





Spacious Labs State-of-the-Art Equipment Certified Instructors New Equipment Demos

# Announcing the EMS Microscopy Academy

We are excited to announce that our new academy is now open! We are now offering training courses and workshops led by our certified faculty. Located next to our extensive warehouse in Hatfield, PA, just minutes from Philadelphia, we are now also offering demonstrations of new equipment. Take advantage of the knowledge Electron Microscopy Sciences is now able to provide and the valuable information you will gain.

"An abundance of practical info, built on the necessary theoretical background!" – Aurion Workshop Attendee

### **COURSES**

Aurion Immuno Gold Biological SEM Biological TEM Cryosectioning/Immunogold Cryo SEM Materials Ultramicrotomy Pharma Applications Pharma Polymorphism X-Ray Microanalysis

## SIGN UP FOR A CLASS TODAY, OR SUGGEST A COURSE THAT YOU WANT...





VISIT OUR WEBSITE TO MAKE A COURSE REQUEST...

www.emsdiasum.com



P.O. Box 550 • 1560 Industry Rd. Hatfield, Pa 19440 Tel: (215) 412-8400 Fax: (215) 412-8450 email: sgkcck@aol.com *or* stacie@ems-secure.com

# Microscopyand Microanalysis

#### An International Journal for the Biological and Physical Sciences

| THE OFFICIAL JOURNAL OF   | MICROSCOPY SOCIETY OF AMERICA<br>MICROANALYSIS SOCIETY<br>MICROSCOPICAL SOCIETY OF CANADA /<br>SOCIÉTÉ DE MICROSCOPIE DU CANADA<br>MEXICAN MICROSCOPY SOCIETY<br>BRAZILIAN SOCIETY FOR MICROSCOPY AND MICROANALYSIS<br>VENEZUELAN SOCIETY OF ELECTRON MICROSCOPY<br>EUROPEAN MICROBEAM ANALYSIS SOCIETY<br>AUSTRALIAN MICROSCOPY AND MICROANALYSIS SOCIETY<br>PORTUGUESE SOCIETY FOR MICROSCOPY<br>ROYAL MICROSCOPICAL SOCIETY<br>GERMAN SOCIETY FOR ELECTRON MICROSCOPY<br>BELGIAN SOCIETY FOR MICROSCOPY<br>MICROSCOPY SOCIETY OF SOUTHERN AFRICA |  |  |
|---|---|--|--|
| PUBLISHED IN AFFILIATION WITH   |   |  |  |
| Editor-in-Chief<br>John Mansfield<br>4304 Spring Lake Blvd.<br>Ann Arbor, MI 48108-9657<br>e-mail: jfmjfm@umich.edu   | <b>Cultural Heritage Applications Editor</b><br>Cathy Selvius DeRoo<br>Detroit Institute of Arts<br>5200 Woodward Avenue<br>Detroit, Michigan 48202   | Yoosuf N. Picard<br>Materials Science & Engineering<br>Carnegie Mellon University<br>Pittsburgh, PA 15213<br>e-mail: ypicard@cmu.edu   |  |
| Administrative Editor<br>John Shields<br>University of Georgia<br>Athens, GA 30602<br>e-mail: jpshield@uga.edu<br>Biological Sciences Applications Editors<br>W. Gray (Jay) Jerome<br>Department of Pathology, Microbiology and<br>Immunology<br>U-2206 MCN | e-mail: cselviusderoo@gmail.com<br><b>Materials Sciences Applications Editors</b><br>Vinayak Dravid<br>Materials Science and Engineering<br>Northwestern University<br>Evanston, IL 60208-3105<br>e-mail: v-dravid@nortwestern.edu<br>Georg E. Fantner<br>Interfaculty Institute for Bioengineering<br>École Polytechnique Fédéral de Lausanne<br>Lausanne, 1015 Switzerland.   | Masashi Watanabe<br>Dept. of Mater. Sci. & Eng.<br>Lehigh University<br>Bethlehem, PA 18015<br>e-mail: masashi.watanabe@lehigh.edu<br><b>Special Issues and Reviews Editor</b><br>David J. Smith<br>Department of Physics<br>Arizona State University<br>Tempe, AZ 85287-1504<br>e-mail: david.smith@asu.edu |  |
| Nashville, TN 37232-2561<br>e-mail: jay.jerome@vanderbilt.edu<br>Heide Schatten<br>Department of Veterinary Pathobiology<br>University of Missouri-Columbia<br>Columbia, MO 65211   | e-mail: georg.fantner@epfl.ch<br>David J. Larson<br>CAMECA<br>5500 Nobel Drive<br>Madison, WI 53711<br>e-mail: david.larson@ametek.com  | <b>Book Review Editor</b><br>Cynthia Goldsmith<br>Centers for Disease Control<br>Atlanta, GA 30333<br>e-mail: csg1@cdc.gov   |  |
| e-mail: SchattenH@missouri.edu<br>Rosemary White<br>CSIRO Plant Industry<br>Canberra, ACT 2601, Australia<br>e-mail: Rosemary.white@csiro.au  | Ross Marceau<br>Institute for Frontier Materials<br>Deakin University<br>Geelong, VIC 3216, Australia<br>e-mail: r.marceau@deakin.edu.au  | <i>M&amp;M Program Guide</i> Editor<br>Richard L. Martens<br>1013 Bevill Building<br>Box 870164<br>Tuscaloosa, AL 35487-0164<br>e-mail: rmartens@caf.ua.edu  |  |
| Elizabeth Wright<br>Department of Pediatrics<br>School of Medicine  | Joseph Michael<br>Sandia National Laboratories  | <b>Proceedings Editor</b><br>Gail Celio  |  |

Albuquerque, NM 87185

e-mail: jrmicha@sandia.gov

P.O. Box 5800

Gail Celio University of Minnesota St. Paul, MN 55108 e-mail: celio001@umn.edu

Emory University

Atlanta, GA 30322 e-mail: erwrigh@emory.edu



© MICROSCOPY SOCIETY OF AMERICA 2017

#### **Editorial Board**

Ralph Albrecht Ilke Arslan Mary Grace Burke Barry Carter Wah Chiu Marc De Graef Niels de Jonge Elizabeth Dickev Mark Ellisman Pratibha Gai Marija Gajdardziska-Josifovska Paul Kotula William Landis Charles Lyman Dale Newbury **Robert Price** Jean-Paul Revel David Smith Nan Yao Nestor Zaluzec

University of Wisconsin, Madison, Wisconsin Pacific Northwest Laboratory, Richland, Washington University of Manchester, Manchester, UK University of Connecticut, Storrs, Connecticut Baylor College of Medicine, Houston, Texas Carnegie Mellon University, Pittsburgh, Pennsylvania INM Institute for New Materials, Saarbrücken, Germany North Carolina State University, Raleigh University of California at San Diego, San Diego, California University of York, United Kingdom University of Wisconsin-Milwaukee, Milwaukee, Wisconsin Sandia National Labs, Albuquerque, New Mexico University of Akron, Akron, Ohio Lehigh University, Bethlehem, Pennsylvania National Institute of Standards and Technology, Gaithersburg, Maryland University of South Carolina, Columbia, South Carolina California Institute of Technology, Pasadena, California Arizona State University, Tempe, Arizona Princeton University, Princeton, New Jersey Argonne National Laboratory, Argonne, Illinois

#### **Editorial Board Representatives from Affiliated Societies**

| Masashi Watanabe    | Lehigh University (MAS)                                       |
|---------------------|---|
| Gautam Kumar Dey    | Bhabha Atomic Research Centre (EMSI)                          |
| Gema Gonzalez       | Venezuelan Institute for Scientific Investigation (Venezuela) |
| Michael Robertson   | Acadia University, Wolfville, Nova Scotia (Canada)            |
| Brendan Griffin     | University of Western Australia (AMMS)                        |
| Guillermo Solorzano | Pontificia Universidade Catolica, Rio de Janeiro (Brazil)     |
| Mike Matthews       | Atomic Weapons Establishment, Reading, Great Britain (EMAS)   |
| Miguel Yacaman      | Mexico Institute for Nuclear Research (Mexico)                |
| Henrique Almeida    | Universidade do Porto (Portugal)                              |
|                     |   |

#### **Founding Editor**

Jean-Paul Revel California Institute of Technology, Pasadena, California

#### **Previous Editors-in-Chief**

| Dale Johnson    | University of South Florida, Tampa, Florida            |
|-----------------|--|
| Charles Lyman   | Lehigh University, Bethlehem, Pennsylvania             |
| Robert L. Price | University of South Carolina, Columbia, South Carolina |

This journal is part of the **Cambridge Core** service. Access to online tables of contents and article abstracts is available to all researchers at no cost. Access to full-text articles online is provided to those with online subscription. Online subscriptions must be activated. Once your subscription is activated, free access to past, present, and forthcoming articles is available at:

#### Microscopy and Microanalysis website: cambridge.org/MAM.

Instructions for authors submitting manuscripts may be found at cambridge.org/MAM. Select "Further Information" then select "Instructions for Contributors." An abbreviated version of these instructions will be published in the first issue (February) of each volume.



## EDAX EDS Systems with New SDD Options Advanced Analysis with Superior Results

- Choice of optimized SDDs to suit your materials analysis needs
- Best light element sensitivity with silicon nitride ( $Si_3N_4$ ) window
- Vacuum encapsulated module
- Highest throughput SDD available, with unparalleled resolution
- Safe for plasma cleaning





#### **Microscopy** AND **Microanalysis**

*Microscopy and Microanalysis* publishes original research papers dealing with a broad range of topics in microscopy and microanalysis. These include articles describing new techniques or instrumentation and their applications, as well as papers in which established methods of microscopy or microanalysis are applied to important problems in the fields of biology or materials science. Microscopy and microanalysis are defined here in a broad sense, and include all current and developing approaches to the imaging and analysis of microstructure. The criteria for acceptance of manuscripts are the originality and significance of the research, the quality of the microscopy or microanalysis involved, and the interest for our readership.

Four types of communications are published in the Journal. **Regular Articles** are of substantial length and describe the findings of an original research project that satisfies the aims and scope of the Journal, described above. **Review Articles** summarize the current status of an important area within the aims and scope of the Journal. **Letters to the Editor** usually contain comments on recent articles that have appeared in the Journal. **Book Reviews** are also published, but these are solicited only through the Book Review Editor.

#### **Instructions for Contributors**

Instructions for authors contributing manuscripts may be found at http://mc.manuscriptcentral.com/mam under "Resources: Instructions and Forms." Authors may also visit cambridge.org/mam, select "Information," and then select "Instructions for Contributors." An abbreviated version of these instructions will be published in the first issue (February) of each volume.

#### **Copyright Information**

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, review, or thesis); that it is not under consideration for publication elsewhere; that its publication has been approved by all coauthors, if any, as well as by the responsible authorities at the institute where the work has been carried out; that, if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the Microscopy Society of America; that the manuscript will not be published elsewhere in any language without the consent of the copyright holders; and that written permission of the copyright holder is obtained by the authors for material used from other copyrighted sources.

All articles published in this journal are protected by copyright, which covers the exclusive rights to reproduce and distribute the article (e.g., as offprints), as well as all translation rights. No material published in this journal may be reproduced photographically or stored on microfilm, in electronic data bases, video disks, etc., without first obtaining written permission from the publisher.

The use of general descriptive names, trade names, trademarks, etc., in this publication, even if not specifically identified, does not imply that these names lack protection by the relevant laws and regulation.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Cambridge University Press, provided that the appropriate fee is paid directly to Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923, USA (Tel: (508) 750-8400), stating the ISSN (1431-9276), the volume, and the first and last page numbers of each article copied. The copyright owner's consent does not include copying for general distribution, promotion, new works, or resale. In these cases, specific written permission must first be obtained from the publisher.

#### Disclaimer

The Microscopy Society of America, the other societies stated, and Cambridge University Press cannot be held responsible for errors or for any consequences arising from the use of the information contained in this journal. The appearance of scientific reports and/or workshops, or any other material in *Microscopy and Microanalysis* does not constitute an endorsement or approval by The Microscopy Society of America of the findings, data, conclusions, recommendations, procedures, results, or any other aspect of the content of such articles. The appearance of advertising in *Microscopy and Microanalysis* does not constitute an endorsement or approval by The Microscopy Society of America of the quality or value of the products advertised or any of the claims, data, conclusions, recommendations, procedures, results, or any other information included in the advertisements.

While the advice and information in this journal is believed to be true and accurate at the date of its going to press, neither the authors, the editors, nor the publisher can accept any legal responsibility for any errors or omissions that may be made.

#### **Subscription Information**

*Microscopy and Microanalysis* is published bimonthly in February, April, June, August, October, and December by Cambridge University Press. Three supplements (*Meeting Guide, Program Guide, and Proceedings*) are published in June and August.

**Society Rates:** Members of the Microscopy Society of America should contact the MSA Business Office for all subscription inquiries: Microscopy Society of America, 11130 Sunrise Valley Dr, Suite 350, Reston, VA 20191, Tel.: (703) 234-4115, Email: associationmanagement@microscopy.org, URL: www.microscopy.org. Members of other affiliated societies should contact their respective society business offices for all subscription inquiries.

Subscription Rates: Institutions print and electronic: US \$2251.00 in the USA, Canada, and Mexico; UK £1354.00 + VAT elsewhere. Institutions online only: US \$1460.00 in the USA, Canada, and Mexico; UK £883.00 + VAT elsewhere. Individuals print plus online: US \$658.00 in the USA, Canada, and Mexico; UK £400.00 + VAT elsewhere. Prices include postage and insurance.

USA, Canada, and Mexico: Subscribers in the USA, Canada, and Mexico should send their orders, with payment in US dollars or the equivalent value in Canadian dollars, to: Cambridge University Press, Customer Services Department (Journals), 1 Liberty Plaza, New York, NY 10006, USA. Tel: (845) 353-7500. Fax: (845) 353-4141. Orders may be phoned direct (toll free): (800) 872-7423. E-mail: journals\_ subscriptions@cup.org.

**Outside North America:** Subscribers elsewhere should send their orders, with payment in sterling, to: Customer Services Department (Journals), Cambridge University Press, University Printing House, Shaftesbury Road, Cambridge, CB2 8BS, UK. Tel: +44 (0)1223 326070. Fax: 44 (0)1223 325150. E-mail: journals@cambridge.org

**Change of address:** Allow six weeks for all changes to become effective. All communications should include both old and new addresses (with postal codes) and should be accompanied by a mailing label from a recent issue. Society members should contact their respective society business offices to inform them of address changes.

#### **Editorial Office**

John Mansfield, Editor in Chief, 4304 Spring Lake Blvd., Ann Arbor, MI 48108-9657, USA; Tel: (734) 936-3352; Fax: (734) 763-2282; E-mail: jfmjfm@umich.edu.

#### Office of Publication

Cambridge University Press, 1 Liberty Plaza, New York, NY 10006, USA; Tel: (212) 337-5000; Fax: (212) 337-5959.

#### **Advertising Sales & Production**

Kelly Miller, M.J. Mrvica Associates, Inc., 2 West Taunton Avenue, Berlin, NJ 08009, USA; Tel: (856) 768-9360; Fax: (856) 753-0064.

© 2017 by Microscopy Society of America. Printed in the United States on acid-free paper. Periodicals postage paid at New York, NY, and additional mailing offices. Return postage guaranteed. Postmaster: Send address changes in the U.S.A. and Canada to *Microscopy and Microanalysis*, Subscription Department, Cambridge University Press, 1 Liberty Plaza, New York, NY 10006.





## thermo scientific

## Single Particle Analysis

A workflow that is pushing the boundaries of modern science

Cryo-EM structure of F-actin decorated with tropomyosin at 3.7Å resolution. Courtesy of Max Planck Institute of Molecular Physiology, Dortmund, Germany.

#### Find out more at thermofisher.com/FEI

© 2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.



## Microscopy and Microanalysis

#### An International Journal for the Biological and Physical Sciences

#### **R**EVIEW ARTICLES

Volume 23, Number 4 August 2017

| The Conjunctiva-Associated Lymphoid Tissue in Chronic Ocular Surface Diseases             | 697 |
|---|-----|
| Rodolfo Mastropasqua, Luca Agnifili, Vincenzo Fasanella, Mario Nubile, Agbeanda A. Gnama, |     |
| Gennaro Falconio, Paolo Perri, Silvio Di Staso, and Cesare Mariotti                       |     |

#### **MATERIALS SCIENCE APPLICATIONS**

| Focused Ion Beam Preparation of Specimens for Micro-Electro-Mechanical System-<br>based Transmission Electron Microscopy Heating Experiments<br>Sriram Vijayan, Joerg R. Jinschek, Stephan Kujawa, Jens Greiser, and Mark Aindow | 708 |
|--|-----|
| Atomic Scale Structural Characterization of Epitaxial (Cd,Cr)Te Magnetic<br>Semiconductor<br>Bastien Bonef, Hervé Boukari, Adeline Grenier, Isabelle Mouton, Pierre-Henri Jouneau,   | 717 |
| Hidekazu Kinjo, and Shinji Kuroda  |     |
| Precipitation of (Si <sub>2-x</sub> Al <sub>x</sub> )Hf in an Al-Si-Mg-Hf Alloy<br>Xueli Wang, Zhiqiang Xie, Huilan Huang, Zhihong Jia, Guang Yang, Lin Gu, and Qing Liu   | 724 |
| Reconstruction of Laser-Induced Surface Topography from Electron Backscatter<br>Diffraction Patterns<br>Patrick G. Callahan, McLean P. Echlin, Tresa M. Pollock, and Marc De Graef   | 730 |
|  |     |
| Practical Aspects of Electrochemical Corrosion Measurements During <i>In Situ</i><br>Analytical Transmission Electron Microscopy (TEM) of Austenitic Stainless Steel in<br>Aqueous Media   | 741 |
| Sibylle Schilling, Arne Janssen, Nestor J. Zaluzec, and M. Grace Burke   |     |
| <i>In Situ</i> Thermal Annealing Transmission Electron Microscopy (TEM) Investigation of III/V Semiconductor Heterostructures Using a Setup for Safe Usage of Toxic and Pyrophoric Gases   | 751 |
| Rainer Straubinger, Andreas Beyer, Thomas Ochs, Wolfgang Stolz, and Kerstin Volz   |     |
| INSTRUMENTATION AND SOFTWARE   |     |
| Nitrogen Gas Field Ion Source (GFIS) Focused Ion Beam (FIB) Secondary Electron<br>Imaging: A First Look  | 758 |
| Marek E. Schmidt, Anto Yasaka, Masashi Akabori, and Hiroshi Mizuta   |     |
| A Comprehensive Approach Towards Optimizing the Xenon Plasma Focused Ion<br>Beam Instrument for Semiconductor Failure Analysis Applications<br>Srinivas Subramaniam, Jennifer Huening, John Richards, and Kevin Johnson          | 769 |
| A Small Spot, Inert Gas, Ion Milling Process as a Complementary Technique to Focused Ion Beam Specimen Preparation   | 782 |
| Paul E. Fischione, Robert E.A. Williams, Arda Genç, Hamish L. Fraser, Rafal E. Dunin-Borkowski,<br>Martina Luysberg, Cecile S. Bonifacio, and András Kovács  |     |
| Simplifying Electron Beam Channeling in Scanning Transmission Electron<br>Microscopy (STEM)  | 794 |
|  |     |

Ryan J. Wu, Anudha Mittal, Michael L. Odlyzko, and K. Andre Mkhoyan

*Microscopy and Microanalysis* website: http://www.journals.cambridge.org/MAM Indexed in Chemical Abstracts, Current Contents, BIOSIS, and MEDLINE (PubMed)



**On the Cover:** Digital optical micrograph of a *Tff3* knock-out mouse epiphyseal plate used to count chondrocytes and calculate chondrocyte density. The tissue is stained with Masson's trichrome stain. For further details, see Bijelić et al., pp. 818–825.

https://doi.org/10.1017/S1431927617012429 Published online by Cambridge University Press

| Automated Image Acquisition for Low-Dose STEM at Atomic Resolution<br>Andreas Mittelberger, Christian Kramberger, Christoph Hofer, Clemens Mangler, and<br>Jannik c. Meyer   | 809 |
|--|-----|
| Histomorphometric Parameters of the Growth Plate and Trabecular Bone in<br>Wild-Type and Trefoil Factor Family 3 ( <i>Tff3</i> )-Deficient Mice Analyzed by<br>Free and Open-Source Image Processing Software<br>Nikola Bijelić, Tatjana Belovari, Dunja Stolnik, Ivana Lovrić, and Mirela Baus Lončar | 818 |
| Quantification of Cardiomyocyte Alignment from Three-Dimensional (3D) Confocal<br>Microscopy of Engineered Tissue<br>William J. Kowalski, Fangping Yuan, Takeichiro Nakane, Hidetoshi Masumoto, Marc Dwenger,<br>Fei Ye, Joseph P. Tinney, and Bradley B. Keller                                       | 826 |
| The Composition of Poly(Ethylene Terephthalate) (PET) Surface Precipitates<br>Determined at High Resolving Power by Tandem Mass Spectrometry Imaging<br>Gregory L. Fisher, John S. Hammond, Scott R. Bryan, Paul E. Larson, and Ron M. A. Heeren   | 843 |
| <b>BIOLOGICAL SCIENCE APPLICATIONS</b>   |     |
| Protective Effects of Coenzyme Q10 on Developmental Competence of Porcine Early Embryos  | 849 |
| Shuang Liang, Ying Jie Niu, Kyung-Tae Shin, and Xiang-Shun Cui   |     |
| Design and Fabrication of a Three-Dimensional <i>In Vitro</i> System for Modeling Vascular Stenosis  | 859 |
| Rebecca S. Jones, Pin H. Chang, Tzlil Perahia, Katrina A. Harmon, Lorain Junor, Michael J. Yost,<br>Daping Fan, John F. Eberth, and Richard L. Goodwin   |     |
| Micrographia   |     |
| Teaching an Old Material New Tricks: Easy and Inexpensive Focused Ion Beam (FIB)<br>Sample Protection Using Conductive Polymers  | 872 |
| Joshua A. Taillon, Valery Ray, and Lourdes G. Salamanca-Riba   |     |
| Swiss Stained-Glass Panels: An Analytical Study  | 878 |
| Andreia Machado, Sophie Wolf, Luis C. Alves, Ildiko Katona-Serneels, Vincent Serneels,<br>Stefan Trümpler, and Márcia Vilarigues   |     |



## ACCELERATE YOUR IN SITU RESEARCH

Whether you are researching new materials or discovering new phenomenon in the electron microscope, nothing should delay your experiment. That's why our service team is always ready to help you with topics ranging from product installation and training to troubleshooting and upgrades. And if you need help with an experiment, we have an applications team with decades of *in situ* experience ready to answer your most challenging questions. We provide all of this for you, because our goal is to accelerate your *in situ* research. Discover more at:

www.protochips.com/contact





## QUANTAX EBSD - Featuring OPTIMUS<sup>™</sup> TKD, ARGUS<sup>™</sup>, ESPRIT QUBE and PicoIndenters<sup>®</sup>

e<sup>-</sup>Flashes

## Unique Solutions for EBSD and TKD

e<sup>-</sup>Flash<sup>HD</sup>

- Fastest simultaneous EBSD/EDS analysis
- OPTIMUS<sup>™</sup> EBSD and TKD with one detector
- Unique ARGUS<sup>™</sup> FSE/BSE imaging system
- ESPRIT QUBE for advanced 3D analysis of EBSD/EDS data
- NEW Quantitative in-situ nanomechanical testing with Hysitron SEM PicoIndenters<sup>®</sup>



Innovation with Integrity