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Editorial overview: The new microscopy

Ariane Briegel and Stephan Uphoff

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Ariane Briegel

Department of Biology, Leiden University, 2333 BE Leiden, Netherlands
e-mail: a.briegel@biology.leidenuniv.nl

Ariane Briegel is a professor at the Leiden University (The Netherlands). She has over 15 years of experience using cryo-electron microscopy to study bacterial and archaeal ultrastructure. The Briegel laboratory focuses on investigating how microbes sense and respond to their environment. In order to gain insight into the structure and function of the molecular complexes involved in these behaviors, the lab uses electron cryotomography and correlative microscopy methods.

Stephan Uphoff

Department of Biochemistry, University of Oxford, Parks Road, Oxford OX1 3QU, UK
e-mail: stephan.uphoff@bioch.ox.ac.uk

From the time Antoni van Leeuwenhoek described the 'animalcules' using a simple single lens light microscope, to today's high-powered multi-million dollar instruments, we continue to rely on microscopy to gain insights into structure and function of microbial life.

Much of our current understanding on essential processes for survival of microbes, such as establishing viral and cellular architecture, growth, proliferation, motility or the formation of complex communities in biofilms, stems from studies utilizing microscopy. The quest to understand life at the molecular level relies on high-resolution imaging, minimally invasive sample preparation and the ability to observe living processes over time. To provide the necessary tools to understand molecular structures and their function in cells, many microscopy techniques were innovated in the past decades. The importance of these new methods was recognized with several recent Nobel Prizes, such as for the discovery and development of the green fluorescent protein (GFP) that allows the spatial and temporal observation of proteins in living cells, tissues or organisms (Osamu Shimomura, Martin Chalfie and Roger Y Tsien, Nobel Prize for Chemistry, 2008). The development of super-resolution microscopy overcomes the resolution limit of the wavelength of light and provides molecule localization information with an accuracy down to a few nanometers (Eric Betzig, Stefan W Hell and William E Moerner, Nobel Prize for Chemistry, 2014). Most recently, the 2017 Nobel Prize for Chemistry was awarded for the development of cryogenic electron microscopy, which allows imaging intact biological structures with atomic resolution (Jacques Dubochet, Joachim Frank and Richard Henderson).

The beauty of microscopy lies in the direct interpretability of the images. The old idiom 'A picture is worth a thousand words' holds true, as the many contributions to answering microbiological questions demonstrate. Both conventional fluorescence and electron microscopy have contributed greatly to our current understanding of cellular architecture and both methods are still invaluable tools for microbiologists today. However, artifacts due to sample preparation, alteration of the native protein behavior due to labels, and mistakes in applying image-processing procedures gave rise to persistent erroneous models of microbial life and ultimately damaged the trust in microscopic studies. For example, artifacts introduced by harsh fixation methods to prepare samples for traditional electron microscopy studies resulted in folded invaginations of the inner membrane. These structures were termed 'mesosomes' and were implied to play a role in several cellular processes [1,2]. The majority of such observed membrane invaginations could later be clearly attributed to a sample preparation artifact, and the 'mesosomes' are not considered as a part of the bacterial cell morphology anymore [3–5]. But not only traditional transmission electron microscopy is susceptible to misinterpretations due to experimental artifacts. The advent of fluorescent tags (besides an impressive amount of deep insight into microbial biology), also resulted in the generation of false models. A prominent example is the architecture of the cytoskeletal protein MreB, which is a structural homolog to eukaryotic actin [6]. Early fluorescence light microscopy resulted in a model where MreB forms an extended and stable cytoskeletal filament bundle running along the inside of the plasma

Stephan Uphoff is a group leader at the Department of Biochemistry of the University of Oxford. His lab investigates mechanisms of DNA repair and mutagenesis in bacteria. In particular, the research addresses the role of stochastic events and cellular heterogeneity in bacterial genome dynamics. The experimental approach utilizes a combination of single-molecule imaging, super-resolution microscopy, and microfluidic single-cell analysis.

membrane in a helical pattern in *Bacillus subtilis* [7] and later other bacteria [8–11]. However, subsequent work revealed that MreB does not form such an extended structure, but instead forms short, dynamic filament bundles that treadmill around the cell body [12–14]. It was later shown that the fluorophore fused to MreB itself caused an aggregation artifact and resulted in the formation of the extended filaments [15], and these filaments are not seen in wildtype cells [16].

Another source of controversial results in microscopy stems from the improper application of image processing procedures. The publication of a 6 Å resolution cryoEM structure of the glycoprotein trimer on the HIV-1 virus envelope [17] caused a heated debate in the scientific community, and many leaders in the field strongly doubted the validity of this map. More specifically, they attribute the result to images of just noise, where the final structure was extracted due to a model bias from applying improper image processing methods [18].

Besides avoiding artifacts, microscopy faces multiple other challenges as a central tool for microbiological research: in order to answer our research questions, we constantly push the methodology to its limit. In this edition of *Current opinion in Microbiology* we have brought together scientists that are overcoming the limits of what is currently possible with microscopy. They demonstrate that the field of microscopy is thriving with the many developments in instrumentation, labels, sample handling, automation, image analysis, and data management. Not long after the first proof-of-principle studies by specialist labs, these methods are now driving mainstream research into the biology of microorganisms.

The new electron microscopy

Earl, Falconieri and Subramaniam give an overview on how cryoEM allows high-resolution structural analysis of a wide spectrum of macromolecular assemblies in prokaryotic and eukaryotic cells, as well as in viruses. It clearly illustrates the power and potential of this methodology for microbiological questions.

Achieving ever-higher resolutions and resolving smaller and smaller biological structures is not the only necessity to gain a deeper understanding of the structural basis of microbial life. High-resolution cryoEM methods such as single particle analysis (SPA) and electron cryotomography (ECT) are inherently limited by the sample size that can be analyzed. Even larger microbial cells are often too thick for meaningful analysis by ECT. However, the development of new imaging modalities such as cryo-scanning transmission electron tomography (CSTET), soft X-ray tomography (SXT) and serial surface imaging using focused ion beam-scanning electron microscopy (FIB-SEM) can overcome these inherent limitations. Elbaum gives a concise overview of the possibilities and limitations of each of these new imaging methods. Furthermore, Medeiros, Böck and Pilhofer illustrate how sample-thinning using cryo-focussed ion beam milling can render a too-thick specimen suitable for ECT analysis and allows for an in-depth study of bacterial ultrastructure inside their host.

Besides the impressive advances in developing the various flavors of electron microscopy, the pairing with other methods such as computer modeling or other imaging modalities have greatly expanded the applicability of EM to address a wide range of microbiological questions. Cassidy and colleagues illustrate how cryoEM data can be used to construct and refine high-fidelity atomistic models that provide insight into structure and