Biology across scales: from atomic processes to bacterial communities through the lens of the microscope

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Many human genetic diseases originate from single DNA mutations. These mutations can lead to functional changes at multiple scales. For instance, single amino-acid alterations can perturb enzymatic function, affect the subcellular localization of proteins or alter the stoichiometry and dynamics of multi-protein complexes. At the multicellular scale, single DNA mutations can also drive large transcriptional changes that dramatically affect cellular function, or that alter cell identity leading to changes in cell-cell interactions and in tissue function. Critically, addressing molecular processes at multiple scales requires tractable biological models amenable to genetic manipulation and displaying analogous multiscale properties as well as appropriate technologies.

In the past, bacteria have been often viewed as an archetypal unicellular model, where single cells behave independently and autonomously. However, bacterial communities have been recently recognized as multicellular organisms able to display emerging collective properties and spatial organization. Over the last decade, fluorescence and electron imaging technologies have seen revolutionary improvements in spatial resolution, labeling, throughput, imaging depth, and analysis capabilities. This special issue compiles a series of articles showcasing how novel imaging technologies can be applied to unveil new biology from the atomic to the community scales.

High-throughput, atomic-scale imaging. The development of direct electron detectors has considerably improved the resolution of Cryo-Electron Microscopy (Cryo-EM), reaching resolutions that are now comparable to those attainable by x-ray crystallography and nuclear magnetic resonance (NMR). An important advantage of Cryo-EM relies on its ability to restore the structures of large macromolecular machineries that could not be obtained by x-ray crystallography due to their size or constraints for their crystallization. In their review, de Valle and Innis (Herrero Del Valle and Innis 2020) show how combined Cryo-EM and 3D particle reconstruction algorithms open the path to a structurally-resolved view of the bacterial translation cycle, determining ribosome-protein interactions and transitions for every step. Because Cryo-EM studies do not require large amounts of biological materials, structural studies can be conducted at high throughput, for example to study how antibiotics affect ribosome function and perhaps to improve drug design based on ribosome-inhibitor structures (a process called structure-guided drug design).

Single-molecule imaging. A critical limitation of most structural methods (e.g. cryo-EM) is that they cannot detect single molecules in cells or perform measurements on living cells, thus limiting their ability to dissect dynamic states or study single-molecule dynamics. Two reviews in this issue (Rombouts and Nollmann 2020; Singh and Kenney 2020) show how single-molecule detection can be used to image processes as diverse as messenger RNA dynamics, intracellular signaling of proteins and virulence factor injection by bacterial pathogens. Rombouts and Nöllmann describe a wealth of sophisticated techniques that can be used to label and image single mRNA molecules in bacteria, particularly at high-throughputs and with multiplexing abilities. These methods will be central to determine whether mRNAs are mostly resident at the site of transcription due to transcription-translation coupling or whether, like in eukaryotic cells, the mRNAs are targeted to precise subcellular locations before the encoded protein is made.

Single Molecule Localization Microscopy (SMLM) has been increasingly used to localize and to track single molecules in live cells (Betzig *et al.* 2006; Rust, Bates and Zhuang 2006; Manley *et al.* 2008), In Singh and Kenney's review, we learn that SMLM revealed unsuspected new features of signaling proteins, from the dynamic assembly of chemoreceptor to potential physical linkages between bacterial membrane and DNA via signaling protein bridges. In addition, the authors illustrate the use of unnatural amino acids for SMLM-based labeling, which might be a solution to label protein with complex interaction pathways such as virulence proteins secreted by pathogens in host cells.

Dissection of single-cell heterogeneity. In the late 1990s, the first live single cell tracking experiments revealed that exponentially growing cells are not identical and that cell-to-cell variations exist, in some conditions, to the point that several distinct cell populations co-exist within a single cell culture. In their review, Lagage and Uphoff discuss the power of combined microfluidics and single cell studies to investigate stress-induced regulation dynamics and reveal the design of the underlying genetic pathways (Lagage and Uphoff 2020). These methods reveal critical properties that could not be identified in bulk cultures. Depending on the presence of noise or feedback loops (positive and negative) in the genetic circuits, gene expression may be pulsatile, delayed and even in some occasions anticipated, a mechanism whereby heterogeneity generated by fluctuations (or noise) can adapt a subpopulation of cells to an environmental change that is yet to come (bet-hedging). However, Lagage and Uphoff report on studies that make use of gene reporter fusions, potentially missing additional regulations which may occur at the mRNA level. As discussed by Rombouts and Nöllmann, a number of recent live RNA labelling methods are now available to directly count specific mRNA molecules in single cells and thus allow direct quantitative analyses of transcription in live cells.

Single-cell physiology. Combination of single-cell observation and microfluidics manipulation open new perspectives to address very old problems that have yet to find a molecular explanation. One of them, the so-called nutrient growth law (Vadia and Levin 2015), formulated that bacterial cell size increases exponentially with growth rate, a remarkable property that has tentatively been explained by a number of theoretical models. In their review, Meunier et al. explain how microfluidics-based single cell measurements over very large numbers can measure variance and small fluctuations, which has invalidated and refined existing models (Meunier, Cornet and Campos 2021). Given that molecular mechanisms can be directly observed by fluorescence, the route is now open to identify the molecular checkpoints that regulate cell size. This potential is further underscored by Joseph and Badrinarayanan as they describe how imaging low fidelity DNA polymerases, either when they are in contact with the replication machinery at replication forks or after the passage of the replication fork, clarified mechanisms of DNA repair lesions by synthesis (Joseph and Badrinarayanan 2020). Remarkably, these polymerases were observed to function independently from replication for the first time. For this special issue, we exceptionally invited one original research paper (which was reviewed with appropriate standards) to show how direct imaging of a new cellular process can be developed. Reuter et al. took advantage of the natural fluorescence of Tetracycline to image its import into single bacterial cells, showing that its intracellular accumulation is a balance between uptake and efflux in single cells (Reuter *et al.* 2020). Combined imaging of the TetA influx pump, demonstrated that, as expected, TetA expression leads to efflux in a dose-dependent manner. Thus, the cellular response to Tetracycline can now be observed at single cell levels, opening new perspectives for quantitative analyses. For instance, the authors observe that under repression, basal levels

of TetA are nevertheless variable (perhaps due to promoter noise as discussed by Lagage and Uphoff), which likely dictates differential efflux responses across cells.

Towards multicellular communities. In specific ecological niches, such as the forest floor or the gut, cell-cell interactions within bacterial communities (e.g. biofilm) can yield emergent collective properties such as: enhanced resistance to stresses, enhanced environmental colonization, division of labor, and as Singh and Kenney suggest, enhanced survival in the host. Several new methods have recently been developed to enable the imaging of biofilms at different scales. For instance, SMLM was applied to image the spatial 3D structures of the *Vibrio cholerae* biofilm exopolysaccharide matrix. However understanding microbial community dynamics will require powerful computational tools to connect specific cellular processes to the larger spatial organization of cells within the complex biofilm structure. In their review, Jeckel and Drescher flesh out the limitations of traditional intensity-based cell segmentation procedures and suggest that machine learning-based approaches and in particular Convolutional Neural Networks (CNN) may provide powerful alternatives to segment bacterial cells at high throughput and with high accuracy in dense populations (Jeckel and Drescher 2020). Even when single-cell resolution cannot be achieved, the complex architecture of bacterial biofilms can now be analyzed by a method called cube-cytometry, which segments the biofilm 3D volumes in bricks allowing the profiling of multiple parameters and thus testing their correlation in space.

Conclusions. Molecular and cell biology in bacteria has been deeply impacted by the recent developments in quantitative imaging. The reviews compiled in this special issue illustrate how improvements in detectors and optics, genetic and chemical probes, computational power, and artificial intelligence are now breaking major barriers towards multi-scale analysis of complex multicellular specimens. Uniquely, these novel quantitative methods should enable detection of protein organization and biochemical events within cells, as well as observation of cell identity, behavior and spatial dynamics in the context of native communities. For example, the emergence of combinatorial RNA imaging methods able to quantify and spatially localize hundreds of messenger RNAs in single cells will soon enable the determination of complete transcriptional programs spatially within a biofilm. A major challenge will be to design, build, and deploy creative computational pipelines to analyze, extract and synthesize the large amounts of information that these new technologies generate. It has never been more exciting to study the bacterial cell.

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