A comparison of super-resolution microscopy approaches to undertake deep learning-based 3D shape analysis of *Orientia tsutsugamushi* bacteria inside mammalian cells

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Abstract

Orientia tsutsugamushi is an obligate intracellular bacterium that causes the life-threatening vector-borne human disease scrub typhus. This affects at least one million people per year, but our understanding of the fundamental biology of the bacterium lags other pathogens of similar severity and prevalence. One of the reasons for this neglect is the technical difficulties associated with working with this organism, which include it being a BLS3 level organism and a fastidious, obligate intracellular bacterium. Microscopy-based approaches are essential for understanding the fundamental life cycle of the bacterium, including its growth and division, mechanisms of motility, and mechanisms of host cell egress. Confocal microscopy is commonly used to image *Orientia tsutsugamushi* but this suffers from an inability to resolve single bacteria when they are present in tightly-packed clumps inside mammalian cultured cells (e.g., Atwal et al., 2022).

Here we compared five super-resolution microscopy modalities with each other and with conventional confocal microscopy imaging, to identify the most suitable approach to investigate these specific samples. The microscopy techniques compared were:

- Confocal microscopy using a Zeiss LSM 880 system (“Confocal”);
- Airyscan confocal microscopy using a Zeiss LSM 880 with an Airyscan 2 detector (“Airyscan”);
- 3D-Structured Illumination Microscopy using an Applied Precision/Leica OMX microscope (“3D-SIM” or “OMX”);
- InstantSIM using a Visitech Vt-iSIM system (“iSIM”);
- Stimulated Emission Depletion microscopy using an Abberior Facility Line system (“STED”);
- Stochastic Optical Reconstruction Microscopy using a homebuilt TIRF system based upon a Nikon stand (“STORM”).

Bacteria were labelled using a monoclonal antibody against the autotransporter surface protein ScaA, followed by a secondary antibody conjugated to fluorochromes optimal for each microscope modality, in order to give the highest possible resolution. The resolution achieved by each system was compared in 2D using Full Width Half Maximum (FWHM) measurements across the bacterial membrane labelling. XZ and YZ planes were also examined to establish which techniques would provide sufficient resolution to permit successful 3D segmentation.

While all super-resolution modalities but STORM could resolve the bacterial outlines near the periphery of the clumps, and the consistently best FWHM measurements in 2D were achieved using the OMX 3D-SIM system, only the STED instrument operated in 3D-STED mode proved capable of providing sufficient resolution throughout the Z-axis to permit subsequent 3D segmentation and bacterial shape analysis. An image analysis pipeline based on the use of a deep learning 3D segmentation method called Cellpose (Stringer et al., 2021) allowed the volume, sphericity and ellipticity of the bacteria to be compared when grown in 2 different mammalian cell lines, HeLa and HUVEC. This method provided sufficient sensitivity to distinguish subtle but statistically significant changes in bacterial shape, depending on the host cell line, though these differences were not apparent from visual examination of the images. We propose that a combination of 3D-STED imaging, using the Abberior microscope system, with the image analysis pipeline presented here, can be readily applied by other researchers investigating the biological properties of intracellular bacteria.

References

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