The smaller the better

Biologists are increasingly interested in single-molecule approaches. In this issue, a Focus provides a biologist's guide to this relatively new field, and two papers present advances in nanoscale visualization.

The ability to analyze biological systems at the single-molecule level opens avenues of investigation that are not possible using techniques that measure aggregate properties of molecular populations. This new vantage point can yield important insights.

A textbook example is that of molecular motors. Although classical biological assays for motor function show that these molecules support constant-velocity movement, studies of individual molecules revealed that they take discrete individual steps. The technique that allowed these crucial biological observations came from the physics field in 1986—a momentous year that saw the first demonstration not only of the optical tweezers technique used in the subsequent molecular motor study but also of the atomic force microscope. Created by physicists, these force-spectroscopy methods form much of the backbone of the field of research now devoted to studying biological systems at the single-molecule level.

Physicists continue to have a leading role in conducting experiments and moving the field forward. This is partly because some technologies are still maturing and require extensive expertise in the physical and mathematical sciences. This situation is clearly changing though.

Atomic force microscopes suitable for valuable biological experiments are commercially available, and protocols are well established. Optical tweezers capable of single-molecule experimentation must still be custom-built, but here also protocols are being standardized. Moreover, fluorescence imaging methods, which use concepts and equipment more familiar to biologists, are now capable of localizing individual molecules and detecting nanometer-scale distance changes. Single-molecule fluorescence resonance energy transfer (FRET), for example, extends the familiar FRET technique involving large population measurements to visualizing the transfer of energy between just two fluorescent molecules. Consequently, discrete molecular movements and distances can be discerned.

In addition, until very recently single-molecule experiments were limited to carefully controlled *in vitro* environments, preferred by physicists but not generally by biologists. The desire to keep the systems as simple as possible comes from the difficulty in discriminating specific single-molecule signals from background noise or nonspecific signals. But with improvements in the technology, observations of single molecules in cells are becoming possible.

Notably, a new set of super-resolution imaging methods has captured the imagination of cell biologists intrigued by the possibility of watching cell functions at the single-molecule level. By cleverly exploiting the properties of fluorescent molecules, these new imaging techniques have broken the resolution limit imposed by the wavelength of light in conventional light microscopy.

These imaging methods are only just beginning to address real biological questions though. One of the big limitations has been moving them from the two-dimensional to the three-dimensional realm. Two original research reports in this issue (p. 527 and p. 539) describe methods for achieving this in micrometer-thick specimens for two major classes of super-resolution imaging, greatly expanding the range of potential studies.

In addition to these new reports, in the Focus starting on page 475, experts in the field explore some of the technical options available for biologists willing to venture into the single-molecule field. Nils Walter and colleagues provide an overview of the many choices available in the modern single-molecule toolkit and of the biological questions they can answer. Keir Neuman and Attila Nagy describe force spectroscopy techniques, and highlight capabilities and limitations so new users can make informed decisions on applying the techniques to their biological system. Taekjip Ha and colleagues provide a practical guide to single-molecule FRET for biologists, with information on how to assemble a reasonably priced experimental system as well as descriptions of established protocols.

When working with single molecules, one of the biggest concerns is whether or not the experimental procedure is perturbing the molecules or introducing artifactual signals. Ha and colleagues describe effective surface immobilization strategies for single-molecule studies and a simple sample chamber design. Laurence Brewer and Piero Bianco describe the construction and use of more complex laminar-flow-cell sample chambers, suitable for a wider variety of applications, which allow the experimentalist to seamlessly add and remove reaction components.

The increased interest of biologists in these approaches is illustrated by the emergence of research centers devoted to bringing biologists and physicists together to explore biological questions using single-molecule techniques and for collaborative development of new methods. One example is the Nano/Bio Interface Center at the University of Pennsylvania (p. 569). Single-molecule analysis of biological systems has become a field of its own, and clearly one that will benefit from such collaborative environments. We hope this Focus also encourages the kind of cross-discipline exchange that will propel the field forward.