ParB-mVenus (green). Dotted square highlights the magnified region shown in (B) side by side with Icy look-up table showing the color adjustments used to show ParB foci reside at high DNA density regions within the nucleoid **C-D**. 3D-SIM visualisation with Matlab. (C) Left: 3D volume of an *E.coli* nucleoid stained with HU-mCherry**.** Nucleoid DNA density in the volumetric representation and side projections is color coded from blue to red. High-density chromosomal regions (HDRs) stretch from pole to pole and are joined by a semi-continuous filamentous density. Right top panel: 3D-cut of a DAPI-stained *B.subtilis* nucleoid (top) allows to see the nucleoid interior. Right lower panel: same nucleoid from top represented using Iso-levels (Light-gray to red color-scale represents low to high DNA densities, respectively). (D) 3D volume of an *E.coli* nucleoid stained with HU-mCherry (solid red) and ParB-mVenus foci (green) with orthogonal 2D projections of nucleoid (red contour) and ParB (green spot) densities. Figures from panels A, C and D were reproduced with permission from (*4*, *12*)

4. Notes

1. Fluorescent beads may have a tendency to adhere to bacteria cells. To circumvent this, beads can be stuck to the microscope coverslip prior to the agar pad assembly (see section 3.2).
2. Beware not to deform microscope coverslips as you pass them under the flame. Deformed coverslips introduce non-even flatness of the sample as well as optical aberrations significantly degrading the quality of the data.
3. The plasma cleaning step can be substituted by incubating the microscope glass slides and coverslips in a KOH bath at 1M, under sonication for 15 minutes, followed by repeated washes with water.
4. More than one sample can be extruded on the same slide. By adjusting the channels widths and the amount of agarose, up to 3 samples can be prepared in the same coverslip without cross contamination.
5. In case the agarose pad is in contact with the adhesive tape, extrude carefully furrows using a clean razor blade prior to depositing bacteria to prevent bacterial suspension to spread on the adhesive tape.
6. Adding beads to the coverslip surface prior to final pad mounting provides a means of ensuring final multichannel 3D-SIM alignment and reconstruction process described in section 3.4. This also provides the means for assessing the final resolution of the reconstructed data.
7. Use the “mosaic” acquisition option of the OMX acquisition software to find a dense region of bacteria faster.
8. After assigning the fluorescent filter set to the camera, the imaging order for each fluorescent channel can be selected when employing sequential acquisition mode. The most energetic excitation wavelength should then be the last to avoid excitation crosstalks and limit phototoxicity of the sample during acquisition.
9. GE Healthcare OMX platform offers a fixed grid spacing. Note however that other commercially available SIM platforms such as Elyra (Zeiss) offers the possibility of adjusting this spacing depending on the fluorescent channel.
10. Due to inherent microscope chromatic aberrations the optimum focus will be a compromise between each fluorescent channel.
11. Typical acquisition parameters used are: DAPI, 5-10 ms exposure with 10% transmission of the 405 nm excitation line; ParB-mVenus, 10 ms exposure with 31.3% transmission of the 488 nm excitation line and HU-mCherry, 10 ms exposure with 31.3% transmission of the 568 nm excitation line.

12. Imaging bacterial chromosomes stained with DAPI requires careful controls of imaging conditions to avoid photodamage. In our hands, extensive illumination with UV light on DAPI-stained *B. subtilis* nucleoids leads to artefactual expansion followed by shrinking of the nucleoid volume.
13. Three different angles (-60°, 0° and +60°) as well as five phase steps are used to reconstruct each 3D-SIM image plane. Thus, imaging 17 sections of your sample requires a total of 255 images.
14. Other commercially available SIM platforms may offer the possibility to adjust the Z step size depending on the fluorescence wavelength to fulfil the Nyquist sampling criterion.
15. Fluorescence intensity variations between frames may arise from different sources with the most commons being photobleaching (if possible keep photobleaching under <~30%) and intensity differences between illumination angles (see Figure 2, panel B).
16. Open-source SIM packages can be found elsewhere (*14–16*).
17. Wiener filtering settings can be adjusted to attenuate reconstruction artifacts. It permits a smoother final image reconstruction but at the cost of a lower resolution.
18. SIMcheck utilities permit to reconstruct pseudo widefield volumes from raw 3D-SIM data by averaging images of all phases and angles from the same imaging planes.
19. Artifacts in reconstructed images may arise from different sources that include sample photobleaching, refractive index mismatch between immersion oil, coverslip or immersion media, or insufficient modulation contrast.
20. Photobleaching can be minimized by changing imaging conditions. This includes lowering the number of Z slices or balancing excitation intensities with camera integration times.
21. Spherical aberrations can be caused by refractive index mismatch. They can be attenuated by optimizing the immersion oil refractive index to reduce the haloing (asymmetric axial shape) of the point spread function of fluorescent beads. In our hands, oils with refractive index of ~1.510 give the best results when imaging nucleoids stained with DAPI or HU-GFP, though bear in mind that this optimization is wavelength dependent.
22. Poor modulation contrast may be caused by low signal to noise ratios, diffusing samples or high background masking the excitation modulation.
23. SimCheck Motion and Illumination Variation checking: All images corresponding to the same illumination angle are averaged and intensity-normalized. The three resulting images are then assigned to distinct false color (Cyan, Magenta and Yellow) and merged into a single output CMY-merged image. Any colorization of the output image indicates potential motions of the sample or uneven illumination during the acquisition. Sample motion can be minimized by reducing the number of optical sections of the sample. Also, ensure that the acquisition mode is set to “all Z then channels” and not “all channels then Z”.
24. SimCheck Modulation Contrast (MCN) checking depicts the ratio of the modulation amplitude to noise amplitude in the acquired data.
25. SimCheck Modulation Contrast Map displays the ratio of the modulation amplitude to noise amplitude in the acquired data multiplied with the intensity for each pixel in the reconstructed data, normalized to the maximum intensity in the image.
26. The size of the ROI will depend on the configuration of your computer. We recommend 64-bits operating systems equipped with multi-core processors and 8 GB of RAM.

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References

1. L. Postow, C. D. Hardy, J. Arsuaga, N. R. Cozzarelli, Topological domain structure of the Escherichia coli chromosome. *Genes Dev.* **18**, 1766–1779 (2004).

2. S. Deng, R. a. Stein, N. P. Higgins, Organization of supercoil domains and their reorganization by transcription. *Mol. Microbiol.* **57**, 1511–1521 (2005).

3. T. B. K. Le, M. V. Imakaev, L. a. Mirny, M. T. Laub, High-resolution mapping of the spatial organization of a bacterial chromosome. *Science*. **342**, 731–734 (2013).

4. M. Marbouty *et al.*, Condensin- and Replication-Mediated Bacterial Chromosome Folding and Origin Condensation Revealed by Hi-C and Super-resolution Imaging. *Mol. Cell*. **59**, 588–602 (2015).

5. X. Wang *et al.*, Condensin promotes the juxtaposition of DNA flanking its loading site in Bacillus subtilis. *Genes Dev.* **29**, 1661–1675 (2015).

6. O. Espéli, F. Boccard, Organization of the Escherichia coli chromosome into macrodomains and its possible functional implications. *J. Struct. Biol.* **156**, 304–310 (2006).

7. E. Betzig *et al.*, Imaging intracellular fluorescent proteins at nanometer resolution. *Science*. **313**, 1642–1645 (2006).

8. S. T. Hess, T. P. K. Girirajan, M. D. Mason, Ultra-high resolution imaging by fluorescence photoactivation localization microscopy. *Biophys. J.* **91**, 4258–4272 (2006).

9. M. J. Rust, M. Bates, X. Zhuang, Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat. Methods*. **3**, 793–795 (2006).

10. M. Heilemann *et al.*, Subdiffraction-resolution fluorescence imaging with conventional fluorescent probes. *Angew. Chem. Int. Ed.* **47**, 6172–6176 (2008).

11. M. G. L. Gustafsson *et al.*, Three-Dimensional Resolution Doubling in Wide-Field Fluorescence Microscopy by Structured Illumination. *Biophys. J.* **94**, 4957–4970 (2008).

12. A. Le Gall *et al.*, Bacterial partition complexes segregate within the volume of the nucleoid. *Nat. Commun.* **in press** (2016), doi:10.1038/ncomms12107.

13. G. Ball *et al.*, SIMcheck: a Toolbox for Successful Super-resolution Structured Illumination Microscopy. *Sci. Rep.* **5**, 15915 (2015).

14. A. Lal, C. Shan, P. Xi, Structured illumination microscopy image reconstruction algorithm. *IEEE J. Sel. Top. Quantum Electron.* **PP**, 1–1 (2016).

15. P. Křížek, T. Lukeš, M. Ovesný, K. Fliegel, G. M. Hagen, SIMToolbox : a MATLAB toolbox for structured illumination fluorescence microscopy OS-SIM e SR-SIM g MAP-SIM, 2–3 (2015).

16. M. Müller, V. Mönkemöller, S. Hennig, W. Hübner, T. Huser, Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ. *Nat.*

Commun. **7**, 10980 (2016).