Foreword

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The rise of the field of superresolution imaging over the past 10 years is reminiscent of the mythical phoenix being reborn to new life. The phoenix's rebirth, in this case, relates to microscopy's new found capability of seeing objects below the diffraction limit of light, which permits imaging of biological structures with spatial resolutions more than an order of magnitude finer than those accessible by conventional light microscopes. As discussed in the articles of this themed issue, this is revolutionizing the study of biological processes and structures at the nanoscale.

A major strategy for superresolution imaging involves single molecule localization via stochastic activation of fluorescence. In this approach, the temporally adjustable emission of either photocontrollable fluorescent proteins (as in photoactivation localization microscopy, PALM) or photoswitchable dyes (as in stochastic optical reconstruction microscopy STORM) is used to obtain localizations of single molecules. The localizations are then summed up to create a pointillistic 'superresolution' image that can achieve ~20 nm resolution when tens of thousands of localizations are collected within a densely labeled sample. Alternatively, the localizations can be used to follow that path and diffusion characteristics of single molecules over time with unprecedented precision. The articles in this issue all focus on different aspects of this methodology, either detailing ways for the methodology's improvement or demonstrating its usefulness for achieving mechanistic insights into important biological questions.

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The first article in the volume is an overreview of the field of single molecule superresolution imaging by Marcus Sauer, who details the history of this field, its basic principles and continual improvements. Following this are several reviews by distinguished researchers that discuss the requirements for successful implementation of single molecule superresolution imaging, including the ability to precisely estimate the position of single molecules in close proximity. This is important for determining whether a cluster of localizations represents the same molecule observed repeatedly or reflects the distribution of different molecules. Other technical articles in the volume address factors affecting achievable resolution, how to choose fluorescent proteins for optimal imaging and how to obtain meaningful biological information from data sets. In the later article, different approaches are presented for pinpointing protein spatial configurations and numbers. Several methodological articles in the volume present new techniques for correlating superresolution images with other imaging modalities or detail new strategies for performing co-cluster analysis of dense populations of molecules. Some of the methods papers also provide reconstruction algorithms that enable background signals in images to be reduced or offer approaches for obtaining better localization precision of single molecules.

In addition to presenting the latest methodological achievements, papers in this volume report new biological insights obtained from using single molecule superresolution imaging. These papers include investigations into the nanoscale distribution of viral proteins at the cell surface; examination of the clustering patterns of signaling molecules in membranes; and analysis of the spatial organization of amino acid receptors. The articles illustrate the diversity of questions that can be rigorously addressed using single molecule superresolution imaging.

In summary, the studies gathered in this special issue all incorporate the fundamentals of what is becoming a groundbreaking new approach to biology, single molecule superresolution imaging, in which processes and structures of cells are examined at the nanoscale. The hope is that these new methodologies will inspire other researchers from all corners to adopt such sophisticated, powerful approaches to persuasively address their specific biological questions in new and innovative ways.