

METHODS IN MOLECULAR BIOLOGY™

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Confocal Microscopy

Methods and Protocols

Second Edition

Edited by

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Preface

Confocal microscopy has become an essential technique in all fields of contemporary biomedical research where a light microscope is required for imaging fixed or living fluorescently labeled cells and tissues. The approach is especially useful in cell biology, developmental biology, neurobiology, and pathology for visualizing the spatial distribution of labeled cells within tissues or the spatial distribution of specific macromolecules within cells.

The field of confocal microscopy has grown exponentially since the publication of the first edition of this book some 10 years ago. While confocal instruments were typically confined to multiuser facilities since they required specialist training and maintenance, the modern instruments have become much more user-friendly and reliable, and are now commonly found in both multiuser microscopy facilities and in individual laboratories.

Before practical confocal microscopes became available to biologists some 30 years ago, fluorescently labeled cells and tissues were imaged using conventional epifluorescence light microscopes. Using these instruments, resolution was compromised by fluorescence from out of the focal plane of interest especially when tissues made up of multiple cell layers such as developing embryos and adult brains were imaged.

In order to attain acceptable resolution in their images at this time, microscopists were forced into all manner of tricks to prepare their specimens for optimal viewing in the microscope. Such techniques include cutting sections of tissues with a microtome, growing cells on a glass coverslip, flattening cells under agar or squashing embryos between a slide and coverslip. While most of these methods produced acceptable images, they ran the risk of introducing artifacts, and as a consequence, the validity of the results could be questioned.

Moreover, the detection of weakly labeled structures was often impossible since long exposure times were necessary to produce an image, and significant photobleaching occurred. Often labeled structures could be observed by eye simply by focusing up and down within the specimen, but it was impossible to document any detailed structural information for further analysis and publication. This was extremely frustrating for the researcher since the exquisite specificity of immunolabeling was lost in the fluorescent haze.

After laser scanning confocal microscopes became commercially available in 1985, the out-of-focus fluorescence was eliminated by the optics of the instrument leaving the in-focus information in the final image. Images produced in this way were reminiscent of those of physically sectioned tissues and were called “optical sections” since light rather than a knife was used to produce them.

The current generation of modern confocal instruments produces optical sections of cells and tissues that are free of out-of-focus fluorescence with reduced chances of artifacts from the techniques of specimen preparation. In addition to imaging fixed specimens, overall improvements to the light efficiency of modern instruments (especially improvements in the sensitivity of the photodetectors) coupled with the introduction and improvement of fluorescent reporter probes over the years has enabled routine imaging of living specimens. Moreover, the measurement of physiological events within cells is now a possibility with faster image acquisition times.

The optical sections collected using the current generation of microscopes are in a digital format, and are easily reproduced on a journal page or a Web site at the exact same resolution and color balance as the data collected by the confocal microscope itself. The images are further processed using various visualization software and analyzed using bioinformatics techniques.

As in the first edition, the second one is aimed primarily, but not exclusively, at the novice user with suggestions for and information about more advanced techniques. The current volume is relatively light on the technical details of the microscopes themselves as these can be found elsewhere, and quickly change as new technology is incorporated into modern confocal systems. As in the previous volume, the aim of the current one is to take the researcher from the benchtop through the imaging process to the journal page for a few protocols.

Emphasis has again been placed on the laser scanning confocal microscope since this instrument continues to be used for most routine applications. There are now many alternatives to the laser scanning confocal microscope for producing optical sections, however. These instruments include the strictly confocal together with instruments such as multi-photon microscopes that have evolved from the confocal approach. These additional methods of collecting optical sections have been listed in our introductory chapter together with some of their applications and possible advantages over the confocal approach.

Protocols for the preparation of tissues from many model organisms including worms, flies, and mice have been included as well as chapters on confocal imaging of living cells, three-dimensional analysis, and the measurement and presentation of confocal images for publication. It is now impossible to include a comprehensive list of protocols in a single volume. Further information on where to obtain additional protocols can be gleaned from Chapter 3 together with the many Web references and links scattered throughout the book.

In addition, we are honored to include an introductory chapter from John White, one of the inventors of the first laser scanning confocal microscopes made available to the biomedical research community in 1985. John epitomizes the spirit of adventure, excitement and cooperation that has largely percolated through the confocal field over the years, and provides us with a flavor of those early years of confocal instrument development and treats us to some of his insight for the future.

Finally, this has been a long project, and has gone through several rounds of revision. We would like to thank all of the authors who have persevered in order to complete their chapters and updates to their chapters in a timely fashion. Finally, we would like to thank our colleagues (past and present) for presenting us with such a variety of stimulating imaging challenges. The series editor, John Walker, has provided expert editorial advice and shown great patience with us. The staff at Humana Press and Springer (and especially Tamara Cabrero) have performed to an extremely high standard of professionalism throughout the project.

Madison, WI, USA

*Stephen W. Paddock
Kevin W. Eliceiri*

Contents

<i>Preface</i>	<i>v</i>
<i>Contributors</i>	<i>ix</i>
1 Reflecting on Confocal Microscopy: A Personal Perspective..... <i>John White</i>	1
2 Laser Scanning Confocal Microscopy: History, Applications, and Related Optical Sectioning Techniques <i>Stephen W. Paddock and Kevin W. Eliceiri</i>	9
3 Confocal Microscopy on the Internet <i>Kristin L. Hazelwood, Christopher S. Murphy, Zachary C. Cunningham, Matthew J. Parry-Hill, Richard L. Ludlow, Ericka B. Ramko, Anna P. Ozarowska, Adam M. Rainey, and Michael W. Davidson</i>	49
4 Using Photoshop with Images Created by a Confocal System..... <i>Jerry Sedgewick</i>	97
5 Clearing Up the Signal: Spectral Imaging and Linear Unmixing in Fluorescence Microscopy..... <i>Timo Zimmermann, Joanne Marrison, Karen Hogg, and Peter O'Toole</i>	129
6 Low Magnification Confocal Microscopy of Tumor Angiogenesis <i>George McNamara, Anna Yanai, Vazgen Khankaldyyan, Walter E. Laug, Jeff Boden, Keith Webster, Yiwen Li, and Rong Wen</i>	149
7 Confocal Imaging of Butterfly Tissue..... <i>Craig R. Brunetti</i>	177
8 Confocal Microscopy of Cardiac Myocytes <i>Robert L. Price, Stephen T. Haley, Tara Bullard, Jeffrey Davis, Thomas K. Borg, and Louis Terracio</i>	185
9 Confocal Imaging of Fluorescently Labeled Proteins in the <i>Drosophila</i> Larval Neuromuscular Junction..... <i>Ian P. Coyle</i>	201
10 Confocal Imaging and Three-Dimensional Visualization of Thick Autofluorescent Specimens..... <i>Angela V. Klaus, Valerie Schawaroch, and Kevin J. Frischmann</i>	213
11 Vital Imaging of Multicellular Spheroids <i>Paulo J. Oliveira, Edward L. Perkins, and Jon Holy</i>	227
12 Live Confocal Analysis of Mutant- and Drug-Treated <i>Drosophila</i> Embryos <i>Barbara Fasulo and William Sullivan</i>	243

13	Confocal Imaging of the Microtubule Cytoskeleton in <i>C. elegans</i> Embryos and Germ Cells	257
	<i>Kevin F. O'Connell and Andy Golden</i>	
14	Measurement in the Confocal Microscope.....	273
	<i>Guy Cox</i>	
15	A Method for Quantifying Blood Flow Distribution Among the Alveoli of the Lung.....	297
	<i>Robert L. Conhaim, Dennis M. Heisey, and Glen E. Levenson</i>	
16	Imaging Tools for Analysis of the Ureteric Tree in the Developing Mouse Kidney	305
	<i>Luise A. Cullen-McEwen, Richard J. Young, Gabriel Ericout, Dominique Jeulin, Ian S. Harper, Frank Costantini, and John F. Bertram</i>	
17	Evaluating Confocal Microscopy System Performance	321
	<i>Robert M. Zucker</i>	
	Erratum.....	E1
	<i>Index</i>	375

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