

Atomic Force Microscopy
Biomedical Methods and Applications

METHODS IN MOLECULAR BIOLOGY™

John M. Walker, SERIES EDITOR

260. **Mobile Genetic Elements: Protocols and Genomic Applications**, edited by Wolfgang J. Miller and Pierre Capy, 2004
259. **Receptor Signal Transduction Protocols, Second Edition**, edited by Gary B. Willars and R. A. John Challiss, 2004
258. **Gene Expression Profiling: Methods and Protocols**, edited by Richard A. Shimkets, 2004
257. **mRNA Processing and Metabolism: Methods and Protocols**, edited by Daniel R. Schoenberg, 2004
256. **Bacterial Artificial Chromosomes, Volume 2: Functional Studies**, edited by Shaying Zhao and Marvin Stodolsky, 2004
255. **Bacterial Artificial Chromosomes, Volume 1: Library Construction, Physical Mapping, and Sequencing**, edited by Shaying Zhao and Marvin Stodolsky, 2004
254. **Germ Cell Protocols, Volume 2: Molecular Embryo Analysis, Live Imaging, Transgenesis, and Cloning**, edited by Heide Schatten, 2004
253. **Germ Cell Protocols, Volume 1: Sperm and Oocyte Analysis**, edited by Heide Schatten, 2004
252. **Ribozymes and siRNA Protocols, Second Edition**, edited by Mouldy Sioud, 2004
251. **HPLC of Peptides and Proteins: Methods and Protocols**, edited by Marie-Isabel Aguilar, 2004
250. **MAP Kinase Signaling Protocols**, edited by Rony Seger, 2004
249. **Cytokine Protocols**, edited by Marc De Ley, 2004
248. **Antibody Engineering: Methods and Protocols**, edited by Benny K. C. Lo, 2004
247. **Drosophila Cytogenetics Protocols**, edited by Daryl S. Henderson, 2004
246. **Gene Delivery to Mammalian Cells: Volume 2: Viral Gene Transfer Techniques**, edited by William C. Heiser, 2004
245. **Gene Delivery to Mammalian Cells: Volume 1: Nonviral Gene Transfer Techniques**, edited by William C. Heiser, 2004
244. **Protein Purification Protocols, Second Edition**, edited by Paul Cutler, 2004
243. **Chiral Separations: Methods and Protocols**, edited by Gerald Gibitz and Martin G. Schmid, 2004
242. **Atomic Force Microscopy: Biomedical Methods and Applications**, edited by Pier Carlo Braga and Davide Ricci, 2004
241. **Cell Cycle Checkpoint Control Protocols**, edited by Howard B. Lieberman, 2004
240. **Mammalian Artificial Chromosomes: Methods and Protocols**, edited by Vittorio Sgaramella and Sandro Eridani, 2003
239. **Cell Migration in Inflammation and Immunity: Methods and Protocols**, edited by Daniele D'Ambrosio and Francesco Sinigaglia, 2003
238. **Biopolymer Methods in Tissue Engineering**, edited by Anthony P. Hollander and Paul V. Hatton, 2003
237. **G Protein Signaling: Methods and Protocols**, edited by Alan V. Smrcka, 2003
236. **Plant Functional Genomics: Methods and Protocols**, edited by Erich Grotewold, 2003
235. **E. coli Plasmid Vectors: Methods and Applications**, edited by Nicola Casali and Andrew Preston, 2003
234. **p53 Protocols**, edited by Sumitra Deb and Swati Palit Deb, 2003
233. **Protein Kinase C Protocols**, edited by Alexandra C. Newton, 2003
232. **Protein Misfolding and Disease: Principles and Protocols**, edited by Peter Bross and Niels Gregersen, 2003
231. **Directed Evolution Library Creation: Methods and Protocols**, edited by Frances H. Arnold and George Georgiou, 2003
230. **Directed Enzyme Evolution: Screening and Selection Methods**, edited by Frances H. Arnold and George Georgiou, 2003
229. **Lentivirus Gene Engineering Protocols**, edited by Maurizio Federico, 2003
228. **Membrane Protein Protocols: Expression, Purification, and Characterization**, edited by Barry S. Selinsky, 2003
227. **Membrane Transporters: Methods and Protocols**, edited by Qing Yan, 2003
226. **PCR Protocols, Second Edition**, edited by John M. S. Bartlett and David Stirling, 2003
225. **Inflammation Protocols**, edited by Paul G. Winyard and Derek A. Willoughby, 2003
224. **Functional Genomics: Methods and Protocols**, edited by Michael J. Brownstein and Arkady B. Khodursky, 2003
223. **Tumor Suppressor Genes: Volume 2: Regulation, Function, and Medicinal Applications**, edited by Wafik S. El-Deiry, 2003
222. **Tumor Suppressor Genes: Volume 1: Pathways and Isolation Strategies**, edited by Wafik S. El-Deiry, 2003
221. **Generation of cDNA Libraries: Methods and Protocols**, edited by Shao-Yao Ying, 2003
220. **Cancer Cytogenetics: Methods and Protocols**, edited by John Swansbury, 2003
219. **Cardiac Cell and Gene Transfer: Principles, Protocols, and Applications**, edited by Joseph M. Metzger, 2003
218. **Cancer Cell Signaling: Methods and Protocols**, edited by David M. Terrian, 2003
217. **Neurogenetics: Methods and Protocols**, edited by Nicholas T. Potter, 2003
216. **PCR Detection of Microbial Pathogens: Methods and Protocols**, edited by Konrad Sachse and Joachim Frey, 2003
215. **Cytokines and Colony Stimulating Factors: Methods and Protocols**, edited by Dieter Körholz and Wieland Kiess, 2003
214. **Superantigen Protocols**, edited by Teresa Krakauer, 2003
213. **Capillary Electrophoresis of Carbohydrates**, edited by Pierre Thibault and Susumu Honda, 2003
212. **Single Nucleotide Polymorphisms: Methods and Protocols**, edited by Pui-Yan Kwok, 2003
211. **Protein Sequencing Protocols, Second Edition**, edited by Bryan John Smith, 2003
210. **MHC Protocols**, edited by Stephen H. Powis and Robert W. Vaughan, 2003
209. **Transgenic Mouse Methods and Protocols**, edited by Marten Hofker and Jan van Deursen, 2003
208. **Peptide Nucleic Acids: Methods and Protocols**, edited by Peter E. Nielsen, 2002
207. **Recombinant Antibodies for Cancer Therapy: Methods and Protocols**, edited by Martin Welschof and Jürgen Krauss, 2002
206. **Endothelin Protocols**, edited by Janet J. Maguire and Anthony P. Davenport, 2002

METHODS IN MOLECULAR BIOLOGY™

Atomic Force Microscopy

Biomedical Methods and Applications

Edited by

Pier Carlo Braga

*Department of Pharmacology
School of Medicine, University of Milan
Milan, Italy*

Davide Ricci

*Department of Biophysical and Electronic Engineering
University of Genoa
Genoa, Italy*

HUMANA PRESS  TOTOWA, NEW JERSEY

© 2004 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

www.humanapress.com

All rights reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher. Methods in Molecular Biology™ is a trademark of The Humana Press Inc.

The content and opinions expressed in this book are the sole work of the authors and editors, who have warranted due diligence in the creation and issuance of their work. The publisher, editors, and authors are not responsible for errors or omissions or for any consequences arising from the information or opinions presented in this book and make no warranty, express or implied, with respect to its contents.

This publication is printed on acid-free paper. 

ANSI Z39.48-1984 (American National Standards Institute) Permanence of Paper for Printed Library Materials.

Cover design by Patricia F. Cleary.

Cover illustrations: *Background*: low-force contact mode AFM image of fresh rabbit corneal endothelium treated with neuraminidase (Fig. 7, Chapter 6; *see* full caption on p. 80 and discussion on p. 78). *Inset*: scanning electron micrograph of an array of eight cantilevers with individual thicknesses of 500 nm (Fig. 3, Chapter 4; *see* discussion on pp. 42, 43).

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel: 973-256-1699; Fax: 973-256-8341; E-mail: humana@humanapr.com or visit our website at <http://humanapress.com>

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients is granted by Humana Press, provided that the base fee of US \$25.00 per copy is paid directly to the Copyright Clearance Center (CCC), 222 Rosewood Dr., Danvers MA 01923. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to the Humana Press. The fee code for users of the Transactional Reporting Service is 1-58829-094-8/04 \$25.00.

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

ISSN: 1064-3745

E-ISBN: 1-59259-647-9

Library of Congress Cataloging-in-Publication Data

Atomic force microscopy : biomedical methods and applications / edited
by Pier Carlo Braga, Davide Ricci.

p. ; cm. -- (Methods in molecular biology ; 242)

Includes bibliographical references and index.

ISBN 1-58829-094-8 (alk. paper)

1. Atomic force microscopy.

[DNLM: 1. Microscopy, Atomic Force--methods. 2. Molecular Biology--methods. QH 212.A78 A881 2004] I. Braga, Pier Carlo. II. Ricci, Davide. III. Series: Methods in molecular biology (Totowa, N.J.) ; v. 242.

QH212.A78A855 2004

570'.28'2--dc22

2003015100

Preface

The natural, biological, medical, and related sciences would not be what they are today without the microscope. After the introduction of the optical microscope, a second breakthrough in morphostructural surface analysis occurred in the 1940s with the development of the scanning electron microscope (SEM), which, instead of light (i.e., photons) and glass lenses, uses electrons and electromagnetic lenses (magnetic coils). Optical and scanning (or transmission) electron microscopes are called “far-field microscopes” because of the long distance between the sample and the point at which the image is obtained in comparison with the wavelengths of the photons or electrons involved. In this case, the image is a diffraction pattern and its resolution is wavelength limited.

In 1986, a completely new type of microscopy was proposed, which, without the use of lenses, photons, or electrons, directly explores the sample surface by means of mechanical scanning, thus opening up unexpected possibilities for the morphostructural and mechanical analysis of biological specimens. These new scanning probe microscopes are based on the concept of near-field microscopy, which overcomes the problem of the limited diffraction-related resolution inherent in conventional microscopes. Located in the immediate vicinity of the sample itself (usually within a few nanometers), the probe records the intensity, rather than the interference signal, thus significantly improving resolution. Since the most well-known microscopes of this type operate using atomic forces, they are frequently referred to as atomic force microscopes (AFMs). Given the progressive spread of commercial atomic force microscopes, their biomedical applications have increased greatly, and they are now used by researchers in many different fields.

Pioneering AFM studies were carried out in the natural sciences and biological and medical fields in the 1990s. Nevertheless, although the literature in this area is steadily increasing, the vast majority of biomedical researchers still know little about the possibilities of AFM. This is probably because of its particular technical background, which is more familiar to physicists or engineers, but unusual to biologists or physicians.

In comparison with the more familiar, simpler, and in some cases more intuitive forms of microscopy used by researchers in the life sciences, AFM offers a number of special features: very high magnification with very high resolution; minimal tissue or cell preparation (no dyes, as in optical microscopy; no vacuum, critical point, or gold sputtering, as in scanning electron microscopy); the ability to obtain different views of the sample from a single data collection; the ability to

work in an aqueous environment in real time using a nonintrusive local probe, thus making possible a study of the dynamic phenomena of live cells in their biofluid environment and under near physiological conditions with nanometer resolution; and finally (a breakthrough in the field of microscopy), the possibility of using the exploring probe in order to interact with the microscopic biological sample when measuring its electromagnetic and mechanical properties (stiffness, viscosity, elasticity, etc.). The limitations of the technique are still the relatively small scan size, the low scanning speeds, and the difficulties in imaging very soft biological samples.

A number of commercial instruments are on the market and various techniques have now been standardized. Although the number of articles in this field is increasing exponentially, they are mainly found in technical rather than more general biomedical journals, which is why this book is aimed at scientists beginning to use these techniques for the first time and with no prior knowledge.

The purpose of *Atomic Force Microscopy: Biomedical Methods and Applications* is to equip researchers in the life sciences with hands-on knowledge and to offer “recipes” like a cookbook to show the many-sided possibilities of different biomedical applications. As a volume of the *Methods in Molecular Biology* series, this book describes detailed practical procedures, accompanied by extensive practical details (how to do it). Each protocol is enhanced by Notes that help identify the problems that may be encountered and how they can be overcome. Each chapter in this volume is written in such a way that a competent scientist unfamiliar with the method can carry out the technique successfully at the first attempt by simply following the detailed descriptions of the practical procedures. Though biologists and physicians not yet working in this field may think the technical methodology complicated at first sight, careful reading of the various sections of this book and their schematic materials and methods should soon enable them to understand the basic approaches.

We decided to bring together a wide range of applications in order to provide examples of different subjects in different fields to first stimulate curiosity and then the interest of researchers in the life sciences in applying ingenuity to their specific fields, thus broadening and opening up new perspectives in ultrastructural biomedicine. We hope that this volume will help researchers approach this new microscopic world, find novel ideas and applications, and use AFM to add significant originality to their studies.

We gratefully acknowledge the contributions by our colleagues each of whom donated their experience in order to help us catalyze the development of this new and fascinating technology.

**Pier Carlo Braga
Davide Ricci**

Contents

Preface	v
Contributors	ix

PART I THE BASICS OF ATOMIC FORCE MICROSCOPY

1 How the Atomic Force Microscope Works <i>Davide Ricci and Pier Carlo Braga</i>	3
2 Imaging Methods in Atomic Force Microscopy <i>Davide Ricci and Pier Carlo Braga</i>	13
3 Recognizing and Avoiding Artifacts in AFM Imaging <i>Davide Ricci and Pier Carlo Braga</i>	25
4 Advanced Biosensing Using Micromechanical Cantilever Arrays <i>Martin Hegner and Youri Arntz</i>	39

PART II MORPHOSTRUCTURAL ANALYSIS OF CELLULAR STRUCTURES

5 Analysis of Human Fibroblasts by Atomic Force Microscopy <i>Gillian R. Bushell, Colm Cahill, Sverre Myhra, and Gregory S. Watson</i>	53
6 Corneal Tissue Observed by Atomic Force Microscopy <i>Stylliani Lydataki, Miltiadis K. Tsilimbaris, Eric S. Lesniewska, Alain Bron, and Iannis G. Pallikaris</i>	69
7 AFM Study of Surface Structure Changes in Mouse Spermatozoa Associated With Maturation <i>Hiroko Takano and Kazuhiro Abe</i>	85
8 Calculation of Cuticle Step Heights from AFM Images of Outer Surfaces of Human Hair <i>James R. Smith</i>	95
9 Imaging Living Chondrocyte Surface Structures With AFM Contact Mode <i>Gerlinde Bischoff, Anke Bernstein, David Wohlrab, and Hans-Joachim Hein</i>	105
10 Growth Cones of Living Neurons Probed by Atomic Force Microscopy <i>Davide Ricci, Massimo Grattarola, and Mariateresa Tedesco</i>	125

- 11 Evaluating Demineralization and Mechanical Properties
of Human Dentin With AFM
**Grayson W. Marshall, Jr., Sally J. Marshall,
Mehdi Balooch, and John H. Kinney** 141
- 12 Applying Atomic Force Microscopy to Studies
in Cardiac Physiology
Jason J. Davis, Trevor Powell, and H. Allen O. Hill 161
- 13 Imaging Bacterial Shape, Surface, and Appendages
Before and After Treatments With Antibiotics
Pier Carlo Braga and Davide Ricci 179

PART III SUBCELLULAR STRUCTURES INVESTIGATION

- 14 Visualizing Nuclear Structure *In Situ*
by Atomic Force Microscopy
**Luis Felipe Jiménez-García
and María de Lourdes Segura-Valdez** 191
- 15 Imaging Surface and Submembranous Structures in Living Cells
With the Atomic Force Microscope: *Notes and Tricks*
Filip Braet and Eddie Wisse 201
- 16 Atomic Force Microscopy of Protein Complexes
Olga I. Kiselyova and Igor V. Yaminsky 217
- 17 Atomic Force Microscopy of Interfacial Monomolecular Films
of Pulmonary Surfactant
**Kaushik Nag, Robert R. Harbottle, Amiyo K. Panda,
and Nils O. Petersen** 231
- 18 High-Resolution Analysis of the 3D Organization
of Human Metaphase Chromosomes
**Stefan Thalhammer, Pietro Gobbi, Mirella Falconi,
Giovanni Mazzotti, and Wolfgang M. Heckl** 245
- 19 Shape and Volume of Living Aldosterone-Sensitive Cells
Imaged With the Atomic Force Microscope
**Stefan W. Schneider, Rainer Matzke, Manfred Radmacher,
and Hans Oberleithner** 255
- 20 Localization of Epithelial Sodium Channels
by Atomic Force Microscopy
Peter R. Smith and Dale J. Benos 281
- 21 High-Resolution Imaging of Bacteriorhodopsin
by Atomic Force Microscopy
Dimitrios Fotiadis and Andreas Engel 291

PART IV FUNCTIONAL INVESTIGATIONS WITH AFM

22 Measurement of Mechanical Properties of Intact Endothelial Cells
in Fresh Arteries
Hiroshi Miyazaki and Kozaburo Hayashi 307

23 Observation of Oxidative Stress on Yeast Cells
Ricardo de Souza Pereira 315

24 Lymphoblastoid Cells Exposed to Low-Frequency
Magnetic Fields: *Study by Atomic Force Microscopy*
Settimio Grimaldi, Marco Girasole, and Antonio Cricenti 323

25 Sample Preparation Method for Observing RNA Polymerase
Activity by Atomic Force Microscopy
Sandor Kasas 341

26 Atomic Force Microscopy of β -Amyloid: *Static and Dynamic
Studies of Nanostructure and Its Formation*
Justin Legleiter and Tomasz Kowalewski 349

27 How to Build Up Biosensors With the Cantilever
of the Atomic Force Microscope
Ricardo de Souza Pereira 365

28 Measurement of Single Molecular Interactions
by Dynamic Force Microscopy
Martin Hegner, Wilfried Grange, and Patricia Bertoncini 369

Index **383**

Contributors

- KAZUHIRO ABE • *Department of Anatomy, Graduate School of Medicine, Hokkaido University, Sapporo, Japan*
- YOURI ARNTZ • *Department of Physics, NCCR Nanoscale Science, University of Basel, Basel, Switzerland*
- MEHDI BALOOCH • *Lawrence Livermore National Laboratory, Livermore, CA*
- DALE J. BENOS • *Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL*
- ANKE BERNSTEIN • *Institut für Analytik und Umweltchemie, Martin-Luther Universität, Halle, Germany*
- PATRICIA BERTONCINI • *Department of Physics, NCCR Nanoscale Science, University of Basel, Basel, Switzerland*
- GERLINDE BISCHOFF • *Institut für Analytik und Umweltchemie, Martin-Luther Universität, Halle, Germany*
- FILIP BRAET • *Laboratory for Cell Biology and Histology, Faculty of Medicine and Pharmacy, Free University of Brussels, Brussels, Belgium*
- PIER CARLO BRAGA • *Department of Pharmacology and Center of Respiratory Pharmacology, School of Medicine, University of Milan, Milan, Italy*
- ALAIN BRON • *Department of Ophthalmology, Medical School, University of Bourgogne, Dijon, France*
- GILLIAN R. BUSHELL • *School of Biomolecular and Biomedical Science, Faculty of Science, Griffith University, Brisbane, Australia*
- COLM CAHILL • *School of Biomolecular and Biomedical Science, Faculty of Science, Griffith University, Brisbane, Australia*
- ANTONIO CRICENTI • *Institute of Struttura della Materia, CNR, Rome, Italy*
- JASON J. DAVIS • *Department of Chemistry, University of Oxford, Oxford, United Kingdom*
- RICARDO DE SOUZA PEREIRA • *Institute of Biomedical Sciences, São Paulo, Brazil*
- ANDREAS ENGEL • *Müller Institute for Microscopy at the Biozentrum, University of Basel, Basel, Switzerland*
- MIRELLA FALCONI • *Department of Human Anatomy, University of Bologna, Bologna, Italy*
- DIMITRIOS FOTIADIS • *Müller Institute for Microscopy at the Biozentrum, University of Basel, Basel, Switzerland*

- MARCO GIRASOLE • *Institute of Struttura della Materia, CNR, Rome, Italy.*
- PIETRO GOBBI • *Institute of Morphological Sciences, University of Urbino, Urbino, Italy*
- WILFRIED GRANGE • *NCCR Nanoscale Science, Department of Physics, University of Basel, Basel, Switzerland*
- MASSIMO GRATTAROLA • *Department of Biophysical and Electronic Engineering, University of Genoa, Genoa, Italy*
- SETTIMIO GRIMALDI • *Institute of Experimental Medicine, CNR, Rome, Italy*
- ROBERT R. HARBOTTLE • *Department of Chemistry, University of Western Ontario, Ontario, Canada*
- KOZABURO HAYASHI • *Division of Bioengineering, Department of Mechanical Science and Bioengineering, Graduate School of Engineering Science, Osaka University, Osaka, Japan*
- WOLFGANG M. HECKL • *Institute of Crystallography, Ludwig Maximilians Universität, Munich, Germany*
- MARTIN HEGNER • *NCCR Nanoscale Science, Department of Physics, University of Basel, Basel, Switzerland*
- HANS-JOACHIM HEIN • *Institut für Analytik und Umweltchemie, Martin-Luther Universität, Halle, Germany*
- H. ALLEN O. HILL • *Laboratory of Physiology, University of Oxford, Oxford, United Kingdom*
- LUIS FELIPE JIMÉNEZ-GARCÍA • *Department of Cell Biology, Faculty of Sciences, National Autonomous University of Mexico, Mexico City, Mexico*
- SANDOR KASAS • *Institut de Biologie Cellulaire et de Morphologie, Lausanne, Switzerland*
- JOHN H. KINNEY • *Lawrence Livermore National Laboratory, Livermore, CA*
- OLGA I. KISELYOVA • *Faculty of Physics, M. V. Lomonosov Moscow State University, Moscow, Russia*
- TOMASZ KOWALEWSKI • *Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA*
- JUSTIN LEGLEITER • *Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA*
- ERIC S. LESNIEWSKA • *Physics Laboratory, UMR CNRS 5027, University of Bourgogne, Dijon, France*
- STYLLIANI LYDATAKI • *Department of Ophthalmology, Medical School, University of Crete, Heraklion, Greece*
- GRAYSON W. MARSHALL, JR. • *Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, CA*

- SALLY J. MARSHALL • *Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, CA*
- RAINER MATZKE • *Center of Nanoscience, Ludwig-Maximilians Universität, Munich, Germany*
- GIOVANNI MAZZOTTI • *Department of Human Anatomy, University of Bologna, Bologna, Italy*
- HIROSHI MIYAZAKI • *Division of Bioengineering, Department of Mechanical Science and Bioengineering, Graduate School of Engineering Science, Osaka University, Osaka, Japan*
- SVERRE MYHRA • *School of Science, Faculty of Science, Griffith University, Brisbane, Australia*
- KAUSHIK NAG • *Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada*
- HANS OBERLEITHNER • *Department of Physiology, Westfälische Wilhelms-Universität, Münster, Germany*
- IANNIS G. PALLIKARIS • *Department of Ophthalmology, Medical School, University of Crete, Heraklion, Greece*
- AMIYO K. PANDA • *Department of Chemistry, Behala College, Kolkata, India*
- NILS O. PETERSEN • *Department of Chemistry, University of Western Ontario, Ontario, Canada*
- TREVOR POWELL • *Department of Chemistry, University of Oxford, Oxford, United Kingdom*
- MANFRED RADMACHER • *Department of Physics, Universität Bremen, Bremen, Germany*
- DAVIDE RICCI • *Department of Biophysical and Electronic Engineering, University of Genoa, Genoa, Italy*
- STEFAN W. SCHNEIDER • *Department of Dermatology, Westfälische Wilhelms-Universität, Münster, Germany*
- MARÍA DE LOURDES SEGURA-VALDEZ • *Department of Cell Biology, Faculty of Sciences, National Autonomous University of Mexico, Mexico City, Mexico*
- JAMES R. SMITH • *Scanning Probe Microscopy Laboratory, School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, United Kingdom*
- PETER R. SMITH • *Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL*
- HIROKO TAKANO • *Department of Anatomy, Graduate School of Medicine, Hokkaido University, Sapporo, Japan*

- MARIATERESA TEDESCO • *Department of Biophysical and Electronic Engineering, University of Genoa, Genoa, Italy*
- STEFAN THALHAMMER • *Institute of Crystallography, Ludwig Maximilians Universität, Munich, Germany*
- MILTADIS K. TSILIMBARIS • *Department of Ophthalmology, Medical School, University of Crete, Heraklion, Greece*
- GREGORY S. WATSON • *School of Science, Faculty of Science, Griffith University, Brisbane, Australia*
- EDDIE WISSE • *Laboratory for Cell Biology and Histology, Faculty of Medicine and Pharmacy, Free University of Brussels, Brussels, Belgium*
- DAVID WOHLRAB • *Institut für Analytik und Umweltchemie, Martin-Luther Universität, Halle, Germany*
- IGOR V. YAMINSKY • *Faculty of Chemistry, M. V. Lomonosov Moscow State University, Moscow, Russia*