CURRENT PROTOCOLS SELECT

Imaging and Microscopy

A COMPENDIUM OF METHODS FROM
Current Protocols

EDITED BY
Simon C. Watkins and Claudette M. St. Croix

WILEY

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Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
Published simultaneously in Canada.

Library of Congress Cataloging-in-Publication Data

Current protocols select : imaging and microscopy / edited by Simon C. Watkins, Claudette M. St. Croix, University of Pittsburgh, Pittsburgh, Pennsylvania.
pages cm
Includes index.
ISBN 978-1-118-04431-5 (pbk.)
1. Microscopy. 2. Microscopes. I. Watkins, Simon C. II. St. Croix, Claudette M.
QH205.2.C864 2013
577–dc23

2013009406

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

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
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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.

CP Select: Imaging & Microscopy xv-xvi

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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CP Select: Imaging & Microscopy xvii-xx

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Table of Contents

Foreword xiii
Preface xv
Contributors xvii

Chapter 1 Fundamentals of the Microscope

Introduction 3

Fluorescence Microscopy: A Concise Guide to Current Imaging Methods 5
Introduction 5
Wide-Field Fluorescence Microscopy (WFFM) Techniques 6
Modern Confocal Microscopy 9
Total Internal Reflection Fluorescence (TIRF) Microscopy 12
Two-Photon Fluorescence Microscopy (TPFM) 14
Stimulated Emission Depletion (STED) Fluorescence Microscopy 16
Final Considerations 18
Acknowledgements 19
Literature Cited 19

Microscope Objectives 21
Introduction 21
Image Fidelity 21
Properties of Microscope Objectives 25
Construction and Types of Microscope Objectives 26
Modern Objectives 28
Objectives for Other Microscopy Applications 32
Other Considerations in Choosing Objectives 33
Literature Cited 34
Key References 34
Internet Resources 34

Light Microscopy Digital Imaging 35
History of Microscopy Image Capture 35
Solid-State Sensors 35
Spectral Sensitivity of Sensors 37
Camera Noise 38
Coupling Digital Cameras to Microscopes 40
Color Imaging 42
Camera and Sensor Characteristics 43
Modes of Image Capture 44
Microscope Optimization for Digital Imaging 45
Care and Maintenance 45
Key References 47

Optical Filters for Wavelength Selection in Fluorescence Instrumentation 49
Introduction 49
Optical Thin-Film Interference Filters 49
Optical Filter Configurations in Fluorescence Instruments 52
Fluorescence Filters Impact Optical System Performance 63
Tunable Optical Filters 71
Conclusion 75
Literature Cited 76

Proper Alignment and Adjustment of the Light Microscope 77
Major Components of the Light Microscope 78
Basic Imaging and Köhler Illumination Light Paths for Bright-Field, Fluorescence, and Dark-Field Microscopy 83
Basic Imaging for Dark-Field Microscopy 85
Basic Protocol 1: Alignment for Köhler Illumination in Bright-Field, Transmitted Light Microscopy 86
Basic Protocol 2: Alignment of the Eyepieces 89
Basic Protocol 3: Alignment for Köhler Illumination in Epifluorescence Microscopy 90
Basic Protocol 4: Alignment for Phase-Contrast Microscopy 92
Basic Protocol 5: Alignment for DIC Microscopy 94
Alignment for Dark-Field Microscopy 98
Basic Protocol 6: Alignment for Low-Power Magnification Dark-Field Microscopy 99
Basic Protocol 7: Alignment for High-Power Magnification Dark-Field Illumination 100
Support Protocol 1: Matching Microscope Magnification to Detector Resolution 101
Support Protocol 2: Calibrating Image Magnification with a Stage Micrometer 102
Tests for the Optical Performance of the Microscope 103
Support Protocol 3: Testing Phase-Contrast and DIC Using Diatom Testing Slide 103
Support Protocol 4: Testing Phase-Contrast, Dark-Field, and DIC Microscopes Using a Squamous Cheek Cell Test Slide 103
Support Protocol 5: Testing Fluorescence Using a Red, Green, and Blue Fluorescent Tissue Culture Cell Test Slide 103
Support Protocol 6: Care and Cleaning of Microscope Optics 105
Commentary 106
Literature Cited 107

Chapter 2 Basic Methods

Introduction **111**

Section I Sample Preparation for Conventional Microscopy

Cryosectioning **113**
Basic Protocol: Specimen Preparation and Sectioning 113
Support Protocol 1: Tissue Fixation and Sucrose Infusion 117
Support Protocol 2: Perfusion of Adult Mice 117
Reagents and Solutions 118
Commentary 119
Literature Cited 120

Immunohistochemistry **121**
Introduction 121
Basic Protocol 1: Immunofluorescent Labeling of Cells Grown as Monolayers 121
Alternate Protocol 1: Immunofluorescent Labeling of Suspension Cells 123
Basic Protocol 2: Immunofluorescent Labeling of Tissue Sections 124
Alternate Protocol 2: Immunofluorescent Labeling Using Streptavidin-Biotin Conjugates 125
Alternate Protocol 3: Immunofluorescent Double-Labeling of Tissue Sections 126
Reagents and Solutions 127
Commentary 127
Literature Cited 131

Section II Dyes and Probes

A Review of Reagents for Fluorescence Microscopy of Cellular Compartments and Structures **133**
Introduction 133
Basic Protocol 1: BacMam Constructs 136
Alternate Protocol 1: Non-Pseudo-Typed BacMam Viruses/Hard-To-Transduce Cell Types 140
Basic Protocol 2: Actin Labeling 141
Basic Protocol 3: Autophagosome Labeling by Transduction of Cells with Premo Autophagy Sensor GFP-LC3B 142
Alternate Protocol 2: Performing Autophagosome Labeling with an Antibody 143
Basic Protocol 4: Wheat Germ Agglutinin Conjugates for Plasma Membrane Labeling 145
Basic Protocol 5: Endoplasmic Reticulum and Nuclear Membrane Labeling Using ER-Tracker Reagents 145
Basic Protocol 6: Labeling Endosomes with pHrodo 10k Dextran 146
Basic Protocol 7: Labeling Golgi Apparatus Using Dye-Labeled Ceramides 147
Basic Protocol 8: Labeling Lysosomes Using LysoTracker Red DND-99 149
Basic Protocol 9: Labeling Mitochondria Using MitoTracker Red CMXRos 150
Basic Protocol 10: Labeling Nucleoli Using SYTO RNASelect Green 152
Basic Protocol 11: Labeling Peroxisomes Using CellLight BacMam 2.0 Peroxisomes-GFP 153
Alternate Protocol 3: Labeling Peroxisomes Using Antibodies 154
Basic Protocol 12: Labeling Tubulin Microtubules with TubulinTracker Green 156
Basic Protocol 13: Labeling Whole Cells or Cytoplasm with 5(6)-CFDA SE 156
Reagents and Solutions 158
Commentary 161
Literature Cited 197
Internet Resources 203

The Fluorescent Protein Color Palette **207**
Introduction 207
Fluorescent Protein Brightness and Maturation 210
Phototoxicity and Photostability 212
Oligomerization 214
The Fluorescent Protein Color Palette 216
Optical Highlighter Fluorescent Proteins 232
The Future of Fluorescent Proteins 239
Literature Cited 239

Photoactivation and Imaging of Optical Highlighter Fluorescent Proteins **247**
Introduction 247
Background 247
Requirements for Highlighting Fluorescent Proteins 252
Optimization Procedures 253
General Photoactivation Experiment 255
Uses of Optical Highlighter Fluorescent Proteins 256
Application of Optical Highlighter Fluorescent Proteins in Cytometry 258
Future Directions of Optical Highlighter Fluorescent Proteins 258
Acknowledgement 259
Literature Cited 259

Section III Optical Sectioning Microscopy

Basic Confocal Microscopy **261**
Introduction 261
Basis of Optical Sectioning 263
Configuration of an LSCM 265
Practical Guidelines 268
Commentary 275
Acknowledgements 278
Literature Cited 278
Key References 280
Internet Resources 280

Evaluation and Purchase of an Analytical Flow Cytometer: Some of the Numerous Factors to Consider **283**
Introduction 283
Applications 285
Hardware 286
Software 288
Quality Assurance (QA) 289
Service, Support, and Company 293
Maintenance/Cleanup Protocol 294
Price 294
Recommendation from Colleagues 294
Summary and Conclusions 294
Disclaimer 295
Resources Listed 295
Acknowledgements 295
Literature Cited 295

3D Deconvolution Microscopy **297**
Introduction 297
Image Formation 297
Resolution and Sampling 301
Estimating and Optimizing the PSF 302
Deblurring and Deconvolution Algorithms 303
Blind Deconvolution 306
Example Deconvolution Results 307
Deconvolution Software 309
Basic Protocol: Data Acquisition and Deconvolution Analysis 312
Concluding Remarks 315
Literature Cited 315
Key References 316
Internet Resources 316

Multi-Photon Imaging **317**
Introduction 317
Multi-Photon Microscopy 317
Multi-Photon Imaging in Practice 323
Concluding Remarks 328
Literature Cited 328

Chapter 3 Applications

Introduction **333**

Section I Basic Live Cell Imaging

Building a Live-Cell Microscope: What You Need and How to Do It **335**
Defining the System 335
Building a Live-Cell Scope: Components and Considerations 337
Transmitted Light Choices 344
Summary 346

Time-Lapse Microscopy Approaches to Track Cell Cycle and Lineage Progression at the Single-Cell Level **347**
Introduction 347
System Setup 348
Basic Protocol 1: Time-Lapse Acquisition Using Adherent Cells 349
Alternate Protocol 1: Time-Lapse Acquisition with Endpoint Assay to Mark S-Phase Cells 350
Alternate Protocol 2: Time-Lapse Acquisition Using Suspension Cells 351
Basic Protocol 2: Sequence Analysis for Mitosis Event or Cell Death 352
Basic Protocol 3: Data Mining—Normalized Event Distribution 353
Basic Protocol 4: Data Mining—Time-to-Event Curves 354
Basic Protocol 5: Data Mining—Duration of Mitotic Event 355
Basic Protocol 6: Data Mining—G2 Checkpoint Breaching 355
Basic Protocol 7: Data Mining—Deriving Basic Lineage Parameters 356
Commentary 356
Literature Cited 359
Internet Resources 360

Analysis of Mitochondrial Dynamics and Functions Using Imaging Approaches **361**
Introduction 361
Strategic Planning 361
Basic Protocol 1: High-Resolution *z*-Stack and Time-Lapse Imaging of Mitochondria 363
Alternate Protocol: Imaging Mitochondrial Morphology Alterations 366
Basic Protocol 2: Fluorescence Recovery After Photobleaching on Mitochondria 367
Basic Protocol 3: Microirradiation Assay to Assess Electrical Continuity in Mitochondria 372
Support Protocol: Staining Mitochondria in Live Cells to Assess Mitochondrial Function by Imaging 375
Commentary 378
Literature Cited 382

Analysis of Protein and Lipid Dynamics Using Confocal Fluorescence Recovery After Photobleaching (FRAP) **385**
Introduction 385
Basic Protocol 1: How to Set Up a FRAP Experiment 387
Basic Protocol 2: Confocal FRAP Measurements of the Lateral Diffusion of Plasma Membrane Proteins and Lipids 391
Alternate Protocol 1: Lateral Diffusion Measurements for a Rapidly Diffusing Soluble Protein 393
Alternate Protocol 2: FRAP Analysis of Intracellular Trafficking Kinetics 395
Basic Protocol 3: Working with FRAP Data 397
Basic Protocol 4: Further Analysis of FRAP Data to Obtain Diffusion Coefficients 399
Commentary 401
Acknowledgements 411
Literature Cited 411

Confocal Imaging of Cell Division **415**
Introduction 415
Spinning Disk Confocal 415
Confocal Imaging of Chromosome Condensation in *C. elegans* Embryos 420
Confocal Imaging of Spindle Assembly and Chromosome Dynamics 421
Confocal Imaging of Cytokinesis 424
Discussion 425
Acknowledgements 426
Literature Cited 426

Total Internal Reflection Fluorescence (TIRF) Microscopy 429
Introduction 429
The Theory Behind the Technique 430
TIRF Objectives 432
Empirically Determining Incident Angle/Penetration Depth 434
TIRF Imaging of Plasma Membrane Receptors in Neurons 436
Multi-Wavelength TIRFM 438
Final Experimental Suggestions 441
Concluding Remarks 442
Literature Cited 442

Total Internal Reflection Fluorescence (TIRF) Microscopy Illuminator for Improved Imaging of Cell Surface Events 445
Introduction 445
Basic Protocol 1: Through-the-Objective TIRF Protocol 445
Alternate Protocol: Improved Uniformity in the Excitation Field Protocol 450
Basic Protocol 2: Through-the-Prism TIRF Protocol 452
Commentary 454
Literature Cited 465

Section II Fluorescence Resonance Energy Transfer

Imaging Protein-Protein Interactions by Förster Resonance Energy Transfer (FRET) Microscopy in Live Cells 467
Commentary 474
Literature Cited 479

Imaging Protein-Protein Interactions by Fluorescence Resonance Energy Transfer (FRET) Microscopy 481
Basic Protocol: FRET Microscopy of Fixed Cells 482
Support Protocol 1: Nuclear and Cytosolic Microinjection 485
Support Protocol 2: Protein Labeling with Cy3 487
Reagents and Solutions 490
Commentary 490
Literature Cited 496

Use of Spectral Fluorescence Resonance Energy Transfer to Detect Nitric Oxide–Based Signaling Events in Isolated Perfused Lung 499
Introduction 499
Strategic Planning 499
Basic Protocol 1: Isolating and Perfusing Mouse Lung 500
Basic Protocol 2: No-Induced Protein Modifications Detected by FRET Using Spectral Confocal Microscopy 503
Reagents and Solutions 506
Commentary 507
Literature Cited 510