UNDER THE LENS

Nanaerobic imaging breathes new life into gut microbiota microscopy

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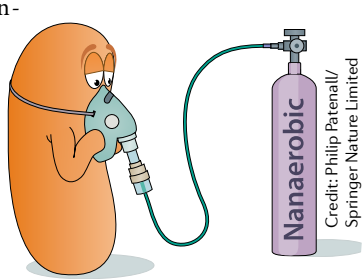
This month's Under the Lens discusses a recent advance in the imaging of anaerobic gut bacteria using nanaerobic growth conditions.

Fluorescent proteins have revolutionized microbiology by allowing real-time visualization of living cells and their constituent parts. However, to be studied under the microscope, the chromophores of conventional fluorescent proteins require oxygen to mature from a non-fluorescent into a fluorescent state. This requirement poses a major constraint on microbiota research, as it limits the study of the physiology and function of anaerobic gut microorganisms.

To tackle this problem, researchers have created oxygen-independent fluorescent proteins for live-cell imaging of anaerobic bacteria. Fluorescent reporters such as UnaG show promise but have yet to be widely adopted, as the dyes are often dim with low signal-to-noise ratios, or require the addition of ligands that can be partially cell-impermeable[1]. Other researchers have forgone live-cell imaging altogether and use endpoint measurements to bring samples into oxygen, allowing the conventional fluorescent proteins to mature[2].

In recent work, García-Bayona et al.[3] solve this dilemma by discovering that oxygen concentrations of 0.10–0.14% — termed nanaerobic — are low enough for certain 'anaerobic' gut-derived microorganisms to thrive and high enough for conventional fluorescent proteins to mature. Because of this, the authors could image physiological processes within one of the most abundant genera of bacteria in the human intestine, the *Bacteroides*. Importantly, rather than being artificial, nanaerobic growth is expected to be a relevant lifestyle approximation for bacteria living along the gut epithelial–mucus interface, where low levels of unconsumed oxygen from host epithelia diffuse into the lumen. The authors not only confirmed that a nanaerobic environment triggers no bacterial stress response and allows for normal growth of different *Bacteroides* species, but also confirmed that these bacteria can use such small quantities of oxygen in an alternative respiration pathway. Further, the authors demonstrated that under nanaerobic conditions, these cells can perform energy-intensive tasks such as operating their type VI secretion systems (T6SS) to kill competitor cells. Motivated by the bright fluorescence of GFP, the authors screened red fluorescent proteins and found that mKate2 displays a similarly bright signal in cells grown under nanaerobic conditions. For ease of use, a custom chamber was used to seal bacteria on agar pads, allowing maintenance of nanaerobic conditions while performing live-cell imaging on a conventional microscope.

Using this assembled setup, García-Bayona et al. also demonstrated the general applicability of nanaerobic imaging by investigating subcellular processes in *Bacteroides* species. Firstly, the authors linked GFP to two proteins both previously reported to be secreted in outer membrane vesicles (OMVs) and followed their localization. Nanaerobic imaging showed that the assayed proteins — an inulin lyase and a MACPF domain antimicrobial toxin — localized to the cell membrane and OMVs, and also allowed tracking of OMV vesiculation events in live *Bacteroides* cells. Secondly, the authors investigated the kinetics of T6SS in *Bacteroides fragilis*. To do this, they fused GFP with TssB (a sheath protein of the T6SS), which, importantly, did not interfere with T6SS function. Using the time-lapse imaging compatibility of nanaerobic growth, this reporter construct allowed the authors to study the kinetics of T6SS sheath assembly, contraction and disassembly processes, which are important for the persistence of *B. fragilis* within the human gut.



Credit: Philip Patenall/ Springer Nature Limited

Finally, the authors searched the genomes of gut bacteria and found Cytochrome *bd* oxidase (a marker for the ability to grow under low oxygen concentrations) in 46.2% of queried strains, thus identifying further species suitable for nanaerobic imaging. However, many gut commensals cannot tolerate even low concentrations of oxygen and therefore cannot be imaged under nanaerobic conditions.

In summary, nanaerobic imaging represents a new method to investigate the physiology of living gut microorganisms by fluorescence microscopy of conventional fluorescent proteins. Therefore, this method has high potential to be transformative in our understanding of previously unseen processes in intestinal bacteria increasingly considered crucial to our health.

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1. Chia, H. E. et al. Extending fluorescence microscopy into anaerobic environments. *Curr. Opin. Chem. Biol.* **51**, 98–104 (2019).
2. Whitaker, W. R. et al. Tunable expression tools enable single-cell strain distinction in the gut microbiome. *Cell* **169**, 538–546 (2017).
3. García-Bayona, L. et al. Nanaerobic growth enables direct visualization of dynamic cellular processes in human gut symbionts. *Proc. Natl Acad. Sci. USA* **117**, 24484–24493 (2020).