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Editorial overview: Advances and future prospects of molecular imaging for studying and quantifying biological processes

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Philipp Kukura is Professor of Chemistry at the University of Oxford and Official Fellow for Physical Chemistry at Exeter College. His research focuses on the use of light scattering to visualise and quantify the energetics and kinetics of biomolecular interactions all the way from small molecules to mesoscopic structures at the single molecule level. Central to these efforts is the universality of light scattering, making it applicable to all forms of matter and its close correlation with mass, which provides direct information on molecular identity.

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Sua Myong is an Associate Professor of Biophysics Department at Johns Hopkins University. Her research focuses on developing and applying single molecule techniques to studies of nucleic acid–protein interactions involved in the central dogma of biology and human diseases. Her lab currently investigates the role of G-quadruplex in gene expression, oxidative damages in telomere processes and the molecular mechanism underlying liquid–liquid phase separation implicated in ALS/FTD.

Almost exactly 60 years ago, Richard Feynman thought about the benefits of everything small: seeing small, writing small, manipulating small. In the context of seeing, Feynman focused on the electron microscope, making the point that the laws of physics allowed for a microscope that can produce images with atomic resolution — so why don't we have it? Sure, there were theorems that supposedly proved that the electron microscope cannot be better than it was at the time, but Feynman's point was: these theorems were based on assumptions, and why should those assumptions be non-negotiable? If they were wrong, we could build a better electron microscope, one that works much closer to how well it could work, rather than just accepting that it isn't good enough.

Fast forward today, and how right he was. We are in the middle of the cryoEM revolution, where solving the structures of biomolecules seems to be more a question of bandwidth rather than experimental feasibility. We are almost used to seeing atoms, be it with electron or atomic force microscopes. We have witnessed the appearance of super-resolution fluorescence, where samples were prepared so as to empower the microscopes, only limited by the basic laws of physics, but we actually have found ways to do even better. Imaging is everywhere. Why? Of course, seeing is believing, and sometimes the only way to understand processes in nature. But there is an additional motivation: seeing is one of the most direct means by which we understand the world around us. It is one thing to explain to a child how to ride a bike — it is another thing for the child to see someone ride a bike.

As a result, we have, and continue to, direct huge efforts and resources to improve existing and develop new ways to see. Fortunately, the electron microscope is not the holy grail, providing answers to all our questions. We are not satisfied by structure alone, we want dynamics, chemical information, different environments, interactions, long observation times, statistics, so on and so forth. This issue of *Current Opinion of Chemical Biology*, contains 10 reviews representative of this need for breadth of information.

The ability to visualise single molecules is a significant challenge all by itself. For starters, the molecule has to stay in place for a sufficient amount of time so that it can be captured and interrogated. While some single molecule techniques provide information on freely diffusing species in solution, many rely on some form of immobilisation. Bespalova et al. review recent advances in passive trapping of single molecules in solution, which are unique in the

sense that they require no feedback to achieve three-dimensional trapping. Frustaci and Vollmer rely on traditional surface immobilisation, but use the unique temporal resolution and label-free detection capability of whispering gallery mode sensors not only to detect single biomolecules, but also to visualise their structural dynamics. The key advances here are the capability of avoiding the need for external labels while accessing faster dynamics than currently possible. Seeing single molecule dynamics gets an entirely new meaning in the context of high-speed AFM, as reviewed by Toshio Ando. While optical techniques generally use indirect measures to extract information about dynamics and interactions, AFM can directly visualise what happens on a molecular scale, and with the advent of high-speed methods, begins to do so at the relevant time scales. Taking single molecule studies from surfaces to the inside of cells, Brouwer et al. review recent advances in using single molecule fluorescence imaging to study, characterise and thereby improve our understanding of gene expression, in particular in the context of how transcriptional bursting is regulated.

The advent of super-resolution fluorescence microscopy has transformed our ability to visualise structure, and to some degree dynamics with light microscopes on the tens of nanometre scale. As previously for single molecule detection, that has been an enormous technological challenge in itself, and much scope for future improvements remains, such as in terms of imaging speed, sensitivity, throughput and applicability. The review by Gregor and Enderlein summarises the concepts, advantages and potential applications of image scanning microscopy, which has provided a somewhat surprising, but simple and elegant improvement to the resolution of confocal microscopes and has rapidly been adopted in commercial instruments enabling broad access and maximising impact. Mahecic et al. in contrast, focus on making super resolution fluorescence approaches quantitative by enabling acquisition of sufficient data to make observations statistically robust. This is a particular challenge for stochastic super resolution methods, largely because they already require large quantities of data to begin with.

These technical advances are complemented by efforts aimed at providing information beyond improved resolution. Yan et al. review recent advances in probe development and application to study intracellular microenvironments, such as pH, hydrophobicity and concentrations, furthering our ability to relate structure to function. Thinking about environments in a slightly different way, Feher et al. discuss the capabilities and limitations of single molecule localisation microscopy to detect and quantify molecular clustering in cells, which has implications for our understanding of signal transduction and beyond.

Broadening the scope of imaging approaches further, Chia et al. focus on addressing an existing bottleneck to the broader use of fluorescent proteins in anaerobic environments, and review the pros and cons of current approaches, providing a framework for future advances. Durrant et al. close the issue with a review on the technological challenges and how they are being addressed when it comes to attempts to obtain chemically specific information in a label-free fashion, specifically through the use of Raman imaging of live cells.

Taken together, these 10 reviews, which are only a small selection of ongoing efforts, illustrate the enormous breadth of technologies, approaches and challenges when it comes to seeing structure, dynamics, mechanism and function on small length scales in complex environments. They represent an excellent overview of how far we have advanced, but also importantly provide a glimpse into the future of where we want to go in terms of our ability to visualise molecular processes in the biological context.