

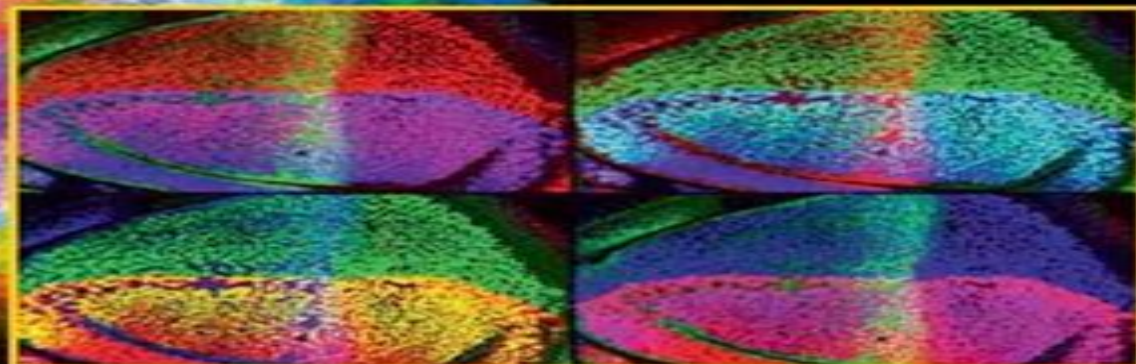
Methods in Molecular Biology™

VOLUME 122

Confocal Microscopy

Methods and Protocols

Edited by
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Confocal Microscopy Methods And Protocols

Stephen W. Paddock



Confocal Microscopy Methods And Protocols:

Confocal Microscopy Stephen W. Paddock, 2016-08-23 *Confocal Microscopy Methods and Protocols* Second Edition takes the researcher from the bench top through the imaging process to the page 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 Emphasis has been placed on the laser scanning confocal microscope since this is still the instrument used for most routine applications 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 This allows the imaging of living specimens and measurements of physiological events within cells Confocal microscopy has become essential in many fields of contemporary biomedical research where a light microscope is required for imaging fluorescently labeled cells and tissues especially cell biology developmental biology neurobiology and pathology Written in the successful *Methods in Molecular Biology* series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible protocols and notes on troubleshooting and avoiding known pitfalls Authoritative and easily accessible *Confocal Microscopy Methods and Protocols* Second Edition is aimed primarily but not exclusively at the novice user with pointers to more advanced techniques

Confocal Microscopy Stephen W. Paddock, 2008-02-03
Methods in Molecular Biology: Confocal microscopy methods and protocols John M. Walker, 1984
Super-Resolution Microscopy Holger Erfle, 2017 This volume presents current advanced technologies and methods used in super resolution microscopy The chapters in this book cover a wide range of topics such as introducing super resolution microscopy into a core facility two photon STED microscopy for nanoscale imaging of neural morphology in vivo correlative SIM STORM microscopy two color single molecule tracking in live cells and correlative single molecule localization microscopy and confocal microscopy Written in the highly successful *Methods in Molecular Biology* series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Cutting edge and comprehensive *Super Resolution Microscopy Methods and Protocols* is a valuable resource for both established and novel researchers and users in this field
Light Microscopy Yolanda Markaki, Hartmann Harz, 2017-04-05 This volume addresses up to date light microscopy approaches and toolsets offered for live or fixed cell observations The imaging strategies discussed in this book include confocal laser scanning and spinning disk confocal microscopy FRET FRAP and laser microsurgery experiments Chapters also describe the use of these imaging methodologies to study properties of a multitude of biomolecular targets in a broad range of model systems ranging from bacteria over tissue to whole animal imaging *Light Microscopy Methods and Protocols* puts special focus on system instrumentation parameters and sophisticated labeling and detection methods Written

in the highly successful Methods in Molecular Biology series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Cutting edge and thorough Light Microscopy Methods and Protocols offers the novice user with straightforward strategies to address biological questions while providing the experienced researcher with the latest applications that can be useful in routine practices This book also serves as a useful teaching manual in practical courses on light microscopy

Connexin Methods and Protocols Roberto Bruzzone, Christian Giaume, 2008-02-05 Direct cell cell communication is a common property of multicellular organisms that is achieved through membrane channels which are organized in gap junctions The protein subunits of these intercellular channels the connexins form a multigene family that has been investigated in great detail in recent years It has now become clear that in different tissues connexins speak several languages that control specific cellular functions This progress has been made possible by the availability of new molecular tools and the improvement of basic techniques for the study of membrane channels as well as by the use of genetic approaches to study protein function in vivo More important connexins have gained visibility because mutations in some connexin genes have been found to be linked to human genetic disorders Connexin Methods and Protocols presents in detail a collection of techniques currently used to study the cellular and molecular biology of connexins and their physiological properties The field of gap junctions and connexin research has always been characterized by a multidisciplinary approach combining morphology biochemistry biophysics and cellular and molecular biology This book provides a series of cutting edge protocols and includes a large spectrum of practical methods that are available to investigate the function of connexin channels Connexin Methods and Protocols is divided into three main parts

Nuclease Methods and Protocols Catherine H. Schein, 2008-02-03 Nucleases enzymes that restructure or degrade nucleic acid polymers are vital to the control of every area of metabolism They range from housekeeping enzymes with broad substrate ranges to extremely specific tools 1 Many types of nucleases are used in lab protocols and their commercial and clinical uses are expanding The purpose of Nuclease Methods and Protocols is to introduce the reader to some well characterized protein nucleases and the methods used to determine their activity structure interaction with other molecules and physiological role Each chapter begins with a mini review on a specific nuclease or a nuclease related theme Although many chapters cover several topics they were arbitrarily divided into five parts Part I Characterizing Nuclease Activity includes protocols and assays to determine general processive distributive or specific mechanisms Methods to assay nuclease products identify cloned nucleases and determine their physiological role are also included here Part II Inhibitors and Activators of Nucleases summarizes assays for measuring the effects of other proteins and small molecules Many of these inhibitors have clinical relevance Part III Relating Nuclease Structure and Function provides an overview of methods to determine or model the 3 D structure of nucleases and their complexes with substrates and inhibitors A 3 D structure can greatly aid the rational design of nucleases and inhibitors for

specific purposes Part IV Nucleases in the Clinic summarizes assays and protocols suitable for use with tissues and for nuclease based therapeutics

Molecular Methods in Developmental Biology Matt Guille, 2008-02-03 The process whereby a single cell the fertilized egg develops into an adult has fascinated for centuries Great progress in understanding that process has ever been made in the last two decades when the techniques of molecular biology have become available to developmental biologists By applying these techniques the exact nature of many of the interactions responsible for forming the body pattern are now being revealed in detail Such studies are a large and it seems ever expanding part of most life science groups It is at newcomers to this field that this book is primarily aimed A number of different plants and animals serve as common model organisms for developmental studies In *Molecular Methods in Developmental Biology* *Xenopus* and Zebrafish a range of the molecular methods applicable to two of these organisms are described these are the South African clawed frog *Xenopus laevis* and the zebrafish *Brachydanio rerio* The embryos of both of these species develop rapidly and externally making them particularly suited to investigations of early vertebrate development However both *Xenopus* and zebrafish have their own advantages and disadvantages *Xenopus* have large robust embryos that can be manipulated surgically with ease but their pseudotetraploidy and long generation time make them unsuitable candidates for genetics This disadvantage may soon be overcome by using the diploid *Xenopus tropicalis* and early experiments are already underway The transparent embryos of zebrafish render them well suited for in situ hybridization and immunohistochemistry and good for observing mutations in genetic screens

Electron Microscopy John Kuo, 2013-12-20 This third edition of *Electron Microscopy Methods and Protocols* expands upon the previous editions with current detailed protocols on biological and molecular research techniques based on TEM and SEM as well as other closely related imaging and analytical methods With new chapters on conventional and microwave assisted specimen cryo specimen preparation negative staining and immunogold labelling techniques DNA and RNA tracking using hybridization in TEM or Atomic Force Microscopy TEM crystallography and cryo TEM 3D tomography 3D tomography of resin embedded tissues using FIB SEM Correlative microscopy using fluorescence microscopy confocal microscopy or immune labelling techniques for both TEM and FIB SEM and Elemental and isotopic identification and their distribution in cells and tissues using TEM SEM Scanning Transmission Electron Microscopy STEM Secondary Ion Mass Spectrometry SIMS and Nano SIMS Written in the highly successful *Methods in Molecular Biology* series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Authoritative and practical *Electron Microscopy Methods and Protocols Third Edition* provides the most up to date and essential information in electron microscopy techniques and methods provided in this edition will assist in advancing future molecular and biological research

Flavoprotein Protocols Steven K. Chapman, Graeme A. Reid, 2008-02-03 As a scientist with an interest in proteins you will at some time in your career isolate an enzyme that turns

out to be yellow or perhaps you already have Alternatively you may identify a polypeptide sequence that is related to known flavin containing proteins This may or may not be your first encounter with flavoproteins However even if you are an old hand in the field you may not have exploited the full range of experimental approaches applicable to the study of flavoproteins We hope that Flavoprotein Protocols will encourage you to do so In this volume we have sought to bring together a range of experimental methods of value to researchers with an interest in flavoproteins whether or not these researchers have experience in this area A broad range of techniques from the everyday to the more specialized is described by scientists who are experts in their fields and who have extensive practical experience with flavoproteins The wide range of approaches from wet chemistry to dry computation has as a consequence demanded a range of formats Where appropriate particularly for analytical methods the protocol described is laid out in easy to follow steps In other cases e g the more advanced spectroscopies and computational methods it is far more apt to describe the general approach and relevance of the methods We hope this wide ranging approach will sow the seeds of many future collaborations between laboratories and further our knowledge and understanding of how flavoproteins work

Principles of Light Microscopy: From Basic to Advanced Volodymyr Nechyporuk-Zloy, 2022-11-29 This textbook is an excellent guide to microscopy for students and scientists who use microscopy as one of their primary research and analysis tool in the laboratory The book covers key microscopy principles and explains the various techniques such as epifluorescence microscopy confocal live cell imaging SIM light sheet microscopy and many more Easy to understand protocols provide helpful guidance for practical implementation in various commercially available imaging systems The reader is introduced to histology and further be guided through advanced image acquisition classification and analysis The book is written by experienced imaging specialists from the UK other EU countries the US and Asia and is based on advanced training courses for master students and PhD students Readers are not expected to be familiar with imaging and microscopy technologies but are introduced to the subject step by step This textbook is intended for biomedical and medical students as well as scientists and postdocs who want to acquire a thorough knowledge of microscopy or gain a comprehensive overview of modern microscopy techniques used in various research laboratories and imaging facilities Chapter 4 is available open access under a Creative Commons Attribution 4.0 International License via link.springer.com

The ELISA Guidebook John R. Crowther, 2008-02-04 John R Crowther provides today's premier practical guide to the understanding and application of ELISA Updating and greatly expanding his widely appreciated earlier publication *ELISA Theory and Practice* 1995 this important work introduces chapters on such major new topics as checkerboard titrations quality control of testing kit production and control novel monoclonal antibodies validation of assays statistical requirements for data examination and epidemiological considerations With its numerous worked examples detailed instructions and extensive illustrations The ELISA Guidebook offers a powerful synthesis of all the basic concepts and practical experimental details investigators need to understand develop and apply the new ELISA methodology

successfully in day to day basic and clinical research Matrix Metalloproteinase Protocols Ian M. Clark,2008-02-05 Research in the matrix metalloproteinase field began with the demonstration by Gross and Lapi re in 1962 that resorbing tadpole tail expressed an enzyme that could degrade collagen gels These humble beginnings have led us to the elucidation of around twenty distinct vertebrate MMPs along with a variety of homologs from such diverse organisms as sea urchin plants nematode worm and bacteria This coupled with four known specific inhibitors of MMPs the TIMPs gives a complex picture Part I of Matrix Metalloproteinase Protocols provides the reader with a selective overview of the MMP arena and a chance to come to grips with where the field has been where it is and where it is going I hope that this complements all of the methodology that comes later Part II presents the reader with a diverse set of methods for the expression and purification of MMPs and TIMPs bringing together the long and often hard earned experience of a number of researchers Part III allows the reader to detect MMPs and TIMPs at both the protein and mRNA level whereas Part IV gives the ability to assay MMP and TIMP activities in a wide variety of circumstances **Basic Confocal Microscopy** W. Gray (Jay) Jerome,Robert L. Price,2018-10-30 Basic Confocal Microscopy Second Edition builds on the successful first edition by keeping the same format and reflecting relevant changes and recent developments in this still burgeoning field This format is based on the Confocal Microscopy Workshop that has been taught by several of the authors for nearly 20 years and remains a popular workshop for gaining basic skills in confocal microscopy While much of the information concerning fluorescence and confocal microscopy that made the first edition a success has not changed in the six years since the book was first published confocal imaging is an evolving field and recent advances in detector technology operating software tissue preparation and clearing image analysis and more have been updated to reflect this Several of these advances are now considered routine in many laboratories and others such as super resolution techniques built on confocal technology are becoming widely available

Proteoglycan Protocols Renato V. Iozzo,2008-02-02 Proteoglycans are some of the most elaborate macromolecules of mammalian and lower organisms The covalent attachment of at least five types of glycosami glycan side chains to more than forty individual protein cores makes these molecules quite complex and endows them with a multitude of biological functions Proteoglycan Protocols offers a comprehensive and up to date collection of prepa tive and analytical methods for the in depth analysis of proteoglycans Featuring st by step detailed protocols this book will enable both novice and experienced researchers to isolate intact proteoglycans from tissues and cultured cells to establish the composition of their carbohydrate moieties to generate strategies for prokaryotic and eukaryotic expression to utilize methods for the suppression of specific proteoglycan gene expression and for the detection of mutant cells and degradation products and to study specific interactions between proteoglycans and extracellular matrix proteins as well as growth factors and their receptors The readers will find concise yet comprehensive techniques carefully drafted by leading experts in the field Each chapter commences with a general Introduction followed by a detailed Materials section and an easy to follow Methods section An

asset of each chapter is the extensive notation that includes troubleshooting tips and practical considerations that are often lacking in formal methodology papers. The reader will find this section most valuable because it is clearly provided by experienced scientists who have first hand knowledge of the techniques they outline. In addition most of the chapters are well illustrated with examples of typical data generated with each method.

Gene Knockout Protocols Martin J. Tymms, Ismail Kola, 2008-02-03 As the major task of sequencing the human genome is near completion and full complement of human genes are catalogued attention will be focused on the ultimate goal to understand the normal biological functions of these genes and how alterations lead to disease states. In this task there is a severe limitation in working with human material but the mouse has been adopted as the favored animal model because of the available genetic resources and the highly conserved gene conservation linkage organization. In just of ten years since the first gene targeting experiments were performed in embryonic stem ES cells and mutations transmitted through the mouse germline more than a thousand mouse strains have been created. These achievements have been made possible by pioneering work that showed that ES cells derived from preimplantation mouse embryos could be cultured for prolonged periods without differentiation in culture and that homologous recombination between targeting constructs and endogenous DNA occurred at a frequency sufficient for recombinants to be isolated. In the next few years the mouse genome will be systematically altered and the techniques for achieving manipulations are constantly being streamlined and improved.

Genomics Protocols Michael P. Starkey, Ramnath Elaswarapu, 2008-02-03 We must unashamedly admit that a large part of the motivation for editing Genomics Protocols was selfish. The possibility of assembling in a single volume a unique and comprehensive collection of complete protocols relevant to our work and the work of our colleagues was too good an opportunity to miss. We are pleased to report however that the outcome is something of use not only to those who are experienced practitioners in the genomics field but is also valuable to the larger community of researchers who have recognized the potential of genomics research and may themselves be beginning to explore the technologies involved. Some of the techniques described in Genomics Protocols are clearly not restricted to the genomics field indeed a prerequisite for many procedures in this discipline is that they require an extremely high throughput beyond the scope of the average investigator. However what we have endeavored here to achieve is both to compile a collection of procedures concerned with genome scale investigations and to incorporate the key components of bottom up and top down approaches to gene finding. The technologies described extend from those traditionally recognized as coming under the genomics umbrella touch on proteomics the study of the expressed protein complement of the genome through to early therapeutic approaches utilizing the potential of genome programs via gene therapy. Chapters 27-30

Capillary Electrophoresis of Nucleic Acids Keith R. Mitchelson, Jing Cheng, 2008-02-05 The development of PCR which enables extremely small amounts of DNA to be amplified led to the rapid development of a multiplicity of analytical procedures that permit use of this new resource for the analysis of genetic variation and for the

detection of disease causing mutations The advent of capillary electrophoresis CE with its power to separate and analyze very small amounts of DNA has also stimulated researchers to develop analytical procedures for the CE format The advantages of CE in terms of speed and reproducibility of analyses are manifold Furthermore the high sensitivity of detection and the ability to increase sample throughput with parallel analysis has led to the creation of a full range of analysis of DNA molecules from modified DNA adducts and single strand oligonucleotides through PCR amplified DNA fragments and whole chromosomes

Capillary Electrophoresis of Nucleic Acids focuses on analytical protocols that can be used for detection and analysis of mutations and modification from precise DNA loci through entire genomes of organisms Important practical considerations for CE such as the choice of separation media electrophoresis conditions and the influence of buffer additives and dyes on DNA mobility are discussed in several key chapters and within particular applications

Confocal Microscopy Stephen W. Paddock, 1998-12-07 Stephen Paddock and a highly skilled panel of experts lead the researcher using confocal techniques from the bench top through the imaging process to the journal page They concisely describe all the key stages of confocal imaging from tissue sampling methods through the staining process to the manipulation presentation and publication of the realized image Written in a user friendly nontechnical style the methods specifically cover most of the commonly used model organisms worms sea urchins flies plants yeast frogs and zebrafish The powerful hands on methods collected here will help even the novice to produce first class cover quality confocal images

Protein Structure, Stability, and Folding Kenneth P. Murphy, 2008-02-04 In Protein Structure Stability and Folding Kenneth P Murphy and a panel of internationally recognized investigators describe some of the newest experimental and theoretical methods for investigating these critical events and processes Among the techniques discussed are the many methods for calculating many of protein stability and dynamics from knowledge of the structure and for performing molecular dynamics simulations of protein unfolding New experimental approaches presented include the use of co solvents novel applications of hydrogen exchange techniques temperature jump methods for looking at folding events and new strategies for mutagenesis experiments Unique in its powerful combination of theory and practice Protein Structure Stability and Folding offers protein and biophysical chemists the means to gain a more comprehensive understanding of some of this complex area by detailing many of the major techniques in use today

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