Basic Confocal Microscopy

Robert L. Price • W. Gray (Jay) Jerome
Editors

Basic Confocal Microscopy

Springer

Editors
Robert L. Price
Department of Cell and
Developmental Biology
School of Medicine
University of South Carolina
6439 Garner's Ferry Road
Columbia, SC
USA
Bob.Price@uscmed.sc.edu

W. Gray (Jay) Jerome
Department of Pathology
Vanderbilt University Medical Center
U-2206 MCN, 1161 21st Avenue,
South Nashville, TN
USA
jay.jerome@vanderbilt.edu

ISBN 978-0-387-78174-7 e-ISBN 978-0-387-78175-4
DOI 10.1007/978-0-387-78175-4
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011925553

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Biological confocal microscopy is still a relatively young field. Most researchers in the field would date the modern era of biological confocal microscopy from the 1985 description of a particularly useful confocal design published by White and Amos in the Journal of Cell Biology. Since that time, the use of confocal microscopes by biologists has increased phenomenally, with new converts joining the ranks daily; many with little or no previous microscopy training. For this reason, in 2001 when we were asked to organize a 1 day session on "basic confocal microscopy" for attendees at the Southeastern Microscopy Society annual meeting in Clemson, SC, we decided to focus not only on the confocal microscope itself, but also on ancillary subjects that are critical for getting the most from confocal microscopy.

Our initial effort seemed to meet a growing need to train new students, technologists, and faculty wishing to use confocal microscopy in their research. Evidence for this need is that each year since 2001, we have been invited by several meeting organizers and microscopy core facility Directors to present our take on what is important to use confocal microscopy successfully for biological exploration. In 2005, we also began teaching a 5-day intensive, hands-on workshop at the University of South Carolina each year. As that course evolved, we invited various colleagues to help with the course. This book is a direct outgrowth of that course and follows the general structure of the didactic portion of the course. In line with the course philosophy, we have not attempted to cover each topic in depth. However, we have maintained a focus on basic information and we have endeavored to cover information that is important for designing, carrying out, and interpreting the results of basic confocal microscopy-based biological experiments completely. We were very fortunate that two of the other course instructors, Drs. Ralph Albrecht and Tom Trusk, have provided chapters for this volume and have embraced the overall philosophy of presenting a basic knowledge base in a complete but concise manner.

Although the forums have been different and the course lengths have varied anywhere from 1 to 5 days, we have always based the workshops on the original concept that there is a group of core issues that must be understood before one can efficiently get the best results from the use of a confocal microscope. The early chapters in this book address these core issues and it is not by accident that after an initial introductory chapter on confocal microscopy, the chapters describing the components of the confocal microscope and how to set the various operating

parameters correctly are located toward the end of the book. Without a well-designed research plan and properly prepared specimen, the data collected by the microscope will not be optimum. Thus, we have devoted Chaps. 2 and 3 to fluorescence and understanding the use of fluorescent microscopy, and Chaps. 4 and 5 to specimen preparation and labeling strategies. These chapters are essential since regardless of the quality of the confocal microscope, if the sample is not prepared properly, the data collected will not be optimal.

Most modern confocal microscope images are digital. Thus, many of the basic operating parameters for confocal microscopy involve setting up the analog to digital conversion of specimen information. It is essential that a confocal microscope operator have a thorough understanding of how digital images for scientific purposes should be collected and analyzed. For this reason, following the chapters on specimen preparation, Chaps. 6 and 7 discuss digital microscopy with respect to confocal imaging.

Although it might seem odd that a book on confocal microscopy contains only two chapters directly devoted to the actual operation of the confocal microscope, these chapters are packed with practical information and, taking advantage of the preliminary information presented in preceding chapters, they provide all that is necessary to begin doing confocal microscopy and optimizing the information obtained. After Chaps. 8 and 9, which discuss the types of confocal instruments and setting up proper operating parameters, the final set of chapters provide information on the 3D analysis and reconstruction of data sets and some ethical considerations in confocal imaging, and provide some resources that we have found useful in our own use of confocal microscopes. After mastering the basic information presented in this book, these resources are great guides for continuing your education into more advanced forms of confocal microscopy.

This book has benefited from our association with numerous colleagues who have challenged and informed us. In particular, numerous debates with one of the course instructors, Dr. John MacKenzie, Jr., have helped hone the information on digital image processing to the most important concepts. We are also grateful to Drs. K. Sam Wells, David Piston, and John Fuseler for stimulating and challenging conversations that have made us better microscopists. We also owe a huge debt to the many students over the years whose enthusiasm and questions have guided our decisions regarding what to include and exclude from the workshops and chapters in this book. We are also thankful to the many companies that have provided resources and applications experts who have significantly enhanced our hands-on workshops at the University of South Carolina.

Finally, we must thank our lab members and families for not only putting up with our obsession for microscopy but also encouraging us in our pursuits.

Columbia, SC Robert L. Price
Nashville, TN W. Gray (Jay) Jerome

List of Abbreviations

2D	Two-dimensional
3D	Three-dimensional
AOBS	Acousto-optical beam splitter
AOTF	Acousto-optical tunable filter
A to D	Analog-to-digital conversion
AVI	Audio-video interleave
BFP	Blue fluorescent protein
CCD camera	Charge-coupled device camera
CDRs	Complementarity-determining regions
CFP	Cyan fluorescent protein
CLAHE	Contrast limited adaptive histogram equalization
CMOS	Complementary metal oxide semiconductor
CMYK	Cyan, magenta, yellow, and black images
CSLM	Confocal scanning laser microscope
CTF	Contrast transfer function
Cy	Cyanine
DABCO	1,4-Diazabicyclo[2,2,2]octane
DIC	Differential interference contrast
DPI	Dots per inch
EMCCD	Electron-multiplied charge-coupled device
EGFP	Enhanced green fluorescent protein
FITC	Fluorescein isothiocyanate
FLIM	Fluorescent lifetime imaging
FRAP	Fluorescent recovery after photobleaching
FRET	Förster resonant energy transfer
FWHM	Full-width half maximum
GFP	Green fluorescent protein
HeNe	Helium–neon laser

IgA	Immunoglobulin class A
IgD	Immunoglobulin class D
IgE	Immunoglobulin class E
IgG	Immunoglobulin class G
IgM	Immunoglobulin class M
IR	Infrared
JPEG	Joint photographic experts group
LASER	Light amplification by stimulated emission of radiation
LED	Light-emitting diode
LM	Light microscopy
LUTs	Look-up tables
MPEG	Moving picture experts group
MSDS	Material safety data sheet
NAD(H)	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate-oxidase
NA	Numerical aperture
NPG	*n*-propyl gallate
PEG	Polyethylene glycol
PerCP	Peridinin-chlorophyll protein
PDF	Portable document format
PMT	Photomultiplier tube
PPD	*p*-Phenylenediamine
PSF	Point-spread function
PPI	Pixels per inch
RESEL	Resolvable element
RFP	Red fluorescent protein
RGB	Red, green, and blue images
RGBA	Red, green, blue, alpha images
ROI	Region of interest
RSOM	Real-time scanning optical microscope
scFv	Single-chain variable fragment
TEM	Transmission electron microscopy
Tf	Transferrin
TIF(F)	Tagged image file format
TRITC	Tetramethylrhodamine-isothiocyanate
TSRLM	Tandem scanning reflected light microscope
UV	Ultraviolet
VaLaP	Vaseline, lanolin, and petroleum jelly
V_H	Variable heavy chain
V_L	Variable light chain
WGA	Wheat germ agglutinin
YFP	Yellow fluorescent protein

Contents

Contributors

Ralph M. Albrecht
Department of Animal Sciences; Pediatrics; and Pharmaceutical Sciences, University of Wisconsin – Madison, 1046 Animal Science Bldg., 1675 Observatory Drive, Madison, WI 53706, USA
albrecht@ansci.wisc.edu

John Fuseler
Department of Cell and Developmental Biology, School of Medicine, University of South Carolina, 6439 Garner's Ferry Road, Columbia, SC 29208, USA
John.Fuseler@uscmed.sc.edu

W. Gray (Jay) Jerome
Department of Pathology, Vanderbilt University Medical Center, U-2206 MCN, 1161 21st Avenue, South Nashville, TN 37232-2561, USA
jay.jerome@vanderbilt.edu

Julie A. Oliver
Department of Biological Sciences, University of Wisconsin – Milwaukee, Lapham Hall N209, 3209 N. Maryland Avenue, Milwaukee, WI 53201, USA
joliver@uwm.edu

Robert L. Price
Department of Cell and Developmental Biology, School of Medicine, University of South Carolina, 6439 Garner's Ferry Road, Columbia, SC 29208, USA
Bob.Price@uscmed.sc.edu

Thomas C. Trusk
Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, BSB 643, 173 Ashley Avenue, Charleston, SC 29425, USA
truskt@musc.edu