Characterizing bacterial infection in epithelial cells at a single-molecule level

Summary

The Nanoimager platform allows visualization and quantification of bacterial infection and host-pathogen interactions at a single-molecule level. This type of research supports assessment of:

- Infection mechanism
- Bacterial propagation within the host
- Host-pathogen interactions
- New diagnostic tools and therapeutic solutions
- Vaccine research and development

Challenge

Some pathogens such as *Salmonella*, *Legionella* or *Mycobacteria* have evolved to evade host detection and replicate inside the infected cells. *Legionella*, which causes about 5% of all pneumonia, avoids degradation by remodeling the phagosome into an ER-like compartment called the Legionella-Containing Vacuole (LCV). Understanding infection mechanisms, through characterizing the subcellular distributions of bacterial protein and points of contact with the host, requires higher resolution than widefield fluorescence or confocal microscopy. dSTORM imaging provides 20nm resolution and single-molecule sensitivity, making it a great tool to detect pathogens and infectious agents that modulate the host.

Working with pathogenic species under stringent sterile conditions (i.e. GMP labs) limits research to confined biosafety cabinets. The Nanoimager, with its compact A4 footprint and unique design, does not require a darkroom or optical table to operate, which makes it a strong choice for BSL3 safety cabinets and BSL4 facilities.

Results

Although sheltered from the host, *Legionella pneumophila* cells within the LCV are able to release molecules into the infected mammalian cell and replicate inside it. The process is mediated through the secretion system (T4SS) that translocates over 300 virulence factors, to manipulate host processes, including host transcription, translation and vesicle trafficking (Figure 1). The T4SS is restricted to both poles of the bacteria and this polarity is essential for *Legionella* virulence.

Two-color dSTORM imaging of lung epithelial cells infected with *Legionella* can distinguish between the effector molecules localized at the vacuolar membrane and those at the bacteria surface with nanometer scale precision. Through quantifying the spatial distribution of the effector molecules (such as PieE), the mechanism of infection is revealed in unprecedented detail.

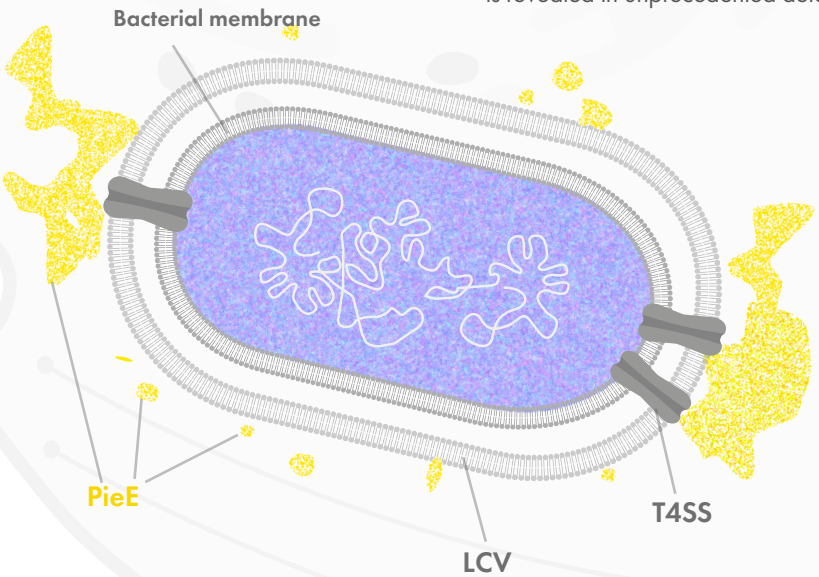

FIGURE 1
Schematic representation of a *Legionella*-containing vacuole (LCV), a cellular phagosome remodelled into an ER-like compartment. Illustration was adapted from G.N.Schroeder, Front. Cell. Infect. Microbiol., 05 January 2018 link: https://doi.org/10.3389/fcimb.2017.00528

In the presented example, *Legionella* was stained with an anti-LPS-AF647. The PieE effector protein, translocated by the T4SS, was immunolabeled with AF488 (Figure 2). The distribution of PieE (yellow) and bacteria (blue) inside the epithelial cells indicates that, in the early infection stage, not all bacterial cells are exporting PieE into the host cytoplasm (compare upper and lower bacterial cells in Figure 2B). Spatial distribution of PieE (Figure 2C) demonstrates that the majority of the protein localizes at the poles of the LCV, in accordance with the polar location of the T4SS complexes.

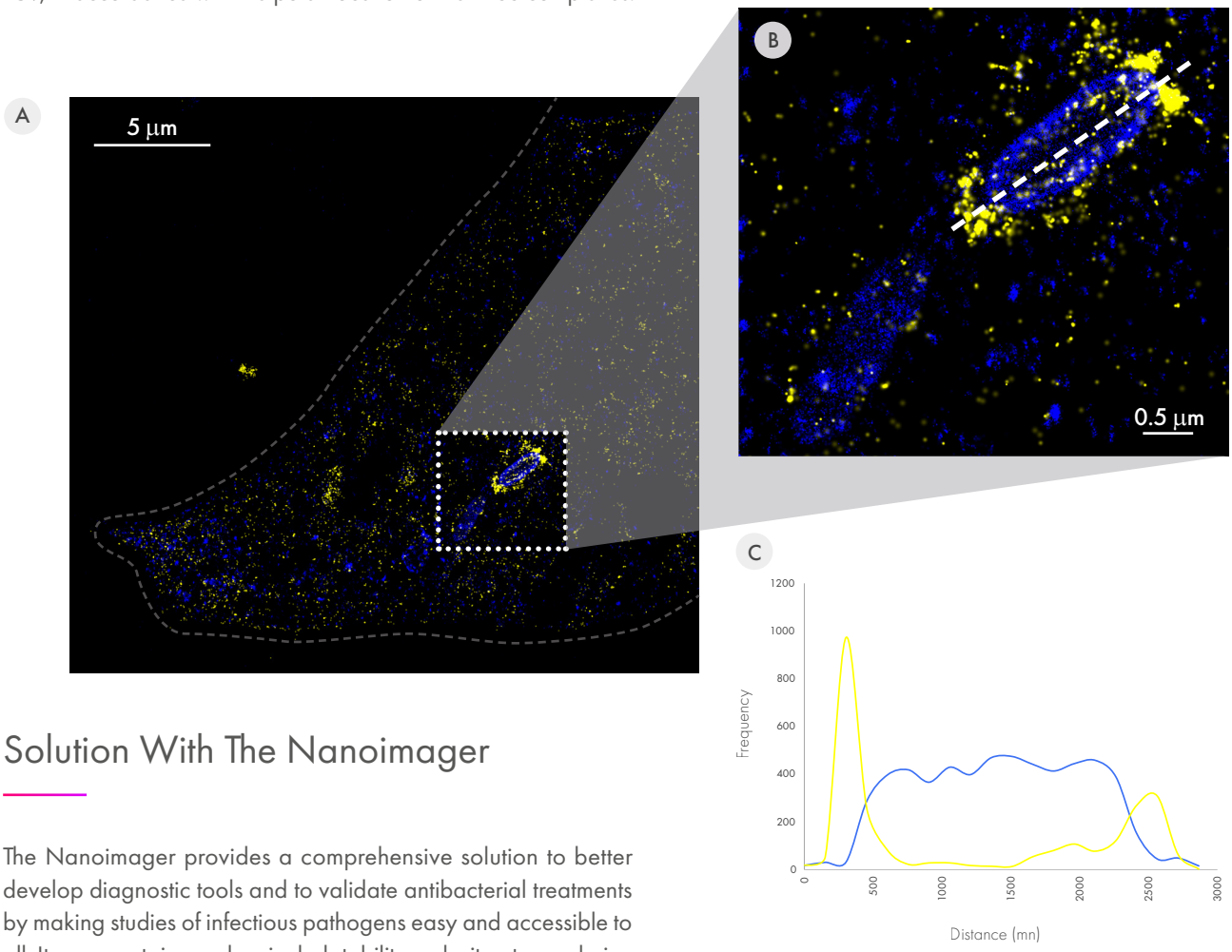

FIGURE 2

dSTORM image of lung epithelial cell infected with *Legionella*. (A) Two bacterial cells (blue, inside the dotted frame) were captured during infection of the host epithelial cell (dashed line); blue background signal in the cell corresponds to non-specific staining (B) The effector PieE protein (yellow) appears to be produced and exported only by the upper bacterial cell. (C) PieE effector spatial organization (yellow) in reference to the longitudinal axis (blue line) of the upper bacterial cell from panel B (the dashed line). Sample prepared by Dr. Gunnar Neels Schroeder's lab, Queen's University Belfast, Ireland.

Solution With The Nanoimager

The Nanoimager provides a comprehensive solution to better develop diagnostic tools and to validate antibacterial treatments by making studies of infectious pathogens easy and accessible to all. Its compact size and unrivaled stability make it a strong choice for BSL3 safety cabinets and BSL4 facilities. It is compatible with both live and fixed cell samples to image infection progression and spatial distribution of key molecules involved in host-pathogen interaction.

To learn more about the microscope features and its different applications at ONI visit **www.oni.bio**.

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