Seeing is believing

Humans are a visual-centric species, so it is no surprise that this partiality extends into the realm of science. Scientists' desire to see ever smaller details helped drive the development of sophisticated microscopes, but their utility for biologists was always constrained by the amount of contrast that could be generated between biological features. Fluorescence provides a powerful means of overcoming the contrast problem in biology, and this power has resulted in an explosion in fluorescence imaging applications, to which we dedicate a Focus this month.

Rather than relying on reflected or transmitted light to produce contrast, a stimulated fluorescent molecule emits light, allowing unprecedented contrast at microscopic scales and even allowing observations inside biological specimens. By using appropriate fluorescent molecules it is possible to track individual molecules in living systems or probe physiological changes within cells and tissue. Until recently, however, the introduction of such molecules was difficult and invasive.

The development of membrane-permeable dyes and the use of fluorescent proteins now allow fluorophores to be introduced more easily and with less perturbation. Combined with powerful imaging modalities such as confocal, multiphoton and fiber-optic methods, these fluorophores allow deeper and more detailed imaging in living tissues and whole organisms.

The power of fluorescence imaging techniques has stimulated their use in biology to the point that they have become an integral part of the basic toolbox of many biologists. The proliferation of fluorescence imaging studies and the relative democratization of access to specialized instrumentation, however, should not obscure the fact that the quality of fluorescence images is affected by many interrelated variables. Variables such as light excitation, fluorescence emission, bleaching, spectral crosstalk and the physical characteristics of imaging equipment all dictate the quality of data that can be obtained in an experiment.

All these phenomena are understood and well described at the level of the underlying physics, allowing the expert microscopist to optimize experimental design and techniques of observation. Although much of this basic physics is taught to all biology undergraduates, it is rarely taught with real-world examples that aspiring biologists will appreciate. As a result, many young biologists do not realize how their work relates to these underlying principles and therefore may not anticipate likely problems or know how to best overcome them.

Signal strength and bleaching, for example, are probably the biggest problems encountered in fluorescence imaging experiments. There are so many variables to consider, however, that a researcher may feel overwhelmed trying to figure out the best way of solving a signal strength problem for example and may settle on a seemingly simple solution such as increasing the time or the intensity of illumination. Although both will lead to increased bleaching, one may be better than the other for a particular imaging system or application, and there may be even better solutions such as averaging approaches that will achieve the same improvement with fewer undesirable results.

Many companies offer sophisticated imaging equipment and reagents that facilitate fluorescence imaging experiments. Unfortunately, this can lead to over-reliance on technology without a clear understanding of how it works. Different imaging systems invariably have advantages and disadvantages for particular applications, and there are many factors that must be considered when choosing equipment and reagents. Therefore, an understanding of the principles of fluorescence and the mechanisms of operation of the different imaging modalities is essential even before a potential user performs their first experiment. Clearly, the importance of educating biologists on these issues is paramount.

Moreover, the development of increasingly useful fluorescence imaging techniques and the convincing beauty of the resulting observations have caused a proliferation of fluorescence images in the scientific literature. Such a technological revolution goes a long way in a visual-centric species, and very often, seeing is believing. Instinctively, people tend to believe what they see and may not question how an image was obtained. Furthermore, it isn't always possible to present all the information necessary to objectively judge the accuracy of an image. Therefore, it is in a researcher's best interest and also a benefit to the community if their images are the highest quality possible while retaining accuracy.

In this Focus, pioneers and leaders in the field of fluorescence imaging discuss the principles and practice of fluorescence microscopy with the aim of giving users an understanding of these techniques. Our hope is that these reviews will help new users of the technology obtain high-quality images that accurately report the results of their studies.