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Methods and Protocols

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Cover design by Patricia F. Cleary.
Cover legend: Foreground panel: A triple labeled *Drosophila* third instar wing imaginal disc imaged using the laser scanning confocal microscope (see Chapter 1, Fig. 4) and displayed so that color is mapped to the expression of three wing patterning genes using methods outlined in Chapter 21. *(Specimen courtesy of Jim Williams and imaged by Steve Paddock, both of the University of Wisconsin.)* Background image: A confocal Z-series collected from groups of cultured rat BN/MSV cells is displayed so that color is mapped to depth in the specimen using methods outlined in Chapter 21 (Note 3). *(Specimen courtesy of Tim Hammond, Tulane University, and imaged by Steve Paddock.)*

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Preface

The confocal microscope is an established tool in many fields of biomedical research where its major application is for improved light microscopic imaging of cells within fluorescently-labeled tissues. Indeed, before the advent of practical confocal microscopy, most fluorescently-labeled tissues were imaged using a conventional light microscope. Resolution was often compromised by fluorescence from outside the focal plane of interest, especially in tissues made up of multiple cell layers. In order to attain acceptable resolution, microscopists were forced into all manner of tricks to prepare their specimens, including cutting sections of the tissues, growing cells on a coverslip, flattening cells under agar, or squashing embryos between coverslips. Most of these methods had the potential of introducing artifacts and therefore led to questions about the validity of the results, especially images of living cells.

The confocal microscope has now enabled the imaging of discrete regions of tissues virtually free of out-of-focus fluorescence, and with reduced chances of artifacts from the techniques of specimen preparation. Indeed, in some cases virtually no preparation of the tissue is required prior to imaging although it is usual to stain tissues with one or more fluorescent probes for confocal imaging of specific macromolecules within cells. The field of confocal microscopy has grown exponentially over the past ten or so years, and it touches on many areas of contemporary biological research where a light microscope is required for imaging, including applications in cell biology, developmental biology, neurobiology, and pathology. It would therefore be impossible to cover all of the protocols in current use in a single book, and references to other sources including microscopy web pages have been included.

An overall effort has been made in recent years to render confocal imaging systems more user friendly. A relatively short training period is now required before a novice, who has experience with a light microscope and a basic working knowledge of a computer, is able to produce acceptable images. In practice, however, resolution of the images collected with a confocal microscope depends upon various properties of the sample itself, which means that a well-prepared specimen is extremely important for achieving images of the highest quality. The old saying "garbage in, garbage out" is a valuable maxim to keep in mind, not only for the novice, but also for the

more-experienced user. Protocols for preparing such specimens are therefore extremely important, and emphasis has been placed on the details of specimen preparation throughout.

The aim of *Confocal Microscopy Methods and Protocols* is to take the researcher from the bench top, through the imaging process, to the journal page. This book is light on the technical details of the microscopes themselves, as these can be found elsewhere and are continually changing as new technology is incorporated into confocal systems. The chapters have been chosen to highlight the biological applications of the confocal microscope and methods for the analysis and the presentation of the images for publication. Protocols for the preparation of tissues from most of the currently popular model organisms, including plants, have been covered, with the addition of chapters on confocal imaging of living cells, three dimensional analysis, and the measurement and presentation of confocal images for publication.

I would like to thank all of the authors, especially those who have persevered through many forms of adversity—both mental and physical (including at least one El-Niño-related incident)—in order to complete their chapters in a timely fashion. I would also like to thank my laboratory colleagues (past and present) for presenting me with such a plethora of imaging questions. A special thank-you goes to Sean Carroll for his encouragement of this project. The series editor, John Walker, has not only provided expert editorial advice, but has also kept me abreast of the cricket scores in the UK! In addition, Tom Lanigan and the staff at Humana Press, especially Patricia Cleary and Fran Lipton, have performed to an extremely high standard of professionalism throughout the project. The color section of the book would not have been possible without the extremely generous sponsorship of Bio-Rad, and I thank Leonard Pulig of Bio-Rad for his help in this matter. Finally, I would like to thank Diana Wheeler for her tolerance and support, especially during the final stages of the project, which happened to coincide with the preparations for our wedding.

Steve Paddock

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Color Plates

Color Plates I–IV appear as an insert following p. 372.

Plate I — *Top Sequence*: Multiple-label *Drosophila* embryos. **(A-B)** Structure of double label images, Fig. 21-2 A-B; **(C-D)** a three-color image, Figs. 10-1, 21-1 B, D. *Lower Left Sequence*: Examples of double labeling of a *Drosophila* embryo and wing imaginal disk using FISH, Fig. 5-1 A-B. *Lower Right Sequence*: Four-dimensional visualization of in situ chondrocytes by color-coded relative volume changes, Fig. 18-13 A-F.

Plate II — Double and triple antibody labelling of *Drosophila* eye imaginal disks, Fig. 4-1.

Plate III — Nuclear and cytosolic calcium dynamics, Fig. 16-8 A.

Plate IV — Three-dimensional illustrations prepared for the parallel-eyed viewing method (standard method for publication), Fig. 22-8 A-C.