UNDER THE LENS

The expanding horizons of host–microorganism imaging are clear to see

Patrick G. Inns and Gideon Mamou

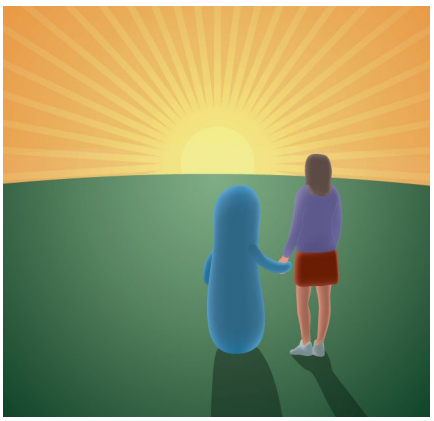
This month's Under the Lens explores how recent developments in sample preparation are aiding and advancing the imaging of host–microorganism interactions.

The populations of microorganisms living on and within the human body are intimately linked with health. Direct imaging of host–microorganism interactions is a key tool in understanding both the biogeography of microbial populations at a single-cell level and for providing insights into how certain species cause or contribute to disease. Two recent studies have used newly developed sample preparation techniques to image the interaction of *Chlamydia trachomatis* and *Helicobacter pylori* with their hosts in substantial detail[1,2].

Kunz et al. used a technique called expansion microscopy (ExM) to investigate the secreted effectors and developmental forms of the pathogen *C. trachomatis*[1,3]. *C. trachomatis* is the most common cause of infectious blindness and the most common sexually transmitted bacterium in humans. ExM involves setting a polyacrylamide gel matrix within a fixed sample, which can then be expanded in an isotropic fashion by dialysis in water, after digestion by a protease. Fluorescent labels fixed to this gel matrix expand with the sample, allowing higher resolution images to be captured owing to the increase in spatial separation of the fluorophores[3]. Kunz et al. achieved a fourfold expansion of *C. trachomatis*-infected HeLa cells, showing that the chlamydial protease, CPAF, is secreted at greater concentrations at later time points during infection. The authors were also able to image the accumulation of a second chlamydial effector protein, Cdu1, at the membrane of the *C. trachomatis*-containing vacuole (the inclusion membrane). Using conventional confocal microscopy, the authors showed that the two developmental forms of *C. trachomatis* (the small elementary bodies and larger reticulate bodies) could not be clearly distinguished by size; however, after expansion, these forms could be unambiguously differentiated[1], demonstrating the improvement that ExM provides over conventional confocal microscopy.

Studying host–microorganism interactions in thick tissues is a difficult task, as exemplified by techniques such as rotating wall vessel bioreactors, developed to recreate 3D tissues on small scales[4]; however, by rendering tissues transparent through a technique called optical clearing, imaging in thick tissue samples becomes possible. Yang et al. developed an optical clearing technique called passive CLARITY technique (PACT)[5]. PACT relies on multiple sample preparation stages to render tissues transparent: crosslinking and fixing using a hydrogel; removal of lipoproteins using ionic detergents; and refractive index matching for microscopy. After these steps, the sample can be fluorescently labelled. In their study, Yang et al. integrate several optical clearing techniques and optimize the reagents used to accelerate the clearing of tissues and render them more accessible to fluorescent labels. Using quantitative 3D confocal microscopy and PACT, Fung et al.[2] investigated the colonization of *H. pylori* in murine stomach tissue. In humans, chronic *H. pylori* infections represent the most significant risk factor for the development of peptic ulcers and stomach cancer. The authors imaged intact murine stomachs and constructed detailed 3D maps of *H. pylori* microcolonies within more than 12,000 gastric glands. Together with imaging longitudinal sections of the stomach, this comprehensive analysis revealed the kinetics of gastric gland infection by *H. pylori* and showed that bacterial microcolonies within these glands serve as a reservoir for chronic infection[2].

In summary, the unprecedented details revealed by these microscopy studies were made possible by innovations in sample preparation techniques. The potential of these techniques is substantial. ExM provides a way for researchers who do not have access to sophisticated superresolution microscopes to capture images of equitable detail on conventional systems, using common laboratory reagents. PACT is a scalable technique[5] and therefore has great potential to be applied to human tissues.



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Patrick G. Inns and Gideon Mamou**
Department of Biochemistry, University of Oxford, Oxford, UK.
**e-mail: underthelens@bioch.ox.ac.uk*

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Competing interests
The authors declare no competing interests.