

Ahmet Yavuz Oral

Zehra Banu Bahsi Oral *Editors*

# 3rd International Multidisciplinary Microscopy and Microanalysis Congress (InterM)

Proceedings, Oludeniz, Turkey, 19–23  
October 2015



Springer

# **Springer Proceedings in Physics**

Volume 186

The series Springer Proceedings in Physics, founded in 1984, is devoted to timely reports of state-of-the-art developments in physics and related sciences. Typically based on material presented at conferences, workshops and similar scientific meetings, volumes published in this series will constitute a comprehensive up-to-date source of reference on a field or subfield of relevance in contemporary physics. Proposals must include the following:

- name, place and date of the scientific meeting
- a link to the committees (local organization, international advisors etc.)
- scientific description of the meeting
- list of invited/plenary speakers
- an estimate of the planned proceedings book parameters (number of pages/articles, requested number of bulk copies, submission deadline).

More information about this series at <http://www.springer.com/series/361>

Ahmet Yavuz Oral · Zehra Banu Bahsi Oral  
Editors

# 3rd International Multidisciplinary Microscopy and Microanalysis Congress (InterM)

Proceedings, Oludeniz, Turkey, 19–23  
October 2015

 Springer

*Editors*

Ahmet Yavuz Oral  
Department of Materials Science and  
Engineering  
Gebze Technical University  
Gebze, Kocaeli  
Turkey

Zehra Banu Bahsi Oral  
Department of Environmental Engineering  
Gebze Technical University  
Gebze, Kocaeli  
Turkey

ISSN 0930-8989

Springer Proceedings in Physics

ISBN 978-3-319-46600-2

DOI 10.1007/978-3-319-46601-9

ISSN 1867-4941 (electronic)

ISBN 978-3-319-46601-9 (eBook)

Library of Congress Control Number: 2016952505

© Springer International Publishing AG 2017

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

This Springer imprint is published by Springer Nature

The registered company is Springer International Publishing AG

The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

# Preface

The 3rd International Multidisciplinary Microscopy Congress (InterM2015) provided all scientists the opportunity to meet, present their work, discuss and mutually interact in order to enhance and promote their research work.

This volume, published by Springer, includes selected papers presented at this congress, held in Oludeniz, Turkey, October 19–23, 2015.

On behalf of the organizing committee we would like to thank all the participants, plenary and invited speakers for their valuable contribution.

We would also like to thank AIGTUR for their support in the organization of the congress as well as the publishers for the quality of this edition.

Gebze, Turkey

Ahmet Yavuz Oral  
Zehra Banu Bahsi Oral

# Organization

## Scientific Committee

George A. Stanciu, University “Politehnica” of Bucharest, Romania  
Seong-Ho Kang, Kyung Hee University, Korea  
Jonas Fransson, Uppsala University, Sweden  
M. Alper Sahiner, Seton Hall University, USA  
Mohamed Bououdina, University of Bahrain, Kingdom of Bahrain  
Cengiz Ozkan, University of California, Riverside, USA  
Kunio Takeyasu, Kyoto University, Japan  
Jiri Nemecek, Czech Technical University in Prague, Czech Republic  
Junsang Doh, Pohang University of Science and Technology, Korea  
Olga Duarte Silva, Universidade de Lisboa, Portuguese  
Ying Feng, The University of Alabama, USA  
Paul Thomas, Matsudaira National University of Singapore, Singapore  
Yoshiaki Uesu, Waseda University, Japan  
Kazuo Umemura, Tokyo University of Science, Japan  
Peter Guttmann, Helmholtz Zentrum Berlin, Germany  
Quanmin Guo, University of Birmingham, UK  
Ivan Stich, Slovak Academy of Sciences, Slovakia  
Golap Kalita, Nagoya Institute of Technology, Japan  
Hideki Kawakatsu, Institute of Industrial Science, the University of Tokyo, Japan  
Masashi Arita, Hokkaido University, Japan

## Organizing Committee

Ahmet Yavuz Oral, Gebze Technical University, Turkey  
Zehra Banu Bahsi Oral, Gebze Technical University, Turkey  
M. Alper Sahiner, Seton Hall University, USA

Ersin Kayahan, Kocaeli University, Turkey  
Tarik Talib Issa Al-Omran, University of Baghdad, Iraq  
Mehmet Sezer, Gebze Technical University, Turkey

## Conference Organizing Company

Aig Turizm Seyahat Kongre ve Org. Tic. Ltd. Őti.

**aigtur**

Atatürk Cad No:1 Tever Apt. D:11  
Sahrayıcedid, Kadıkoy, Istanbul, Turkey  
+90 216 330 80 90  
<http://aigtur.com.tr/>



# Contents

## Part I Applications of Microscopy in the Biological Sciences

<b>Structural Analysis of Long Single-Stranded RNA Molecules with Atomic Force Microscopy Imaging</b> . . . . .	3
Jamie L. Gilmore, Aiko Yoshida, Katashi Deguchi, Suguru Asai, Hideki Aizaki, Masahiro Kumeta, Kiwamu Hyodo, Tetsuro Okuno, Takaji Wakita and Kunio Takeyasu	
<b>Recombinant Fluorescent Ligand of Potassium Kv1.1 and Kv1.3 Channels: Design, Properties and Applications</b> . . . . .	11
Alexey V. Feofanov, Kseniya S. Kudryashova, Anastasiya A. Ignatova and Oksana V. Nekrasova	
<b>Single-Particle FRET Microscopy of Immobilized Nucleosomes: Technique Development</b> . . . . .	17
Alexey V. Feofanov, Oleg V. Chertkov, Kseniya S. Kudryashova, Yaroslav O. Ivanov, Vasily M. Studitsky and Mikhail P. Kirpichnikov	
<b>Post Embryonic Changes in the Eye of an Economic Mango Plant Pest <i>Amritodus atkinsoni</i> Leth. (Hemiptera: Membracoidea: Cicadellidae)</b> . . . . .	25
Seetha Seetha, Sheetal Sahu, Biswa Bhusana Mahapatra and Monalisa Mishra	
<b>Elemental Analysis of Various Feathers of Indian Rose Ringed Parakeet <i>Psittacula krameri</i></b> . . . . .	33
Debabrat Sabat, Sabera Millan, P. Suchismita Sethy, Sandhya Marathe, Harekrushna Sahoo and Monalisa Mishra	
<b>PNIPA Microgel and Alcian Blue Dye Aqueous Solution Interaction (Microscopic Investigation)</b> . . . . .	41
T.G. Baluyan, A.A. Novakova, Yu. B. Mandzhieva and V. Yu. Karaulov	

<b>Cells Shrinkage and Phosphatidylserine Externalization in Post Mortem Muscle by Fluorescence Microscopy</b> . . . . .	53
S. Becila, Y. Boudida, M. Gagaoua, K. Hafid, H. Boudchicha, H. Smili, R. Belachehabe, C.H. Herrera-Mendez, M.A. Sentandreu, R. Labas, T. Astruc, A. Boudjellal, B. Picard and A. Ouali	
<b>Part II Applications of Microscopy in the Physical/Chemical Sciences, at all Dimensional Scales</b>	
<b>Synthesis of Nanostructure Carbon Thin Films by Microwave Plasma-Enhanced Chemical Vapor Deposition</b> . . . . .	67
Ahmed S. Wasfi, Hammad R. Humud and Mohammed E. Ismael	
<b>Microstructural Investigation of SPA-C Steel Sheets Used in Railway Vehicles in Resistance Spot Welding</b> . . . . .	77
Nuri Akkaş, Erman Ferik, Recep Kılıç, Erdinç İlhan and Salim Aslanlar	
<b>Microstructure/Properties Relationship of Advanced Heat-Resistant Intermetallics TiAl(Nb,Cr,Zr) After Casting and Float Zone Processing</b> . . . . .	83
A.V. Kartavykh, M.V. Gorshenkov and A.V. Korotitskiy	
<b>Micro Graphical Analysis and Comparison of MWNT and CNF Reinforced Polymer Composite</b> . . . . .	91
Smrutisikha Bal	
<b>The Effect of ZrO<sub>2</sub> Addition on Sintering and Microstructural Properties of Cordierite Produced from Zeolite</b> . . . . .	99
Betül Çitak, Sunay Ayhan, Abdulkadir Akyol, Tuğba Tunç Parlak and A. Şükran Demirkıran	
<b>Energetics and Scanning Tunneling Microscopy Images of B and N Defects in Graphene Bilayer</b> . . . . .	107
Yoshitaka Fujimoto and Susumu Saito	
<b>Improved, Photon Conversion Efficiency of (SnO<sub>2</sub>) Doped Cesium Oxide (Cs) Nanofibers for Photocatalytic Application Under Solar Irradiation</b> . . . . .	113
K. Kaviyarasu, E. Manikandan, J. Kennedy, R. Ladchumananandasivam, Uilame Umbelino Gomes, M. Maaza and Genene T. Mola	
<b>Microscopy Study of Amorphous/Nanocrystalline Coatings Thermally Sprayed</b> . . . . .	129
Nacer E. Bacha	
<b>Phenotypic Plasticity in Desert Rodents Harderian Glands Under Seasonal Steroids Control</b> . . . . .	135
O. Saadi-Brenkia and N. Haniche	

<b>TEM Investigation of Nanostructures with a High Aspect Ratio</b> . . . . .	143
A.V. Myasoedov, A.E. Kalmykov, D.A. Kirilenko and L.M. Sorokin	
<b>Morphology, Chemical Composition, and Magnetic Properties of Arc Discharge Fe–C Soot</b> . . . . .	149
Sergey A. Novopashin, Marina A. Serebryakova and Alexey V. Zaikovskii	
<b>Exploration of Carbon Based Solid Acid Catalyst Derived from Corn Starch for Conversion of Non-edible Oil into Biodiesel</b> . . . . .	157
Judy R.B. Witono, Ken Hashigata, Herry Santoso and Inge W. Noordergraaf	
<b>Responses of Dendritic Cells to Different Coatings of Titanium</b> . . . . .	165
Natalia G. Plekhova, Irina N. Lyapun, Valentin B. Shumatov, Sergey V. Gnedenkov, Sergey L. Sinebryukhov, Artem V. Puz' and Evgenii V. Pustovalov	
<b>Microscopy of a Goatskin Bag Cheese “Bouhezza”</b> . . . . .	175
O. Aissaoui Zitoun, S. Carpino, N. Fucà, M.L. Mansour, H. Attia and M.N. Zidoune	
<b>N-Hexane Isomerization on Pt-Containing Ti-Pillared Tagan's Montmorillonite</b> . . . . .	183
N.A. Zakarina, A.K. Akurpekova, D.A. Zhumadulaev and O. Dalekhanuly	
<b>Part III Advances in Instrumentation and Techniques</b>	
<b>Analysis of Historical Monuments Through the Lens and Electrons: Case Study: The Monastery Hurezi</b> . . . . .	195
Ioana Gomoiu, Dan Mohanu, Ileana Mohanu, Mădălin Enache and Roxana Cojoc	
<b>Investigation on Switching Operation in Resistive RAM Using In-Situ TEM</b> . . . . .	205
Masashi Arita and Yasuo Takahashi	
<b>Simulation and Verification of Tip-Induced Polarization During Kelvin Probe Force Microscopy Measurements on Film Capacitors</b> . . . . .	215
D.A. Nielsen, V.N. Popok and K. Pedersen	
<b>Estimating 3D Volume of Dirt Particles Using Depth from Shadow</b> . . . . .	223
Peter Frühberger, Thomas Stephan, Jan Burke and Jürgen Beyerer	
<b>Structural/Functional Analyses of Protein-Nucleic Acid Interactions by AFM</b> . . . . .	229
Kunio Takeyasu, Katashi Deguchi and Jamie L. Gilmore	

<b>Dual Energy Microtomography Applied to Oil and Gas Assessments</b> .....	237
A.P. Teles, R.T. Lopes and I. Lima	
<b>Contribution of X-Ray Imaging Microscopy in Metal Bioaccumulation Studies</b> .....	245
S. Pennafirme, R.G. Leitão, R.T. Lopes, I. Lima and M.A.C. Crapez	
<b>Index</b> .....	253

# Contributors

**O. Aissaoui Zitoun** INATAA, Laboratory of Nutrition and Food S Technologies, University Frères Mentouri Constantine 1, Constantine, Algeria

**Hideki Aizaki** Virus Division II, National Institute of Infectious Disease, Tokyo, Japan

**Nuri Akkaş** Department of Metallurgical and Materials Engineering, University of Sakarya, Sakarya, Turkey

**A.K. Akurpekova** D.V.Sokolsky Institute of Organic Catalysis and Electrochemistry, Almaty, Kazakhstan

**Abdulkadir Akyol** Department of Metallurgy and Materials Engineering, Engineering Faculty, Esentepe Campus, Sakarya University, Sakarya, Turkey

**Masashi Arita** Graduate School of Information Science and Technology, Hokkaido University, Kita-ku, Sapporo, Japan

**Suguru Asai** Laboratory of Plasma Membrane and Nuclear Signaling, Kyoto University Graduate School of Biostudies, Kyoto, Japan

**Salim Aslanlar** Department of Metallurgical and Materials Engineering, University of Sakarya, Sakarya, Turkey

**T. Astruc** Theix, QuaPA, INRA de Clermont Ferrand, St Genes Champanelle, France

**H. Attia** Unité d'Analyses Alimentaires - École Nationale d'Ingénieurs, Sfax, Tunisie

**Sunay Ayhan** Department of Metallurgy and Materials Engineering, Engineering Faculty, Esentepe Campus, Sakarya University, Sakarya, Turkey

**Nacer E. Bacha** Lab. of Surface Treatment and Materials, University of Blida, Blida, Algeria

**Smrutisikha Bal** Department of Metallurgical and Materials Engineering, National Institute of Technology, Rourkela, Odisha, India

**T.G. Baluyan** Chair of the Solid State Physics, Department of Physics, Moscow State University, Moscow, Russia

**S. Becila** INATAA, Bothers Mentouri Constantine University, Constantine, Algeria

**R. Belachehabe** INATAA, Bothers Mentouri Constantine University, Constantine, Algeria

**Jan Burke** System Technologies and Image Exploitation IOSB, Fraunhofer Institute of Optronics, Karlsruhe, Germany

**Jürgen Beyerer** System Technologies and Image Exploitation IOSB, Fraunhofer Institute of Optronics, Karlsruhe, Germany; Vision and Fusion Laboratory (IES), Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

**H. Boudchicha** INATAA, Bothers Mentouri Constantine University, Constantine, Algeria

**Y. Boudida** INATAA, Bothers Mentouri Constantine University, Constantine, Algeria

**A. Boudjellal** INATAA, Bothers Mentouri Constantine University, Constantine, Algeria

**S. Carpino** CoRFiLaC, Ragusa Mare, Italy

**Oleg V. Chertkov** Biological Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

**Betül Çıtak** Department of Metallurgy and Materials Engineering, Engineering Faculty, Esentepe Campus, Sakarya University, Sakarya, Turkey

**Roxana Cojoc** Microbiology Department, Institute of Biology Bucharest, Romanian Academy, Bucharest, Romania

**M.A.C. Crapez** Marine Biology Postgraduate Program, Federal Fluminense University, Angra dos Reis, Brazil

**O. Dalelkhanuly** D.V.Sokolsky Institute of Organic Catalysis and Electrochemistry, Almaty, Kazakhstan

**Katashi Deguchi** Laboratory of Plasma Membrane and Nuclear Signaling, Kyoto University Graduate School of Biostudies, Sakyo-ku, Kyoto, Japan

**Mădălin Enache** Microbiology Department, Institute of Biology Bucharest, Romanian Academy, Bucharest, Romania

**Alexey V. Feofanov** Biological Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

**Erman Ferik** Department of Metallurgical and Materials Engineering, University of Sakarya, Sakarya, Turkey

**Peter Frühberger** System Technologies and Image Exploitation IOSB, Fraunhofer Institute of Optronics, Karlsruhe, Germany; Vision and Fusion Laboratory (IES), Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

**N. Fucà** CoRFiLaC, Ragusa Mare, Italy

**Yoshitaka Fujimoto** Department of Physics, Tokyo Institute of Technology, Tokyo, Japan

**M. Gagaoua** INATAA, Bothers Mentouri Constantine University, Constantine, Algeria

**Jamie L. Gilmore** Laboratory of Plasma Membrane and Nuclear Signaling, Kyoto University Graduate School of Biostudies, Sakyo-ku, Kyoto, Japan

**Sergey V. Gnedenkov** Institute of Chemistry Far-Eastern Branch Russian Academy of Sciences, Vladivostok, Russia

**Uilame Umbelino Gomes** Graduate Program in Materials Science and Engineering, Departamento de Fisica, Universidade Federal Do Rio Grande Do Norte, Natal-RN, Brazil

**Ioana Gomoiu** Conservation and Restoration Department, National University of Arts, Bucharest, Romania

**M.V. Gorshenkov** National University of Science and Technology “MISIS”, Moscow, Russia

**K. Hafid** INATAA, Bothers Mentouri Constantine University, Constantine, Algeria

**N. Haniche** Laboratoire de Biologie et Physiologie Des Organismes Equipe de Neurobiologie, USTHB, Bab Ezzouar, Algérie

**Ken Hashigata** Chemical Engineering Department, Parahyangan Catholic University, Bandung, Indonesia

**C.H. Herrera-Mendez** Departamento de Ingeniería Agroindustrial, Universidad de Guanajuato, Salvatierra, Mexico

**Hammad R. Humud** Physics Department, College of Science, University of Baghdad, Jadiriya, Baghdad, Iraq

**Kiwamu Hyodo** Laboratory of Plant Physiology, Kyoto University Graduate School of Agriculture, Kyoto, Japan

**Anastasiya A. Ignatova** Biological Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

**Erdinç İlhan** Department of Metallurgical and Materials Engineering, University of Sakarya, Sakarya, Turkey

**Mohammed E. Ismael** Physics Department, College of Science, University of Baghdad, Jadiriya, Baghdad, Iraq

**Yaroslav O. Ivanov** Biological Faculty, Lomonosov Moscow State University, Moscow, Russia

**A.E. Kalmykov** Ioffe Institute, St. Petersburg, Russia

**V. Yu. Karaulov** SERNIA LLC, Moscow, Russia

**A.V. Kartavykh** National University of Science and Technology “MISIS”, Moscow, Russia

**K. Kaviyarasu** UNESCO-UNISA Africa Chair in Nanosciences/Nanotechnology Laboratories, College of Graduate Studies, University of South Africa (UNISA), Pretoria, South Africa; Nanosciences African Network (NANOAFNET), Materials Research Group (MRG), iThemba LABS-National Research Foundation (NRF), Somerset West, Western Cape Province, South Africa

**J. Kennedy** UNESCO-UNISA Africa Chair in Nanosciences/Nanotechnology Laboratories, College of Graduate Studies, University of South Africa (UNISA), Pretoria, South Africa; National Isotope Centre, GNS Science, Lower Hutt, New Zealand

**D.A. Kirilenko** Ioffe Institute, St. Petersburg, Russia

**Mikhail P. Kirpichnikov** Biological Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

**A.V. Korotitskiy** National University of Science and Technology “MISIS”, Moscow, Russia

**Kseniya S. Kudryashova** Biological Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

**Masahiro Kumeta** Laboratory of Plasma Membrane and Nuclear Signaling, Kyoto University Graduate School of Biostudies, Kyoto, Japan

**Recep Kılıç** Sakarya Metropolitan Municipality, Sakarya, Turkey

**R. Labas** Theix, QuaPA, INRA de Clermont Ferrand, St Genes Champanelle, France



**R. Ladchumananandasiivam** Department of Textile Engineering and Post Graduate Programme in Mechanical Engineering, Centre of Technology, Federal University of the State of Rio Grande Do Norte, Campus Universitario, Natal-RN, Brazil

**R.G. Leitão** Nuclear Instrumentation Laboratory, PEN/COPPE/UFRJ, Rio de Janeiro, Brazil

**I. Lima** Nuclear Instrumentation Laboratory, PEN/COPPE/UFRJ, Rio de Janeiro, RJ, Brazil

**R.T. Lopes** Nuclear Instrumentation Laboratory, PEN/COPPE/UFRJ, Rio de Janeiro, RJ, Brazil

**Irina N. Lyapun** Cell Biology and Histopathology Laboratory, Somov Institute of Epidemiology and Microbiology, Vladivostok, Russia

**M. Maaza** UNESCO-UNISA Africa Chair in Nanosciences/Nanotechnology Laboratories, College of Graduate Studies, University of South Africa (UNISA), Pretoria, South Africa; Nanosciences African Network (NANOAFNET), Materials Research Group (MRG), iThemba LABS-National Research Foundation (NRF), Somerset West, Western Cape Province, South Africa

**Biswa Bhusana Mahapatra** Department of Life Science, National Institute of Technology Rourkela, Rourkela, Odisha, India

**Yu. B. Mandzhieva** Chair of the Solid State Physics, Department of Physics, Moscow State University, Moscow, Russia

**E. Manikandan** UNESCO-UNISA Africa Chair in Nanosciences/Nanotechnology Laboratories, College of Graduate Studies, University of South Africa (UNISA), Pretoria, South Africa

**M.L. Mansour** Faculté des Sciences de la Nature et de la Vie, Université Ferhat Abbas, Setif, Algeria

**Sandhya Marathe** Department of Biological Sciences, Birla Institute of Technology and Science, Pilani, Rajasthan, India

**Sabera Millan** Department of Chemistry, National Institute of Technology, Rourkela, Odisha, India

**Monalisa Mishra** Department of Life Science, National Institute of Technology Rourkela, Rourkela, Odisha, India

**Dan Mohanu** Conservation and Restoration Department, National University of Arts, Bucharest, Romania

**Ileana Mohanu** Binders Materials Research Department, CEPROCIM S.A., Bucharest, Romania

**Genene T. Mola** School of Chemistry and Physics, University of Kwazulu-Natal, Scottsville, Pietermaritzburg, South Africa

**A.V. Myasoedov** Ioffe Institute, St. Petersburg, Russia

**Oksana V. Nekrasova** Biological Faculty, Lomonosov Moscow State University, Moscow, Russia; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

**D.A. Nielsen** Department of Physics and Nanotechnology, Aalborg University, Aalborg, Denmark

**Inge W. Noordergraaf** Chemical Engineering Department, University of Groningen, Groningen, The Netherlands

**A.A. Novakova** Chair of the Solid State Physics, Department of Physics, Moscow State University, Moscow, Russia

**Sergey A. Novopashin** Kutateladze Institute of Thermophysics, Novosibirsk, Russia

**Tetsuro Okuno** Laboratory of Plant Physiology, Kyoto University Graduate School of Agriculture, Kyoto, Japan

**A. Ouali** Theix, QuaPA, INRA de Clermont Ferrand, St Genes Champanelle, France

**Tuğba Tunç Parlak** Department of Metallurgy and Materials Engineering, Engineering Faculty, Esentepe Campus, Sakarya University, Sakarya, Turkey

**K. Pedersen** Department of Physics and Nanotechnology, Aalborg University, Aalborg, Denmark

**S. Pennafirme** Marine Biology Postgraduate Program, Federal Fluminense University, Angra dos Reis, Brazil

**B. Picard** Theix, QuaPA, INRA de Clermont Ferrand, St Genes Champanelle, France

**Natalia G. Plekhova** Central Scientific Research Laboratory, Pacific State Medical University, Vladivostok, Russia; School of Natural Sciences, School of Biomedical, Far Eastern Federal University, Vladivostok, Russia

**V.N. Popok** Department of Physics and Nanotechnology, Aalborg University, Aalborg, Denmark

**Evgenii V. Pustovalov** School of Natural Sciences, School of Biomedical, Far Eastern Federal University, Vladivostok, Russia

**Artem V. Puz'** Institute of Chemistry Far-Eastern Branch Russian Academy of Sciences, Vladivostok, Russia

**O. Saadi-Brenkia** Laboratoire de Biologie et Physiologie Des Organismes Equipe de Neurobiologie, USTHB, Bab Ezzouar, Algérie; Département de Biologie, Université M'hamed Bougara Boumerdes, Boumerdes, Algérie

**Debabrat Sabat** Department of Life Science, National Institute of Technology, Rourkela, Odisha, India

**Harekrushna Sahoo** Department of Chemistry, National Institute of Technology, Rourkela, Odisha, India

**Sheetal Sahu** Department of Life Science, National Institute of Technology Rourkela, Rourkela, Odisha, India

**Susumu Saito** Department of Physics, Tokyo Institute of Technology, Tokyo, Japan

**Herry Santoso** Chemical Engineering Department, Parahyangan Catholic University, Bandung, Indonesia

**Seetha Seetha** Department of Life Science, National Institute of Technology Rourkela, Rourkela, Odisha, India

**M.A. Sentandreu** Instituto de Agroquímica y Tecnología de Alimentos, CSIC, Valencia, Spain

**Marina A. Serebryakova** Kutateladze Institute of Thermophysics, Novosibirsk, Russia

**Valentin B. Shumatov** Central Scientific Research Laboratory, Pacific State Medical University, Vladivostok, Russia

**Sergey L. Sinebryukhov** Institute of Chemistry Far-Eastern Branch Russian Academy of Sciences, Vladivostok, Russia

**H. Smili** INATAA, Bothers Mentouri Constantine University, Constantine, Algeria

**L.M. Sorokin** Ioffe Institute, St. Petersburg, Russia

**Thomas Stephan** System Technologies and Image Exploitation IOSB, Fraunhofer Institute of Optronics, Karlsruhe, Germany; Vision and Fusion Laboratory (IES), Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

**Vasily M. Studitsky** Cancer Epigenetics Program, Fox Chase Cancer Center, Philadelphia, USA; Biological Faculty, Lomonosov Moscow State University, Moscow, Russia

**P. Suchismita Sethy** Department of Life Science, National Institute of Technology, Rourkela, Odisha, India

**A. Şükran Demirkıran** Department of Metallurgy and Materials Engineering, Engineering Faculty, Esentepe Campus, Sakarya University, Sakarya, Turkey

**Yasuo Takahashi** Graduate School of Information Science and Technology, Hokkaido University, Kita-ku, Sapporo, Japan

**Kunio Takeyasu** Laboratory of Plasma Membrane and Nuclear Signaling, Kyoto University Graduate School of Biostudies, Sakyo-ku, Kyoto, Japan

**A.P. Teles** Nuclear Instrumentation Laboratory, PEN/COPPE/UFRJ, Rio de Janeiro, RJ, Brazil

**Takaji Wakita** Virus Division II, National Institute of Infectious Disease, Tokyo, Japan

**Ahmed S. Wasfi** Physics Department, College of Science, University of Baghdad, Jadiriya, Baghdad, Iraq

**Judy R.B. Witono** Chemical Engineering Department, Parahyangan Catholic University, Bandung, Indonesia

**Aiko Yoshida** Laboratory of Plasma Membrane and Nuclear Signaling, Kyoto University Graduate School of Biostudies, Kyoto, Japan

**Alexey V. Zaikovskii** Kutateladze Institute of Thermophysics, Novosibirsk, Russia

**N.A. Zakarina** D.V.Sokolsky Institute of Organic Catalysis and Electrochemistry, Almaty, Kazakhstan

**D.A. Zhumadulaev** D.V.Sokolsky Institute of Organic Catalysis and Electrochemistry, Almaty, Kazakhstan

**M.N. Zidoune** INATAA, Laboratory of Nutrition and Food S Technologies, University Frères Mentouri Constantine 1, Constantine, Algeria

**Part I**  
**Applications of Microscopy in the**  
**Biological Sciences**

# Structural Analysis of Long Single-Stranded RNA Molecules with Atomic Force Microscopy Imaging

Jamie L. Gilmore, Aiko Yoshida, Katashi Deguchi, Suguru Asai, Hideki Aizaki, Masahiro Kumeta, Kiwamu Hyodo, Tetsuro Okuno, Takaji Wakita and Kunio Takeyasu

**Abstract** Characterization of the structure of long RNA molecules (>1 kb) is usually a time-consuming and tedious process. In this study, we have developed an imaging procedure for obtaining images of the extended secondary structures of long RNA molecules combined with automated MATLAB-based data processing algorithms for identification of the domain architecture of the molecules in these images. These algorithms include a molecule autoselection procedure based on height and area thresholding, a morphological thinning procedure to generate skeletons of the molecule in order to analyze the branched structure of the molecules, and a procedure to generate local volume profiles along the main chain of the molecule for identification of domains and prediction of the number of nucleotides comprising each domain. The single-molecule nature of this technique also allows for the identification of varying conformations of the molecule and assessment of the conformational flexibility of the identified domain organization.

## 1 Introduction

Structural characterization of long single-stranded RNA molecules (>1 kb) is a process that often takes many years with each domain identified and studied in independent sets of experiments. In the case the of the Hepatitis C virus (HCV) RNA genome, structural characterization has proceeded gradually, focused mainly

---

J.L. Gilmore (✉) · A. Yoshida · K. Deguchi · S. Asai · M. Kumeta · K. Takeyasu  
Laboratory of Plasma Membrane and Nuclear Signaling, Kyoto University Graduate School of Biostudies, Yoshida-konoe, Sakyo-ku, Kyoto 606-8501, Japan  
e-mail: gilmore.jamielynn.3e@kyoto-u.ac.jp

H. Aizaki · T. Wakita  
Virus Division II, National Institute of Infectious Disease, Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

K. Hyodo · T. Okuno  
Laboratory of Plant Physiology, Kyoto University Graduate School of Agriculture, Yoshida-konoe, Sakyo-ku, Kyoto 606-8501, Japan

on the 5' and 3' untranslated regions (UTRs), with the internal structures remaining largely uncharacterized [1, 2]. Given the tedious nature of these processes, the development of methods to perform high-throughput characterization of RNA structure could greatly advance our ability to recognize the structural features on long RNA molecules such as viral RNA genomes, messenger RNA (mRNA) transcripts, or ribosomal RNA (rRNA).

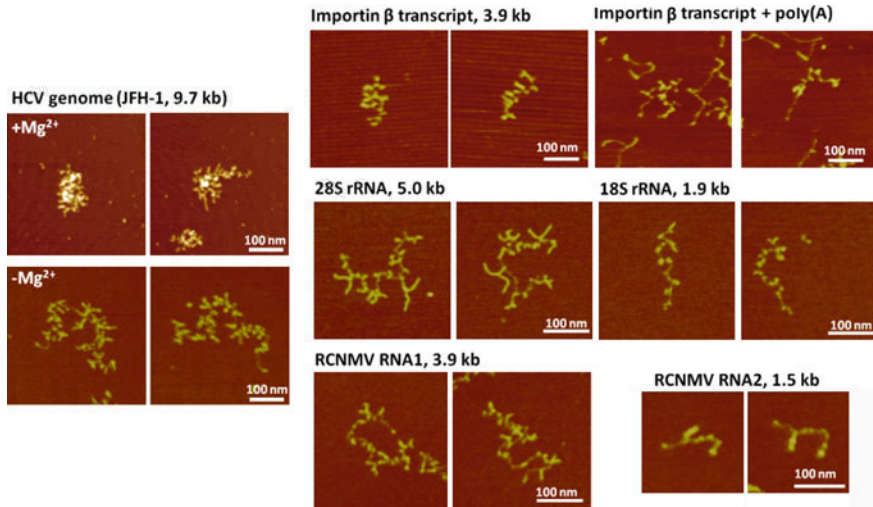
Towards this goal, we are attempting to develop Atomic Force Microscopy (AFM) imaging as a method to characterize the secondary structure of these molecules [3]. The first step of this process is the development of procedures to reproducibly image extended secondary structures of various RNA molecules. The next step is the development of automated procedures to extract structural information from the molecules in these images. To do this, we have developed MATLAB-based algorithms to autoselect the molecules from the images by height and area thresholding, generate skeletons of the molecule to analyze the branches of the RNA molecules, and generate local volume profiles along the morphological backbone of the molecule to predict the location of domains along the chain and the number of nucleotides in each.

## 2 Development of AFM Procedures to Image RNA Secondary Structure

Taking advantage of the fact that  $Mg^{2+}$  is necessary for RNA tertiary structure but not for secondary structure [4, 5], we have developed a method to reproducibly observe secondary structures of RNA molecules on a spermidine-modified mica surface using AFM imaging by omitting  $Mg^{2+}$  from our reactions and briefly heating the RNA to 65 °C (Fig. 1). This technique has proved effective for a variety of RNA molecules, including the 9.7 kb full-length genome of HCV (JFH-1) [6], a 3.9 kb importin  $\beta$  gene transcript, a polyadenylated importin  $\beta$  transcript, a 5.0 kb 28S rRNA, a 1.9 kb 18S rRNA, and the bipartite genome of the Red clover necrotic mosaic virus (RCNMV) [7] comprised of RNA1 and RNA2 (Fig. 1). Due to the hierarchical nature of RNA folding [5], much of this structure is likely to be conserved in the final folded molecule.

## 3 Analysis and Domain Recognition of RNA

After development of procedures to obtain reproducible images of RNA molecules, the next stage is to develop data analysis procedures that can extract structural information from these images. To do this, we have developed a series of automated MATLAB-based algorithms to analyze RNA molecules. For now, these algorithms have been applied to a 1.1 kb deletion mutant of the JFH-1 HCV genome with

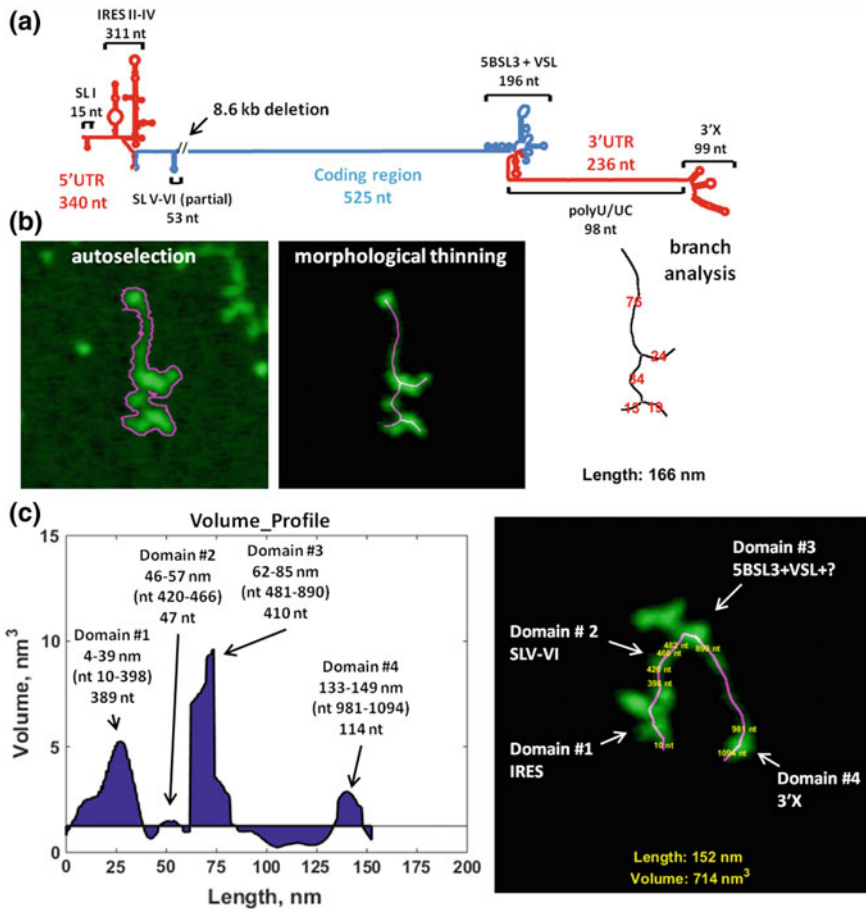


**Fig. 1** AFM images of various RNA molecules. Images of the full-length 9.7 kb JFH-1 Hepatitis C Virus (HCV) genome are displayed on the *left* showing the tertiary structure in the presence of 1 mM  $Mg^{2+}$  and without the addition of  $Mg^{2+}$  (1 mM EDTA). The remaining images all show the secondary structure in  $Mg^{2+}$ -free conditions

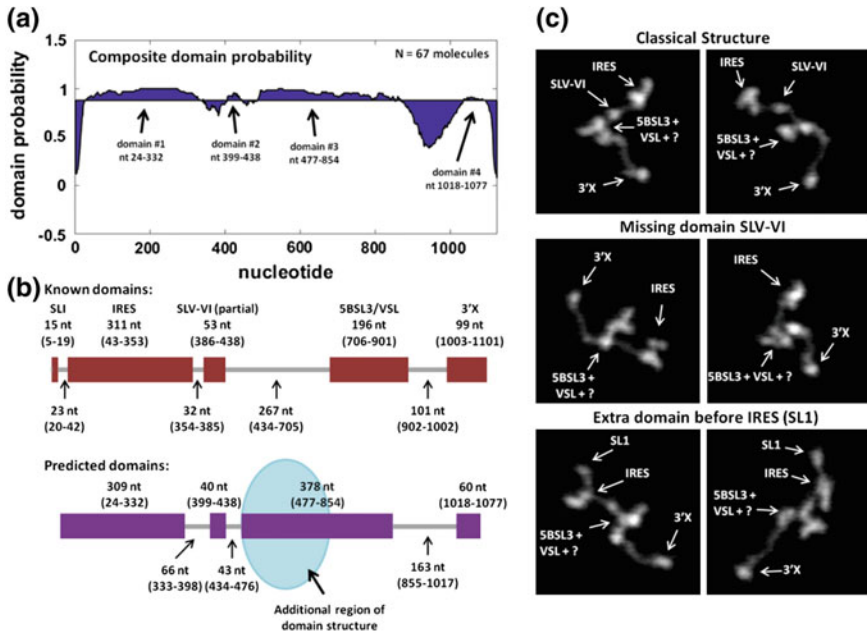
8.6 kb of the coding region removed (JFH-1dC\_5B, Fig. 2a), leaving mainly the 5' and 3' UTRs which are important for guiding the viral translation and viral replication processes, respectively. These regions have been reported to be some of the most structurally conserved regions of the genome [8]. Structures in these regions have been well characterized [1, 2], making it ideal to test the validity of our method.

Our algorithms were developed using the MATLAB image processing toolbox [9]. A detailed description of the algorithms will be reported elsewhere. These include autoselection of the molecules through height and area thresholding (Fig. 2b, left), 2D morphological thinning to generate a skeleton of the molecule to analyze its branched structure (Fig. 2b, middle and right), and the generation of 'local' volume profiles along the length of the molecule (Fig. 2c). By plotting the 'local' volume versus the length along the molecule, we can get an idea of the domain structure (Fig. 2c, left). Furthermore, the nucleotide number of each domain structure could be predicted based on the cumulative volume along the chain (Fig. 2c). For the molecule displayed in Fig. 2c, three of the four domains identified corresponded reasonably well to previously reported domains in the HCV genome, including the internal ribosome entry site (IRES) (domain#1), stem loops V-VI (domain #2), and the 3'X RNA (domain #4) located at the end of the genome which is located at the end of a notable single-stranded polyU/UC region (Fig. 2c, right). However, the 5BSL and VSL regions appeared to be contained within a domain structure that encompasses more of the coding region of the genome (domain #4, Fig. 2c, right).





**Fig. 2** Automated MATLAB-based algorithms to analyze a 1.1 kb JFH-1 HCV deletion mutant with 8.6 kb of the coding region deleted. **a** Model of the HCV deletion mutant with well characterized structures labeled. **b** Automated data analysis processes for the deletion mutant include autoselection of the molecule, morphological thinning to produce a skeleton, and branch analysis. The length of each branch in nanometers is displayed in red and the total length is recorded at the bottom of the image. **c** On the *left*, a profile of the ‘local’ volume along the main chain detects four domains when a threshold of  $1.25 \text{ nm}^3$  is used. The profile was generated by identifying the longest end-to-end chain in the skeleton previously generated by morphological thinning. All pixels in the original autoselected region were then assigned to a pixel in the main chain through an image transformation process which iteratively assigns nearby pixels a value corresponding to the value of the line pixel. The volume was then summed for all pixels which were ‘local’ to the line pixel and the length of the line was determined using a geodesic quasi-Euclidean distance transform and then converted to nanometers according to the image dimensions in order to generate the graph of ‘local’ volume versus length. The corresponding molecule is shown on the *right* with the predicted domains based on the predicted nucleotide number determined according to the cumulative volume along the main chain. Images are displayed using the MATLAB default falsecolor visualization (*green-magenta*) to overlay the *lines* onto the original image. Image dimensions are  $200 \times 200 \text{ nm}^2$



**Fig. 3** Composite domain analysis in a JFH-1 HCV deletion mutant for  $N = 67$  molecules. **a** The probability that a domain will be detected at each nucleotide value was calculated and plotted. The edges of the graph falsely have very low probabilities due to low height at the edge of the molecules. **b** Regions of expected structure based on previously described structures are shown on top and the predicted regions for each domain based on the domain probability in **a** are displayed below. **c** By sorting the molecules in the dataset, examples of conformational flexibility are observed. Image dimensions are  $200 \times 200 \text{ nm}^2$

By calculating the composite probability that a domain will be detected at each nucleotide for  $N = 67$  molecules (Fig. 3a), another  $\sim 230$  nt in addition to the 196 nt reported for the 5BSL and VSL regions (Fig. 3a, b) was predicted by our analysis, although this still remains to be confirmed for the full length HCV genome. Notably, this domain usually had an extended two-lobed structure suggesting that it is composed of ‘subdomains’. The predicted nucleotides for each domain often vary from the predicted structures by as much as 50 nt, however there may be variations in the predicted value depending on how the domains may be oriented on the mica surface, and on the threshold used to define the region of domains. Additional sorting of the molecules also allows for alternative conformations of the molecules to be detected (Fig. 3c). For example, although many of the molecules in our dataset had the classical four domain structure, the second small domain (SLV-VI) was sometimes missing from a subset of the molecules. Additionally, in some molecules, the 15 nt SLI domain which precedes the IRES could be detected as a separate domain.