

CURRENT PROTOCOLS SELECT

# Imaging and Microscopy

A COMPENDIUM OF METHODS FROM  
Current Protocols

EDITED BY

Simon C. Watkins and Claudette M. St. Croix



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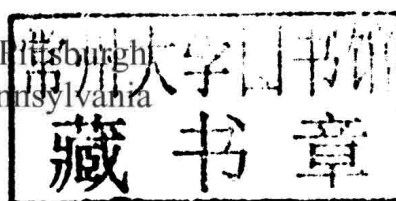
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Edited by

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# Foreword

Microscopy is the most important instrumentation-based technology in all the biomedical sciences. While growth has been dramatic in recent decades, the number of high-quality instructional books remains limited. There are a few single-author volumes; however, these are quite general, and commonly the author has a limited range of specific expertise. The goal of this new text edited by Simon Watkins and Claudette St. Croix is to integrate instructional and review articles from the very best in the field into a single volume. To do this, they have pulled selected units from many of the *Current Protocols* titles and have solicited a large number of new chapters from authors who are versed not only in the theory but also the practice of the published technology. You can think of this book as a “go to” handbook. How does a microscope work? What about a camera, which is the best to choose for a given application? Should I use confocal microscopy, or multiphoton microscopy? How does Fluorescence Resonance Energy Transfer work? The list is long and very deep. The selection of units is very current and treats almost all aspects of microscopy, from the most basic descriptions of microscope components to the most advanced technologies currently in use. What impresses me most is the list of authors for the chapters. They really are the best in the field, and importantly come from both academia and from industry. All in all, this is a must-have laboratory guide, suitable for any and all microscopists from the complete neophyte to the practicing professional imager.

Alan Waggoner  
*Carnegie Mellon University*



# Preface

In the last two decades, the capabilities of scientific tools have exploded. In each case, the unifying change has been the continued expansive integration of new technologies on all fronts, resulting in a synergistic mix of molecular, biochemical, robotic, and computer-based tools. Few fields have embraced and been more enabled by advanced technologies as microscopy. In retrospect, conventional microscopy imaging was almost entirely film based until the early 1990s, and was limited to simple non-quantitative histology or non-digital image collection. Those with long memories will remember the difficulties of collecting fluorescence images on 35-mm film. Objective lenses were examples of the optical craftsman's art, well corrected for spherical and chromatic aberration, but with insufficient light throughput for fluorescence microscopy. The breakthrough tool of the late 1980s, the confocal microscope, had just arrived on the scene in the form of the BioRad MRC 600 (developed by Amos and White), and represented a transformative development in terms of instrumentation. Green fluorescent protein (GFP) and the power of molecular integration of fluorescence into proteins had yet to be implemented, and the capabilities of computing for quantitation were still limited, and certainly not possible with "personal computers."

In the present biomedical research arena, the microscope has reinvented itself and now represents the integration of modern optics, robotics, computing, probes, and cameras. This has transformed it from a device capable of generating principally descriptive data to a primary quantitative research tool capable of addressing questions at all levels of resolution, from the single molecule to the whole organism. Understanding the capabilities, opportunities, and limitations of each facet of microscope- and image-based technology has become a highly specialized and validated research field. The goals of this volume are to provide an in-depth description of each microscope-based imaging technology in a way that is readily understood by the biologist, and present practical examples of the major applications such that researchers can implement the specific methods in their laboratories. In order to achieve these goals, it is necessary to present some material in a purely theoretical way. For example, the discussion of microscope objectives describes the basic physics of how an objective works, because this information is required for a complete understanding of appropriate objective choices for specific tasks. Other approaches, such as fluorescence (or Förster) resonance energy transfer (FRET), demand a description of the technology but also examples of the multiple ways in which FRET can be implemented in the "real world." Finally, some information is presented simply in the form of practical recipes and troubleshooting suggestions (for example, the discussions of cutting sections and immunocytochemistry). Ultimately, the goal of the book is to act as a resource for training and application, and for troubleshooting of experiments.

This was a heady and difficult task, but fortunately the concept had been previously developed within the *Current Protocols* series. As such, this book was initially conceived as an integration of microscopy units from all the different thematic collections represented in *Current Protocols*. To some degree, this did happen, and many of the units come directly from *Current Protocols*, though all were updated for this book. However, we also judiciously solicited multiple new units to fill out the title, such that we hope it represents a well-rounded treatment of the field. Most importantly, we have relied on, and have been successful in, soliciting units from true experts in the field. While several chapters were written by colleagues in industry, the majority of the authors are active researchers who are implementing the approaches in their daily research life.

The book has multiple major thematic foci. The goal of the first section is to give an appreciation of the various components of the standard digital fluorescence microscope. This includes the core components of the microscope—objectives, contrast mechanisms (e.g., DIC, phase contrast), detectors and filters, and cubes for fluorescent microscopy. There are also units covering the basics of digital and fluorescence microscopy, and how to set up the microscope for optimal use. Essentially this is the “go to” section for beginners.

The chapters covering the fundamentals of the microscope are followed by a section describing the basic methods. The first chapters of this section describe how to optimize sample preparation (fixation, sectioning, mounting, and antibody labeling) for standard wide-field microscopy and confocal microscopy of cells and tissue sections. These two chapters and the subsequent three chapters covering probes, optical sectioning, and wide-field imaging are specifically designed as a preamble to the specific applications section that follows. As optical sectioning methods are at the core of modern fluorescence microscopy, this section includes specific subsections on confocal, deconvolution, and multiphoton approaches.

The bulk of this book deals with the application of different optical modalities in specific experimental situations. In each case, we have selected the premier examples from throughout the *Current Protocols* titles. These include basic live cell imaging, TIRF, FRAP, FRET (in all its technical variants), and examples of model system imaging in multiple different animal models. This section concludes with a treatment of super-resolution methods.

Finally, we have multiple chapters on various aspects of image analysis. This is in addition to the significant discussions of image analysis relevant to each specific technology and application within the relevant sections and chapters.

Overall, in this volume we have attempted to develop a practical guide to current microscope-based imaging technologies such that the basic laboratory researcher can plan, implement, and integrate modern imaging approaches in an intelligent way into his/her research strategy.

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