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Overcoming uncertainty through advances in fluorescence imaging of molecular processes in cells

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The uncertainty principle in its popularized form states that the act of observing a process perturbs it, and therefore limits the accuracy at which the unperturbed process can be known. Likewise, in biomedical research there is an apparent tradeoff between the complexity of an experimental system and the level of detail at which the system can be understood (Fig. 1). Thus, the investigation of molecular mechanisms is often thought to require disruption of the organisms and cells in which those processes normally take place, introducing uncertainty whether the observed characteristics reflect those of the unperturbed system. However, unlike the laws of quantum physics that underpin Heisenberg's dictum, there are no natural laws that prevent scientists from investigating molecular processes in ever greater detail without disrupting living cells and organisms. Key enablers in the investigation of molecular events in their native environments are experimental approaches that allow detection and control of molecular events in living cells and organisms with minimal perturbation.

Fluorescence imaging has become the detection method of choice for the visualization of molecular processes in living cells and organisms. The primary reasons for this include the high spatial and temporal resolutions as well as the sensitivity of fluorescence imaging. These factors have driven the development of instrumentation, including microscope optics and electronics, flow cytometers and microfluidic devices, small-animal imaging, and high-throughput microplate imaging systems. In addition to fluorescence imaging, luminescence, magnetic resonance, and Raman imaging have developed rapidly, but these are hampered by their lower resolutions and the limited availability of probes for the detection of specific molecular processes.

To image specific molecular processes, it is essential to develop probes that selectively detect the processes of interest. A variety of small-molecule fluorophores whose fluorescence changes upon binding specific ions or other ligands have been developed. Many molecular processes are directed by macromolecules and macromolecular complexes. For such processes, intrinsically fluorescent proteins, in particular green fluorescent protein derivatives, can provide ideal probes. Fusion of fluorescent proteins to a variety of cellular proteins has enabled determination of their subcellular localizations and mobilities. The use of photo-activatable and photo-convertible proteins has enabled tracking of a subpopulation of proteins. The use of spectrally distinguishable fluorescent protein variants has enabled simultaneous visualization of multiple proteins and comparison of their distributions and mobilities. However, imaging

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of the total population of any protein provides only limited information about its subcellular sites of action since a majority of the protein may not be functionally engaged.

Several approaches have been developed for visualization of macromolecules engaged with specific partners in living cells. Resonance energy transfer (FRET and BRET) methods have been used to detect a variety of interactions in cells. Fluorescence cross-correlation (FCCS) has been recently applied to the analysis of protein interactions in live cells in a few model systems. Bimolecular fluorescence complementation (BiFC) analysis has been used for visualization of protein interactions in a wide variety of cell types and organisms. It is beyond the scope of this editorial to compare these experimental approaches. The availability of multiple experimental approaches allows investigators to choose the strategy most appropriate for the purpose of their experiments and for the available instrumentation.

In this issue, the authors report on new developments in a variety of areas related to imaging and the control of protein interactions and localization. It is not possible within the scope of a single issue to provide comprehensive coverage of all topics in this burgeoning field. Karen Reddy and Harinder Singh compare recent studies of the effects of constraining chromosomally integrated genes at the nuclear periphery on their transcriptional activities [1]. Watt Webb, John Lis, and colleagues report on visualization of the association of transcription factors and RNA polymerase with specific genetic loci on *Drosophila* polytene chromosomes [2]. Ulf Landegren, Lars-Gunnar Larsson, and colleagues describe the visualization of interactions among endogenous proteins using in situ proximity ligation [3]. Atsushi Miyawaki and colleagues report the use of Keima fluorescent protein in FCCS analysis with two-photon excitation [4]. Young-Hwa Song and Matthias Wilmanns describe the use of BiFC analysis in combination with high-resolution structural biology methods for determination of the architectures of multi-protein complexes [5]. Terence Hébert and colleagues report the detection of ternary protein complexes using a combination of complementation and resonance energy transfer assays [6]. Cathy Berlot and colleagues describe the use of multicolor BiFC analysis for quantitative analysis of the relative efficiencies of complex formation among different G protein subunits [7]. Vitaly Citovsky, Yedidya Gafni, and Tzvi Tzfira describe the development of plasmid vectors for BiFC analysis in plants [8]. Yasushi Saka, Anja Hagemann, and James Smith report the use of BiFC analysis for the visualization of protein interactions during frog development [9]. Chang-Deng Hu and colleagues describe the visualization of protein interactions in nematodes using BiFC analysis [10]. These reports provide an up-to-date compendium of recent advances in the visualization and control of molecular interactions.

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Fig. 1.

Overcoming the tradeoff between the complexity of experimental systems and the level of detail at which those systems can be understood through advances in experimental methods. The level of detail at which a system of a given complexity can be understood is often limited by the experimental methods that are available (pink area). The investigation of systems of greater complexity at a higher level of detail requires the development of new experimental methods (red area).