

Quentin Sattentau, PhD
The University of Oxford, Oxford, UK

Q: How has the field of microbiology changed over the past 25 years?

A: Imaging – seeing is believing – and appreciating!

Microbiology is by definition the science of organisms too small to be observed by the naked eye, and so we have always relied upon microscopy to observe and understand microbes. Huge progress has been made since the first light microscopic observations of bacteria and protozoa made by Van Leeuwenhoek in the 17th century. Although viruses were discovered in the late 19th century using filters too small for bacteria to pass, it was the advent of electron microscopy in the 20th century that allowed their visualization.

Electron microscopy (EM) has been central to viral identification and classification, and has seen a quantum leap in the form of cryo-EM. The 2017 Nobel Prize in Chemistry was awarded for work on cryo-EM and this technique is leading the way in describing the organization of macromolecules, many microbial, at atomic or near-atomic resolution. Cryo-EM has advantages over crystallography including absence of requirement for a crystal lattice, the ability to extract structural information from complexes *in situ* such as embedded in membranes, and a predilection for large, often hard to crystallise molecular complexes. Cryo-EM tomography and single particle analysis have already made essential contributions in many areas of microbiology including high-resolution structures of bacterial ribosomes, chemoreceptors and secretion complexes, non-enveloped and enveloped viral architecture, and parasite host cell invasion machines and drug targets. A specific example in my own field has been the amazing breakthrough in understanding the structure and function of the HIV-1 envelope glycoproteins, and its implications for antibody-based vaccine design.

Over the same period light microscopy technologies have been developed that break the diffraction limit of light to yield 'super-resolution'. This again resulted in a Nobel Prize in Chemistry in 2014, and has allowed us to overcome the challenge of imaging both cells (about 10 μm) and microbes (from ~20 nm for some viruses to ~50 μm for some protozoa) using light microscopes. Super-resolution microscopy has revealed exciting new details such as virus–receptor interactions at cell surfaces and changes in cellular organization and architecture during pathogen invasion. Although with lower resolution, two- and multi-photon fluorescence intravital microscopy has revolutionized our understanding of events taking place *in vivo*, since this technology allows light to penetrate tissue and report back on dynamic events, without substantially perturbing the system. Using this approach we have been able to visualize the behaviour of cells and microbes within living tissues, often within the intact host, revolutionizing our understanding of microbial pathogenesis and immune responses to pathogens.

What does the future hold for imaging in microbiology? Cryo-EM will yield higher resolution structures of more and more complex microbial machines facilitating their targeting with drugs and vaccines, intravital microscopy will allow us to better understand host–microbe interactions under the most physiological conditions, and super-resolution microscopy will fill in the gaps between these technologies to allow detailed descriptions of host–pathogen interactions. The future for microbiology looks colorful, detailed and bright!