nature methods

Focus on fluorescence imaging

Principles and practice of microscope techniques

Central brain hemisphere and eye-antenna disc of *Drosophila melanogaster* at the third larval stage stained with three Alexa dyes and imaged on a Zeiss LSM 510 Meta. Image courtesy of Veta Trunova, The Johns Hopkins University.

Fluorescent proteins. Image courtesy of Roger Tsien, University of California at San Diego.

When Sir George Gabriel Stokes first described the phenomenon of fluorescence in 1852 it is doubtful many people ever considered its potential as a tool for biologists. As often happens with new discoveries, however, scientists figured out a way to exploit this physical process and began to use fluorescent molecules as biological labels. In concert with microscopy this permitted previously undreamt-of possibilities for detection and visualization.

Notably, immunofluorescence techniques became powerful tools for inquisitive biologists. Recently, the description of new imaging modalities such as confocal and two-photon microscopy, the creation of new fluorescent molecules, and the discovery and exploitation of fluorescent proteins have triggered an explosion in fluorescence microscopy techniques. This has also resulted in their use within living systems where they are revolutionizing biological imaging. A sampling of seminal papers documenting this advance is available in the 'Classics Library' on the Focus website.

The application of fluorescence techniques to living specimens presents many challenges to users. There is thus a greater need for users to understand the principles of fluorescence and how different imaging systems work so biologists can fully exploit this revolution. Determining the most suitable equipment and reagents for an application, and properly designing and conducting experiments both require a clear understanding of the basic principles involved. This Focus is intended to present this basic information as well as practical advice that biologists need to use these powerful techniques in their work.

We are pleased to acknowledge the support of Carl Zeiss Microscopy as principal sponsor and Intelligent Imaging Innovations, Inc. as supporting sponsor in producing this focus. As always, *Nature Methods* carries sole responsibility for all editorial content and peer review.

Daniel Evanko

CONTENTS