UNDER THE LENS

A clearer picture of microbial biogeography

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This month's Under the Lens discusses recent advances in spatial metabolomics and light-sheet microscopy for imaging the biogeography of host–microbiota interactions.

Interspecies cooperation both at macroscopic and microscopic levels drives species survival in a constantly evolving world. A thorough understanding of host–microbiota interactions is key to differentiating between beneficial and harmful interactions and their contribution to evolution and pathogenesis. The inability to culture many symbiotic microorganisms in vitro underlines the need to discover new technologies to investigate these interactions in situ. In this Under the Lens article, we describe recent advances in visualizing host–microbiota interactions.

In a recent study, Geier et al. demonstrate metaFISH, a spatial metabolomics imaging and analysis pipeline that combines atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-MALDI-MSI) and fluorescence in situ hybridization (FISH)¹. The pipeline can be applied directly to the same animal sample, creating highly resolved abundance maps of metabolites to spatially resolve microbiota and host tissue regions. Spatial metabolomics imaging is performed first, removing only 2–3 µm of 10 µm thick samples. The remaining tissue is then fixed, and 16S ribosomal RNA genes are fluorescently stained to distinguish host and bacterial DNA using confocal laser scanning microscopy. MetaFISH was applied to study symbiosis between the ocean mussel *Bathymodiolus puteoserpentis* and its two endosymbionts, a sulfur oxidizing (SOX) bacterium and a methane oxidizing (MOX) bacterium. AP-MALDI-MSI has a spatial resolution of 3 µm, which is sufficient to image eukaryotic cells but not the endosymbionts. By correlating FISH imaging with MSI, spatial metabolome clusters of metabolites could be assigned to either host only or

endosymbiont-containing tissue. Single pixel MSI resolution of bacterial communities was achieved with this correlation, which would have otherwise been disregarded as noise. Mapping of lipid membrane components showed phenotypic adaption of the MOX bacteria to the host oxygen concentration through the modification of a single hydroxyl group. By applying the metaFISH protocol to 11 other species of ocean mussel, which also harbour SOX or MOX endosymbionts, five specialized metabolites were discovered that directly correlate with MOX endosymbiont–*B. puteoserpentis* symbiosis. This shows the immense potential for discovering host–microbiota metabolic interactions without the need for in vitro culturing.

Another advance in the in situ study of host–microbiota interactions was shown by Amich et al. using three-dimensional light-sheet fluorescence microscopy (3D-LSFM) to visualize the growth of fungal pathogen *Aspergillus fumigatus* in the intact lungs of mice². Traditional methods to study disease progression require immunomodulation of the mice prior to intranasal infection with fungal spores, combined with detection of fungal growth using RT-PCR and flow cytometry. 3D-LSFM is far more effective than PCR at detecting and quantifying fungal infection, with abundant growth detected after only 16 hours. Modulation of the mouse immune system using cortisone or a combination of cyclophosphamide and cortisone or irradiation showed that fungal growth is most aggressive in mice immunosuppressed with the cyclophosphamide–cortisone regimen. The immune responses from alveolar macrophages and polymorphonuclear leukocytes (PMNs) are crucial to regulate fungal growth in the lungs. 3D-LSFM imaging of alveolar macrophage and PMN recruitment during fungal growth revealed that all the immunosuppressive regimens drastically decreased both the number and function of alveolar macrophages and PMNs, with

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cyclophosphamide–cortisone treatment severely inhibiting the ability of PMNs to engulf fungal spores, leading to extensive infection. Taken together, 3D-LSFM is an effective tool for simultaneously detecting and measuring microbial growth and the host immune response.

In sum, these studies highlight the technical advances in combining MSI with FISH and in 3D-LSFM to track, detect and differentiate between host and microorganism growth and their metabolism.

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Competing interests

The authors declare no competing interests.