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Methods and Protocols

Edited by

Douglas J. Taatjes

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Cover illustration: *Background*: whole-cell experiment combined with a pre-embedding method: CLS-RCM image of a 90-nm Epon section (Chap. 18, Fig. 15D, p. 397). *Inset, upper left*: electron micrograph depicting a porosome close to a microvillus at the apical plasma membrane of a pancreatic acinar cell (Chap. 15, Fig. 2B, p. 303). *Upper right*: NSOM image of an antibody-labeled mouse macrophage (Chap. 14, Fig. 3C, p. 282). *Lower left*: metaphase analysis with multiple single-gene probes (Chap. 12, Fig. 1, p. 238). *Lower right*: adeno-associated virus serotype 5 (Chap. 7, Fig. 9, p. 161).

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Preface

In 1665, a book was published that inaugurated the use of the microscope to investigate the natural world. The author was Robert Hooke, a talented artist, architect, and amateur scientist. Hooke wrote *Micrographia: Or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses with Observations and Inquiries Thereupon*, at the behest of the newly chartered Royal Society in London, for whom he was working as curator of scientific experiments. In *Micrographia*, he presented the first detailed observations of everyday objects made with his self-constructed light microscope. Although this book contains a treasure-trove of drawings (in Hooke's own hand) of the appearance of various animate and inanimate specimens as viewed in magnified form, one of the drawings and its associated description stands out as particularly germane to our present topic of cell imaging techniques. In his description of a thin piece of clear cork cut with a penknife and observed with his microscope, Hooke described the honey-comb-like appearance of the cork with "pores" or "cells" representing the basic structural unit. This represents the first printed reference of the term "cell" to describe a unit structure of an organism.

In the 340 years since the publication of *Micrographia*, a multitude of new microscopy-based systems have evolved for the observation of cells. Indeed, many of these techniques have been developed in the past few decades. Recent books have sought to present single volumes detailing methods for specific types of microscopy, such as confocal scanning laser microscopy, atomic force microscopy, and electron microscopy. In the present book, we have sought to present an eclectic collection of what we consider some of the essential state-of-the-art methods for imaging cells and molecules. *Cell Imaging Techniques: Methods and Protocols* has been organized to begin with light microscopic methods to observe molecules such as mRNA, calcium, and collagen. Chapters covering confocal scanning laser microscopy, quantitative computer-assisted image analysis, laser scanning cytometry, laser capture microdissection, microarray image scanning, near-field scanning optical microscopy, atomic force microscopy, and reflection contrast microscopy follow. The book then finishes with chapters on preparative methods for transmission electron microscopy of particles and cells.

We have tried to arrange the chapters in a logical format, beginning with light microscopy techniques, proceeding through scanning probe-type

techniques, and ending with electron microscopy. The chapter on reflection contrast microscopy serves as a link between light and electron microscopy. Although *Cell Imaging Techniques: Methods and Protocols* is primarily intended to convey detailed methods and protocols for cell imaging, we have also included some review-type chapters to set the stage for the protocol-driven chapters. Moreover, given the incredible breadth of microscopy-based imaging techniques available today, we tried to include many that might not have been covered in detail in previous books. By necessity, we have had to exclude many valuable and marvelous techniques (multiphoton confocal microscopy, for one), but are secure in the knowledge that they have been comprehensively treated in other volumes of the *Methods in Molecular Biology* series.

We believe that *Cell Imaging Techniques: Methods and Protocols* will be useful for those involved in seeking a variety of microscopy-based techniques for imaging cells and molecules. With the proliferation of core “cell imaging facilities” at universities, hospitals, and pharmaceutical and biotechnology companies throughout the world, this volume should provide a handy reference or starting point for researchers seeking the latest information and protocols for a wide variety of cell imaging techniques. We hope that readers will find value in the techniques presented herein and might even be tempted to try some techniques they had not considered previously.

Finally, we would like to thank those associated with the production of this book. First, the authors themselves for agreeing to take the time to prepare their chapters in a timely manner and in a form filled with technical details not usually present in a research publication. It was not an easy task, and we thank them for their efforts. Second, we would like to thank Professor John Walker, the series editor, for his helpful insights, interest in the book, and his timely response to our queries. Third, we would like to express our appreciation to Craig Adams and the staff at Humana Press for their patience and editorial efforts in the production of the book, and to Marilyn Wadsworth at the University of Vermont for her invaluable assistance in our editorial tasks. Finally, the color reproduction of images, so important in a volume like this, would not have been possible without the generous financial support of the Optical Analysis Corporation (Nashua, NH), JMAR Technologies, Inc. (South Burlington, VT), and the Department of Pathology, University of Vermont (Burlington, VT).

Douglas J. Taatjes
Brooke T. Mossman

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