

PREFACE

Fluorescence techniques are uniquely suitable for probing living cells because of their sensitivity and specificity. Since fluorescence from a single cell can be detected with a microscope both as an image and as a photometric signal, fluorescence microscopy has great potential for qualitative and quantitative studies on the structure and function of cells. However, due to previous technical limitations, fluorescence has been used primarily for staining fixed cells for many years. It has not been until recently that the true power of the techniques has evolved for use with single living cells. The most important advances that have made this possible include the development of (1) probes for specific structures or environmental parameters; (2) methods for delivering fluorescent probes into living cells; (3) methods for detecting weak fluorescence signals from living cells; and (4) methods for acquiring, processing, and analyzing fluorescence signals with microscopes.

The primary purpose of this and the accompanying volume of *Methods in Cell Biology* is to provide readers with detailed descriptions of methods in these four areas. While techniques for fluorescence spectroscopy in solution are described in various sources, there has been no convenient source for the methods specifically applied to living cells. Even with an extensive literature search, one often finds crucial technical details, including instrumentation, sample handling, and precautions, left out in many research articles. It is our hope that these volumes will provide enough detail to make the new developments approachable by most investigators. Although some biological perspectives are provided in many chapters, the main emphasis of the volumes is practical laboratory methods; the job of biological reasoning and experimental design is left to individual investigators. The books are thus targeted primarily at experienced cell biologists who wish to apply modern fluorescence techniques. However, they should also be of great interest to biochemists and molecular biologists who attempt to correlate results in test tubes with activities in living cells. In addition, many chapters should be valuable to those specializing in instrumentation, including microscopy, electronic imaging, and digital image processing.

The two volumes represent a collective effort of many investigators. The chapters were assembled by specific areas which, in our view, were important or held great promise in the future. We then invited those researchers with extensive experience in the particular area to make contributions. There was a certain degree of subjectiveness in choosing the topics. On the one hand, we have included topics crucial to, but not specific for, fluorescence microscopy of living cells, including microscope cell culture, microinjection, microscope photometry, and low light level imaging. On the other hand, we

decided to sacrifice several useful topics that were either not in a mature stage of development or where we were unable to obtain a commitment from an authority.

The first volume (Volume 29) deals with the preparation, delivery, and detection of fluorescent probes. The first half is focused on the preparation of specific structural probes, including fluorescent analogs that can be utilized by living cells in structural assembly, fluorescent molecules that bind to specific cellular components, and probes that can be used to label particular cellular compartments. There are special challenges in the preparation of each class of probes, including proteins, small peptides, heterocyclic compounds, lipids, and polysaccharides. Subsequent chapters discuss factors that determine the destination of probes and methods for delivering probes to specific sites in living cells. The second half of the first volume discusses the detection of fluorescent probes in living cells, including issues related to sample physiology (microscope cell culture), optics (basic fluorescence microscopy), and signal detection (electronic photometry and imaging, immunoelectron microscopic detection of fluorophores). The last few chapters introduce modern techniques in image detection and provide a continuity to quantitative, analytical methods covered in Volume 30.

The second volume (Volume 30) explores a combination of the theoretical and technical issues related to the quantitation of fluorescence signals in the living cell with a light microscope. The first section explores the engineering principles required in the characterization of the performance of an imaging system. The use of system validation procedures and quantitative fluorescent standards are explored in detail. The remainder of Volume 30 is devoted to specific applications and optical methods. A mix of theoretical and practical issues is discussed, including the measurement of membrane potential, ionic concentrations, tracer diffusion coefficients, total internal reflection, fluorescence polarization, and three-dimensional reconstruction. Thus, the two-volume set defines a technical continuum from organic chemistry, through biochemistry, cell biology, physics, and engineering, to computer science. The present status of the field reflects the occurrence of a revolution in cell biological research.

We would like to thank all contributing authors for providing us with their extensive experience in various areas. Most of them have worked closely with us in planning their chapters and minimizing overlaps, then submitting excellent manuscripts in a timely fashion and answering questions which arose during editing.

YU-LI WANG
D. LANSING TAYLOR