UNDER THE LENS

Light sheets unveil host–microorganism interactions

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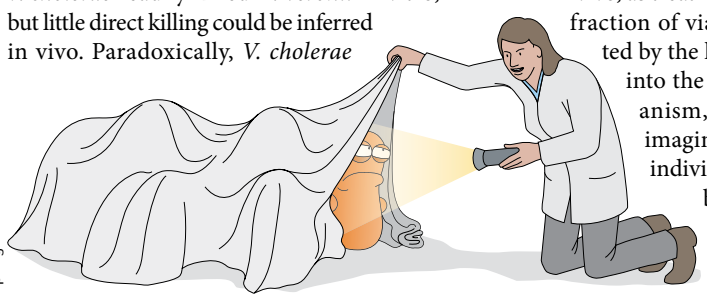
This month's Under the Lens discusses the application of light sheet fluorescence microscopy to observe the intestinal microbiota of live zebrafish, revealing unexpected host–microorganism interactions.

In light sheet fluorescence microscopy (LSFM), a laser beam is reshaped into a thin sheet that simultaneously illuminates an entire, but specific, optical plane of a sample. LSFM enables much faster 3D imaging of samples than confocal microscopy, while capturing a large field of view and causing low phototoxicity to samples, owing to short exposures[1]. These advantages make LSFM capable of imaging fluorescently tagged bacteria within a live organism; in small vertebrates such as zebrafish larvae, imaging can even span the entire (>1 mm) intestinal tract, offering unprecedented insights into interactions between living hosts and their intestinal bacteria.

Logan et al.[2] used LSFM to examine interactions between the zebrafish gut symbiont *Aeromonas veronii* and pathogenic *Vibrio cholerae*. Armed with toxin-injecting type VI secretion systems (T6SSs), *V. cholerae* might be expected to invade zebrafish guts by simply killing off *A. veronii* competitors. Indeed, *V. cholerae* readily killed *A. veronii* in vitro, but little direct killing could be inferred in vivo. Paradoxically, *V. cholerae* invaders still required an active T6SS to eliminate *A. veronii* from the zebrafish gut. To resolve this paradox, LSFM was employed to track labelled *A. veronii* populations across the entire zebrafish intestine, while differential interference contrast microscopy (DICM) was used to image unlabelled intestinal tissue. In combination, these complementary techniques allowed simultaneous tracking of the host's intestinal contractions and their impact on the gut microbiota. This revealed that, by injecting hosts with an actin crosslinking toxin, *V. cholerae* increased the strength of the host's intestinal contractions, purging the gut of *A. veronii*. LSFM therefore had a vital role in establishing that *V. cholerae* pathogens can invade zebrafish gut communities through manipulation of host behaviour, instead of through direct competitor killing.

More recently, Schlomann, Wiles et al.[3] used LSFM to study how zebrafish gut microorganisms respond to small doses of antibiotics. Surprisingly, sub-lethal ciprofloxacin exposure depleted intestinal populations of *V. cholerae* or *Enterobacter cloacae* to a greater extent than in liquid culture, suggesting that the zebrafish host somehow potentiates antibiotic treatment. This effect could not be attributed to increased bacterial killing in vivo, as treatment did not alter the fraction of viable bacteria excreted by the host. To gain insight into the potentiation mechanism, high-speed LSFM imaging was used to track individual and aggregated bacteria across the entire volume of the gut, over extended time periods. Unexpectedly, this revealed that the antibiotic treatment caused bacteria to clump together following an SOS-induced biofilm response, making them more susceptible to expulsion through gut peristalsis. By tracking microbiota dynamics within living hosts, LSFM imaging showed that bacterial defence mechanisms can prove counterproductive in the gut, such that even small antibiotic doses can cause a collapse of the microbiota.



Credit: Philip Patenall/ Springer Nature Limited

Host–microorganism interactions are of immense importance in many diseases and for the host's response to treatment. However, examining these interactions directly is technically challenging: how does one image both host and microbiota when they operate on vastly different length and time scales? These studies highlight how LSFM overcomes this problem, enabling cell-resolution imaging of bacteria over the entire length of the gut, over multiple hours, within living zebrafish hosts. The resulting insights emphasize the crucial — and often unexpected — roles that hosts can have in modulating interactions between gut microbiota and their environment.

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Competing interests
The authors declare no competing interests.