Cell Biology
Protocols

Editors Robin Harris
John Graham
David Rickwood

WILEY

Cell Biology Protocols

Editors

J. Robin Harris
Institute of Zoology,
Johannes Gutenberg-Universität, Mainz, Germany

John Graham
JG Research Consultancy, Upton, Wirral, UK

David Rickwood
Department of Biological Sciences, University of Essex, Colchester, UK

John Wiley & Sons, Ltd

Telephone (+44) 1243 779777

Email (for orders and customer service enquiries): cs-books@wiley.co.uk
Visit our Home Page on www.wiley.com

Other Wiley Editorial Offices

John Wiley & Sons Inc., 111 River Street, Hoboken, NJ 07030, USA

Jossey-Bass, 989 Market Street, San Francisco, CA 94103-1741, USA

Wiley-VCH Verlag GmbH, Boschstr. 12, D-69469 Weinheim, Germany

John Wiley & Sons Australia Ltd, 42 McDougall Street, Milton, Queensland 4064, Australia

John Wiley & Sons (Asia) Pte Ltd, 2 Clementi Loop #02-01, Jin Xing Distripark, Singapore 129809

John Wiley & Sons Canada Ltd, 22 Worcester Road, Etobicoke, Ontario, Canada M9W 1L1

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Library of Congress Cataloging-in-Publication Data:

Cell biology protocols / editors, J. Robin Harris, John Graham, David Rickwood.
p. cm.
ISBN-13: 978-0-470-84758-1
ISBN-10: 0-470-84758-1
1. Cytology – Laboratory manuals. I. Harris, James R. II. Graham, J. M. (John M.), 1943- III. Rickwood, D. (David)
QH583.2.C47 2005
571.6078 – dc22

2005029713

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN-13: 978-0-470-84758-1
ISBN-10: 0-470-84758-1

Typeset in 10.5/12.5pt Times by Laserwords Private Limited, Chennai, India
Printed and bound in Great Britain by Antony Rowe Ltd, Chippenham, Wiltshire
This book is printed on acid-free paper responsibly manufactured from sustainable forestry in which at least two trees are planted for each one used for paper production.

Cell Biology Protocols

Preface

Cell biology is a rapidly expanding discipline that is dependent upon continual technical development. We have attempted to compile an exciting and broadly useful cell biology techniques book, containing tried-and-tested procedures as well as newly established ones. Thus, this book contains an extensive series of routine and up-to-date protocols of value for those studying diverse aspects of present-day cell biology. The book commences with the presentation of several essential light microscopical procedures and leads on to the basic procedures required for producing a range of different cellular, subcellular and macromolecular specimens for transmission electron microscopical study. Then follows a chapter dealing with cell culture and cell separation procedures that are widely used to provide starting material for cellular research. The numerous techniques needed to study subcellular organelles and isolated cellular membranes are presented in the next two chapters, thereby providing the main thrust of the book. A series of 44 more specialist techniques used for *in vitro* studies and reassembly approaches in cell biology appear in the next chapter, each contributed by authors knowledgeable and experienced in their field of study. Finally, a reference chapter contains useful information on chemical hazard/safety aspects, centrifugation and radioisotopes. The book has a strong practical content and is directed to those at all levels who perform research in cell biology.

J. Robin Harris
John Graham
David Rickwood

June, 2005

List of Contributors

Judie B. Alimonti
Special Pathogens Program, National Microbiology Laboratory, H2380, 1015 Arlington Ave. Winnipeg, Manitoba, Canada, R3E 3R2

Geneviéve Almouzni
Institut Curie, CNRS, UMR 218, Section Recherche, 26 rue Ulm, F-75248 Paris 05, France

Alicia Alonso
Universidad del Pais Vasco, EHU, CSIC, Unidad Biofis, Aptdo 644, E-48080, Spain

Susan L. Bane
Department of Chemistry, SUNY, Binghamton, NY 13902-6016, USA

Julie Benesova
Lehrstuhl für Biochemie der Pflanzen, Ruhr-Universität Bochum, D-447780 Bochum, Germany

Eric Bertrand
Novartis Pharma AG, CH-4002, Switzerland

Stephanie Boggasch
Institut für Allgemeine Botanik des Johannes-Gutenberg-Universität, Müllerweg 6, D-55099 Mainz, Germany

Igor Bronstein
BBSRC Institute for Animal Health, High Street, Crompton RG20 7NN, UK

William J. Brown
Biochemistry, Molecular and Cell Biology Sections, Cornell University, Ithaca, NY 14853, USA

Koert N.J. Burger
Department of Biochemical Physiology, Institute of Biomembranes, Room W210, Padualaan 8, 3584 CH Utrecht, The Netherlands

K. Chambers
Biochemistry, Molecular and Cell Biology Sections, Cornell University, Ithaca, NY 14853, USA

C. Yan Cheng
Population Council, Center of Biomedical Research, 1230 York Avenue, New York NY 10021, USA

Richard Chi
Department of Biological Science, Florida State University, Tallahassee, FL 32306-4370, USA

Anton I.P.M. de Kroon
Department Biochemistry of Membranes, Centre for Biomembranes and Lipid Enzymology, Institute of Biomembranes, Padualaan 8, 3584 CH Utrecht, The Netherlands

Ben de Kruijff
Department Biochemistry of Membranes, Centre for Biomembranes and Lipid Enzymology, Institute of Biomembranes, Padualaan 8, 3584 CH Utrecht, The Netherlands

Daniela S. Dimitrova
Center for Single Molecule Biophysics and Department of Microbiology, 304 Sherman Hall, SUNY at Buffalo, Buffalo, NY 14214, USA

A. Doody
Biochemistry, Molecular and Cell Biology Sections, Cornell University, Ithaca, NY 14853, USA

H. Dariush Fahimi
Institute of Anatomy and Cell Biology, University of Heidelberg, INF 307, Neuenheimer Feld 307, D-69120 Heidelberg, Germany

Paul G. Fitzgerald
Department of Cell Biology and Human Anatomy, School of Medicine, 1 Shields Avenue, Davis, CA 95616-8643, USA

Roland Foisner
Department of Molecular Cell Biology, Institute of Medical Biochemistry, Vienna Biocenter, University of Vienna, Dr. Bohr Gasse 9/3, A-1030 Vienna, Austria

Barbara Gajkowska
The Laboratory of Cell Ultrastructure, Polish Academy of Sciences, Warsaw, Poland

Ya-sheng Gao
Department of Pathology, Duke University Medical Center, Box No. 3020, Rm 225, Jones Bldg, Durham, NC 27, USA

Robert Gniadecki
University of Copenhagen, Bispebjerg Hospital, Department of Dermatology D92, Bispebjerg Bakke 23, DK-2400 Copenhagen NV, Denmark

Félix M. Goñi
Universidad del País Vasco, EHU, CSIC, Unidad Biofis, Aptdo 644, E-48080, Spain

John Graham
JG Research Consultancy, 34 Meadway, Upton Wirral CH49 6IQ, UK

Arnold H. Greenberg†
University of Manitoba, Department of Medical Microbiology, 539-730 William Avenue, Winnipeg, MB, R3E OV9, Canada

†deceased

J. Robin Harris
Institute of Zoology, University of Mainz, D-55099 Mainz, Germany

John F. Hess
Department of Cell Biology and Human Anatomy, School of Medicine, 1 Shields Avenue, Davis, CA 95616-8643, USA

Martin Hetzer
Molecular and Cell Biology Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

Matthew K. Higgins
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Shin-ichi Hisanaga
Department of Biology, Tokyo Metropolitan University, Graduate School of Science, Hachioji, Tokyo 1920397, Japan

David F. Holmes
Wellcome Trust Centre for Cell Matrix Research, School of Biological Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester MI3 9PT, UK

Karl E. Kadler
Wellcome Trust Centre for Cell Matrix Research, School of Biological Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester MI3 9PT, UK

Thomas C.S. Keller
Department of Biological Sciences, Florida State University, Tallahassee, FL 32306-4370, USA

Helmut Kirchhoff
Institut für Botanik, Westfälische Wilhelms-Universität Münster, Schlossplatz 2, D-48149 Münster, Germany

Doris Kirschner
Institut Carie, 26 rue d'Ulm, 75248 Paris Cedex 05, France

Barbara Korbei
Department of Molecular Cell Biology, Institute of Medical Biochemistry, Vienna Biocenter, University of Vienna, Dr. Bohr Gasse 9/3, A-1030 Vienna, Austria

Marina Kriajevska
University of Leicester, Clinical Sciences Unit, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW, UK

Sven-T. Liffers
Lehrstuhl für Biochemie der Pflanzen, Ruhr-Universität Bochum, D-447780 Bochum, Germany

Yuechueng Liu
Department of Pathology, University of Oklahoma Health Services Center, Oklahoma City, OK 73104, USA

Eugene Lukanidin
Danish Center Society, Institute of Cancer Biology, Department of Molecular Cancer Biology, Strandblvd 49, 4-3, DK-2100 Copenhagen, Denmark

Ian G. Mills
Dept. of Neurobiology, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Nathaniel G.N. Milton
Department of Molecular Pathology & Clinical Biochemistry, Royal Free Hospital Campus, Rowland Hill Street, London NW3 2PF, UK

Dolores D. Mruk
Population Council, Center of Biomedical Research, 1230 York Avenue, New York, NY10021, USA

Luis Eduardo Soares Netto
Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277; Sala 327, Cidade Universitária, CEP 05508-900, São Paulo-SP, Brazil

Jeffrey A. Nickerson
Department of Cell Biology, School of Medicine, University of Massachusetts, 55 Lake Avenue N., Worcester, MA 01655, USA

Minnie O'Farrell
Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, UK

Jacques Paiement
Département de Pathologie et Biologie Cellulaire, Université de Montréal N-813, Pavilion Principal, 2900 Edouard-Montpetit, Montréal, Québec H3T 1J4, Canada

Harald Paulsen
Institut für Allgemeine Botanik der Johannes-Gutenberg-Universität, Müllerweg 6, D-55099 Mainz, Germany

Brian J. Peter
McMahon Laboratory, Neurobiology Division, MRC-LMB, Hills Road, Cambridge CB2 2QH, UK

Reiner Peters
Institut für Medizinische Physik und Biophysik, Universität Münster, Robert-Koch-Straße 31, D-48149 Münster, Germany

Anuradha Pradhan
Department of Pathology, University of Oklahoma Health Services Center, Oklahoma City, OK 73104, USA

David Rickwood
Department of Biological Sciences, University of Essex, Colchester, UK

Matthias Rögner
Lehrstuhl für Biochemie der Pflanzen, Ruhr-Universität Bochum, D-447780 Bochum, Germany

T. Sasaki
Department of Biology, Tokyo Metropolitan University, Graduate School of Science, Hachioji, Tokyo 1920364, Japan

Rutger W.H.M. Staffhorst
Department Biochemistry of Membranes, Centre for Biomembranes and Lipid Enzymology, Institute of Biomembranes, Padualaan 8, 3584 CH Utrecht, The Netherlands

Elizabeth Sztul
Department of Cell Biology, University of Alabama, McCullum Bldg, Rm 668, 1530 S. 3rd Avenue, Birmingham, AL 35294, USA

Jun Tan
The Roskamp Institute, University of South Florida, 3515 E. Fletcher Avenue, Tampa, FL 33613, USA

Meinolf Thiemann
Graffinity Pharmaceuticals AG, Im Neuenheimer Feld 518-519, D-69120 Heidelberg, Germany

Terrence Town
Yale University School of Medicine and Howard Hughes Medical Institute, 310 Cedar St., PO Box 208011, New Haven, CT 06520-8011, USA

Kenji Uéda
Department of Biology, Tokyo Metropolitan University, Graduate School of Science, Hachioji, Tokyo 1920364, Japan

Jean Underwood
Department of Cell Biology, University of Massachusetts Medical School, 55 Lake Avenue, Worcester, MA 01655, USA

Ana-Victoria Villar
Universidad del País Vasco, EHU, CSIC, Unidad Biofis, Aptdo 644, E-48080, Spain

John C. Voss
Department of Biological Chemistry, School of Medicine, 1 Shields Avenue, Davis, CA 95616-8643, USA

Stefan Wagner
Department of Cell Biology, University of Massachusetts Medical School, 55 Lake Avenue, Worcester, MA 01655, USA

Ivan Walev
Institute for Medical Microbiology and Hygiene, University of Mainz, Hochhaus Augustusplatz, D-55131 Mainz, Germany

Tobias C. Walther
EMBL, Gene Expression Programme, Meyerhofstrasse 1, 69117 Heidelberg, Germany

Anne Wilson
Woodbine Terrace, Stanton, Ashbourne Derbyshire DE6 2DA

F.-Xabier Contreras
Universidad del Pais Vasco, EHU, CSIC, Unidad Biofis, Aptdo 644, E-48080, Spain

Jinnan Xiao
Department of Pathology, University of Oklahoma Health Services Center, Oklahoma City, OK 73104, USA

Chunhong Yang
Institut für Allgemeine Botanik der Johannes-Gutenberg-Universität, Müllerweg 6, D-55099 Mainz, Germany

Robin Young
Département de Pathologie et Biologie Cellulaire, Université de Montréal N-813, Pavilion Principal, 2900 Edouard-Montpetit, Montréal, Québec H3T 1J4, Canada

Contents

Cells and membrane systems

Cytoskeletal and fibrillar systems

1

Basic Light Microscopy

Minnie O'Farrell

Introduction

Light microscopy is an indispensable technique for cell and molecular biologists to study cellular structures and biological processes in both living and fixed cells. This chapter provides an overview of light microscopy, describes the important parts of the microscope and goes on to explain how to set up a standard research microscope for bright field and phase contrast microscopy. There is also a short section on confocal microscopy. More comprehensive descriptions of the different forms of light microscopy are found elsewhere [1–4].

Microscopes are instruments that produce an enlarged image of a specimen. The eyepieces and the objectives are the main components of the magnification system of the microscope, the product of the magnification of the objective lens and the ocular lens give the total magnification of the microscope. The visibility of the magnified specimen depends on contrast and resolution. Contrast is the difference in light intensity between an object and its background. Some biological samples contain coloured compounds, for example pigmented animal cells and chlorophyll-containing chloroplasts in plant cells, but most biological samples are colourless and have to be fixed and stained before observation [5]. Such stained specimens are observed using bright field microscopy. Other kinds of microscope systems are available to enhance contrast in living samples; these include phase contrast, dark field, differential interference contrast (DIC) and fluorescence microscopy (Table 1.1). The flow chart in Figure 1.1 will help in the selection of the appropriate microscopic observation method.

Cell Biology Protocols. Edited by J. Robin Harris, John Graham, David Rickwood

Table 1.1 Techniques for producing contrast in light microscopy

Type	Mechanism	Requirements	Fixed cells	Live cells	Appearance
Bright field	Absorption of visible light following staining of specimen	Any light microscope; range of histochemical stains	Yes	No	Coloured image depending on stains
Phase contrast	Variations in refractive index within specimen	Phase objective and phase condenser	Yes	Yes	Many shades of grey
Dark field	Scattered light	Dark field stop in condenser	Yes	Yes	Bright objects against dark background
Differential interference contrast	Gradient of refractive index	Special objective lens	Yes	Yes	3D effect
Fluorescence	Excitation and emission of light by fluorophore	An excitation light source and appropriate filters for emission; range of fluorescent probes including naturally fluorescent proteins	Yes	Yes	Bright colours against a dark background

The resolution of the optical system, that is the ability to distinguish objects separated by small distances, determines the degree of detail observable. The limit of resolution of the light microscope is about 0.2 μm. Enlarging the image too much results in 'empty magnification' and the quality of the image deteriorates. The limits of resolution are determined by the quality of the objective and the condenser.

Key components of the compound microscope

The eyepieces, body tube, nosepiece and objectives are part of the magnification system of the microscope. The condenser, condenser-iris diaphragm, filters, field iris diaphragm and light source are the parts that compose the illumination system of the microscope. To use a microscope properly, and to get the most out of it, it is important to understand the purpose and function of each of the microscope's components (Figure 1.2).

The body and lamp

The binocular body, the arm and the base form the frame of the microscope. This provides the stability and holds the optical and other components rigid and in place. The lamp is in the base of the body; its brightness is controlled by an on/off switch and a rheostat control knob. Just above the lamp is a collector lens with a field diaphragm to control the area of illumination. The field diaphragm also aids focusing and centring of the illumination.