UNDER THE LENS

The power of three spatial dimensions

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This month's Under the Lens discusses how three dimensional fluorescence microscopy techniques are furthering the study of dynamic spatial organisation and confinement in bacterial cells.

Fluorescence microscopy has become one of the workhorse tools for single-cell microbiology. Minimally perturbative, two dimensional (2D) imaging has enabled the study of biomolecules in the highly dynamic and crowded environment of the bacterial cytoplasm. Now, a new set of three dimensional (3D) techniques are shedding light on the internal dynamics and emerging structural organization of the bacterial cytoplasm.

Although axial sectioning techniques such as light-sheet microscopy have become routine in eukaryotic cells and tissues[1], these cannot be applied in bacteria as their size is comparable to the depth resolution. For bacteria, techniques are required that enable 3D reconstruction from either a few 2D images, or from one image which contains the axial information (point spread function shaping). Double helix point spread function (DH-PSF) microscopy engineers the image of a single molecule in the back focal plane such that one image contains positional information to super-resolve the three spatial dimensions. Instead of a symmetric image for each molecule (as in 2D fluorescence microscopy) the light spot becomes two lobes; the centre of the lobes gives the lateral position and the orientation of the centre line between the lobes provides the axial position.

Bayas *et al.*[2] used the photoblinking enhanced yellow fluorescent protein and DH-PSF to study the spatial organisation and dynamics of RNase E (RNA degradosome) and ribosomes in *Caulobacter crescentus*. 3D trajectories with a mean duration of 7 frames (175 ms) revealed that the diffusion coefficients of the ribosomal L1 protein and RNase E increased in transcription-blocked live cells. This agrees with fixed cell 3D localisation microscopy, which showed decreased clustering of the ribosomal L1 protein and RNase E when transcription was blocked. The researchers found that in *C. crescentus*, ribosomes are cytoplasmically clustered and that RNase E is clustered along the central cell axis (with cluster number varying throughout the cell cycle). This could not have been conclusively determined from 2D images. Dual-colour microscopy showed that RNase E colocalises with highly transcribed loci, suggesting that in *C. crescentus*, degradosome processing of rRNA occurs at the synthesis site.

3D imaging is important for studying the spatial organisation of both single molecules and cellular structures, with the bacterial chromosome being a structure of particular interest[3]. Wu *et al.*[4] used 3D and 2D structured illumination microscopy (SIM) to image the toroidal geometry of the *Escherichia coli* chromosome in widened cells. In 3D SIM, three images with striped illumination at different angles are captured at each axial position to produce a 3D super-resolved image.

The narrow diameter of *E. coli* confines the chromosome too tightly for imaging, but the cells were widened using the MreB inhibitor A22. By additionally using the dnaC2(ts) allele to prevent replication above 40˚C, the researchers generated cells with one chromosome. 3D imaging revealed that the DNA density (measured by fluorescence intensity) was heterogeneous and dynamic around the chromosome circumference, forming 3–8 domains. The DNA density between different fluorescently labelled chromosomal loci along the 3D DNA contour showed that some regions were consistently formed into domain boundaries by MatP (a protein involved in spatial organization of the bacterial chromosome terminus region during the cell cycle), which could not have been identified from 2D images.

In these studies, 3D microscopy was essential to remove ambiguities introduced by 2D projection images. 3D imaging techniques will allow a more detailed understanding of the biological mechanisms, spatiotemporal organisation and confinement of biomolecules in live bacterial cells.

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Competing interests

The authors declare no competing interests.