# Single Molecule Spectroscopy and Imaging II

Jörg Enderlein Zygmunt K. Gryczynski Rainer Erdmann Editors

24–25 January 2009 San Jose, California, United States

Sponsored by SPIE

Cosponsored by PicoQuant GmBH Berlin (Germany)

Published by SPIE

Volume 7185

The papers included in this volume were part of the technical conference cited on the cover and title page. Papers were selected and subject to review by the editors and conference program committee. Some conference presentations may not be available for publication. The papers published in these proceedings reflect the work and thoughts of the authors and are published herein as submitted. The publisher is not responsible for the validity of the information or for any outcomes resulting from reliance thereon.

Please use the following format to cite material from this book:

Author(s), "Title of Paper," in *Single Molecule Spectroscopy and Imaging II*, edited by Jörg Enderlein, Zygmunt K. Gryczynski, Rainer Erdmann, Proceedings of SPIE Vol. 7185 (SPIE, Bellingham, WA, 2009) Article CID Number.

ISSN 1605-7422 ISBN 9780819474315

Published by

SPIE

P.O. Box 10, Bellingham, Washington 98227-0010 USA Telephone +1 360 676 3290 (Pacific Time) · Fax +1 360 647 1445 SPIE.org

Copyright © 2009, Society of Photo-Optical Instrumentation Engineers.

Copying of material in this book for internal or personal use, or for the internal or personal use of specific clients, beyond the fair use provisions granted by the U.S. Copyright Law is authorized by SPIE subject to payment of copying fees. The Transactional Reporting Service base fee for this volume is \$18.00 per article (or portion thereof), which should be paid directly to the Copyright Clearance Center (CCC), 222 Rosewood Drive, Danvers, MA 01923. Payment may also be made electronically through CCC Online at copyright.com. Other copying for republication, resale, advertising or promotion, or any form of systematic or multiple reproduction of any material in this book is prohibited except with permission in writing from the publisher. The CCC fee code is 1605-7422/09/\$18.00.

Printed in the United States of America.

Publication of record for individual papers is online in the SPIE Digital Library.



**Paper Numbering:** Proceedings of SPIE follow an e-First publication model, with papers published first online and then in print and on CD-ROM. Papers are published as they are submitted and meet publication criteria. A unique, consistent, permanent citation identifier (CID) number is assigned to each article at the time of the first publication. Utilization of CIDs allows articles to be fully citable as soon they are published online, and connects the same identifier to all online, print, and electronic versions of the publication. SPIE uses a six-digit CID article numbering system in which:

- The first four digits correspond to the SPIE volume number.
- The last two digits indicate publication order within the volume using a Base 36 numbering system employing both numerals and letters. These two-number sets start with 00, 01, 02, 03, 04, 05, 06, 07, 08, 09, 0A, 0B ... 0Z, followed by 10-1Z, 20-2Z, etc.

The CID number appears on each page of the manuscript. The complete citation is used on the first page, and an abbreviated version on subsequent pages. Numbers in the index correspond to the last two digits of the six-digit CID number.

# **Contents**

vii ix	Conference Committee Introduction				
	FRET AND ENERGY TRANSFER				
7185 03	Photoinduced electron transfer probes for the observation of enzyme activities (Best Pa Award) [7185-02] S. Henkenjohann, M. Sauer, Univ. Bielefeld (Germany)				
7185 04	Optical microresonator modifies the efficiency of the fluorescence resonance energy transfer in the autofluorescent protein DsRed [7185-03] F. Schleifenbaum, K. Elgass, Eberhard Karls Univ. Tübingen (Germany); M. Steiner, IBM Thomas J. Watson Research Ctr. (United States); J. Enderlein, Georg-August-Univ. Göttin (Germany); S. Peter, A. J. Meixner, Eberhard Karls Univ. Tübingen (Germany)				
7185 05	Simultaneous monitoring of the two coupled motors of a single F <sub>o</sub> F <sub>1</sub> -ATP synthase by three-color FRET using duty cycle-optimized triple-ALEX (Invited Paper) [7185-04] N. Zarrabi, S. Ernst, M. G. Düser, A. Golovina-Leiker, Univ. Stuttgart (Germany); W. Becker, Becker & Hickl GmbH (Germany); R. Erdmann, PicoQuant GmbH (Germany); S. D. Dunn, Univ. of Western Ontario (Canada); M. Börsch, Univ. Stuttgart (Germany)				
7185 06	Anti-correlation of the emission from different red pools in photosystem I [7185-05] M. Brecht, V. Radics, J. B. Nieder, R. Bittl, Freie Univ. Berlin (Germany)				
	FLUORESCENCE CORRELATION SPECTROSCOPY				
7185 08	Comparison of background corrected fluorescence correlation spectroscopy and fluorescence lifetime correlation spectroscopy: dilution series revisited [7185-07]  S. Rüttinger, Physikalisch-Technische Bundesanstalt (Germany); P. Kapusta, M. Patting, M. Wahl, PicoQuant GmbH (Germany); R. Macdonald, Physikalisch-Technische Bundesanstalt (Germany)				
7185 09	Modulated or alternating excitation in fluorescence correlation spectroscopy [7185-08] G. Persson, T. Sandén, P. Thyberg, J. Widengren, Royal Institute of Technology (Sweden)				
7185 0C	Fluorophore conjugated silver nanoparticles: a time-resolved fluorescence correlation spectroscopic study [7185-11] K. Ray, J. Zhang, J. R. Lakowicz, Univ. of Maryland School of Medicine (United States)				
7185 OD	High volume confinement in two-photon fluorescence correlation spectroscopy with radially polarized light [7185-12]  D. Ivanov, V. Shcheslavskiy, I. Märki, Lab. d'Optique Biomedicale, Ecole Polytechnique Fédérale de Lausanne (Switzerland); M. Leutenegger, Max-Planck-Institut für Biophysikalische Chemie (Germany); T. Lasser, Lab. d'Optique Biomedicale, Ecole Polytechnique Fédérale de Lausanne (Switzerland)				

7185 OF	<b>Photoswitching microscopy with subdiffraction-resolution</b> [7185-14] S. van de Linde, M. Schüttpelz, R. Kasper, B. Seefeldt, M. Heilemann, M. Sauer, Bielefeld Univ (Germany)
7185 OI	Superresolution imaging in live Caulobacter Crescentus cells using photoswitchable enhanced yellow fluorescent protein [7185-16]  J. S. Biteen, M. A. Thompson, N. K. Tselentis, L. Shapiro, W. E. Moerner, Stanford Univ. (United States)
	HIGH RESOLUTION MICROSCOPY II AND SAMPLES
7185 OJ	SPDM: single molecule superresolution of cellular nanostructures (Invited Paper) [7185-17] R. Kaufmann, P. Lemmer, M. Gunkel, Y. Weiland, P. Müller, M. Hausmann, Univ. of Heidelberg (Germany); D. Baddeley, Univ. of Heidelberg (Germany) and Univ. of Auckland (New Zealand); R. Amberger, Univ. of Heidelberg (Germany); C. Cremer, Univ. of Heidelberg (Germany) and Institute for Molecular Biophysics (United States)
	NEW DEVELOPMENTS IN METHODS AND SYSTEMS I
7185 0Q	Recent advances in photon coincidence measurements for photon antibunching and full correlation analysis (Invited Paper) [7185-24] F. Koberling, B. Kraemer, V. Buschmann, PicoQuant GmbH (Germany); S. Rüttinger, Physikalisch-Technische Bundesanstalt (Germany); P. Kapusta, M. Patting, M. Wahl, R. Erdmann, PicoQuant GmbH (Germany)
7185 OR	High-speed low-cost correlator for single molecule fluorescence correlation spectroscopy [7185-25] H-Y. Lee, Institute of Atomic and Molecular Sciences (Taiwan); H-Y. Lin, J. D. White, Yuan Ze Univ. (Taiwan); W. Fann, Institute of Atomic and Molecular Sciences (Taiwan)
7185 OT	Phasor-based single-molecule fluorescence lifetime imaging using a wide-field photon-counting detector [7185-27] R. Colyer, Univ. of California, Los Angeles (United States); O. Siegmund, A. Tremsin, J. Vallerga, Univ. of California, Berkeley (United States); S. Weiss, X. Michalet, Univ. of California, Los Angeles (United States)
	NEW DEVELOPMENTS IN METHODS AND SYSTEMS II
7185 OU	Plasmonic-controlled single molecule fluorescence near defined metallic nanostructures (Invited Paper) [7185-28] Y. Fu, J. Zhang, J. R. Lakowicz, Univ. of Maryland School of Medicine (United States)
7185 OV	Nanoscale distance fluctuations probed by photothermal correlation spectroscopy [7185-46] M. Wähnert, R. Radünz, F. Cichos, Univ. Leipzig (Germany)

## 7185 0W Optofluidic single molecule flow proteometry [7185-30]

N. Jing, Texas A&M Univ. (United States); C.-K. Chou, M.-C. Hung, The Univ. Of Texas M.D. Anderson Cancer Ctr. (United States); J. Kameoka, Texas A&M Univ. (United States)

# 7185 0Y Electro-magnetic free energy transduction by molecular motors [7185-32]

V. V. Yakovlev, Univ. of Wisconsin, Milwaukee (United States)

# Going beyond 2D: following membrane diffusion and topography in the IgE-FcuRI system using 3-dimensional tracking microscopy (Best Paper Award) [7185-33]

N. P. Wells, G. A. Lessard, M. E. Phipps, P. M. Goodwin, Los Alamos National Lab. (United States); D. S. Lidke, B. S. Wilson, Univ. of New Mexico (United States); J. H. Werner, Los Alamos National Lab. (United States)

#### SINGLE MOLECULE SPECTROSCOPY IN BIOLOGY II

7185 16 Effect of spontaneous diffusion in micro/nanoporous chemically crosslinked poly (*N*-vinyl imidazole) gel on the conformational changes of acetylcholine [7185-41]

E. Vaganova, The Hebrew Univ. of Jerusalem (Israel); I. F. Pierola, Univ. Nacional de Educación a Distancia (Spain); H. Ovadia, Hadassah Univ. Hospital (Israel); S. E. Lyshevski, Rochester Institute of Technology (United States); S. Yitzchaik, The Hebrew Univ. of Jerusalem (Israel)

# **POSTER SESSION**

7185 18 Mirror-based broadband scanner with minimized aberration [7185-43]

J.-Y. Yu, Y.-Y. Tzeng, National Taiwan Univ. (Taiwan); C.-H. Huang, National Taiwan Univ. (Taiwan) and National Cheng Kung Univ. (Taiwan); H.-C. Chui, National Cheng Kung Univ. (Taiwan); S.-W. Chu, National Taiwan Univ. (Taiwan)

Author Index

# **Conference Committee**

# Symposium Chairs

James G. Fujimoto, Massachusetts Institute of Technology (United States)

**R. Rox Anderson**, Wellman Ctr. for Photomedicine, Massachusetts General Hospital (United States) and Harvard School of Medicine (United States)

## **Program Track Chairs**

**Ammasi Periasamy**, University of Virginia (United States) **Daniel L. Farkas**, Cedars-Sinai Medical Center (United States)

#### Conference Chairs

Jörg Enderlein, Georg-August-Universität Göttingen (Germany)

Zygmunt K. Gryczynski, The University of North Texas Health Science
Center (United States)

Rainer Erdmann, PicoQuant GmbH (Germany)

# Program Committee

Sabato D'Auria, Consiglio Nazionale delle Ricerche (Italy)
Paul M. W. French, Imperial College London (United Kingdom)
Ewa M. Goldys, Macquarie University (Australia)
Johan Hofkens, Katholieke Universiteit Leuven (Belgium)
Thomas Huser, NSF Center for Biophotonics, University of California,
Davis, (United States)
Gabor Laczko, University of Szeged (Hungary)

Gabor Laczko, University of Szeged (Hungary)

Maria Teresa Neves-Petersen, Aalborg Universitet (Denmark)

Markus Sauer, Universität Bielefeld (Germany)

Benjamin Schuler, Universität Zürich (Switzerland)

Andong Xia, Institute of Chemistry (China)

## Session Chairs

- 1 FRET and Energy Transfer Rainer Erdmann, PicoQuant GmbH (Germany)
- 2 Fluorescence Correlaction Spectroscopy
  Zygmunt K. Gryczynski, The University of North Texas Health Science Center (United States)

- 3 Keynote Presentation
  Rainer Erdmann, PicoQuant GmbH (Germany)
- 4 High Resolution Microscopy I
   Felix Koberling, PicoQuant GmbH (Germany)
- 5 High Resolution Microscopy II and Samples **Markus Sauer**, Universität Bielefeld (Germany)
- Keynote PresentationRainer Erdmann, PicoQuant GmbH (Germany)
- 7 New Developments in Methods and Systems I Rainer Erdmann, PicoQuant GmbH (Germany)
- 8 New Developments in Methods and Systems II
  Jörg Enderlein, Georg-August-Universität Göttingen (Germany)
- 9 Single Molecule Spectroscopy in Biology I Rainer Erdmann, PicoQuant GmbH (Germany)
- Single Molecule Spectroscopy in Biology II
   Thomas Dertinger, University of California, Los Angeles (United States)

Award Presentation

**Zygmunt K. Gryczynski**, The University of North Texas Health Science Center (United States)

# Introduction

During 24–30 January 2009, SPIE organized a special symposium on Biomedical Spectroscopy, Microscopy, and Imaging as part of Photonics West, North America's largest photonics event. The Single Molecule Spectroscopy and Imaging II conference was surely one of the highlights of this symposium, and about 150 scientists enjoyed more than 40 presentations. In particular, the keynote lecture from Prof. Stefan Hell about "Nanoscopy with focused light" attracted significant attention and filled the session hall.

The aim of this conference was to provide a state-of-the-art interdisciplinary forum for spectroscopists, biochemists, and engineers to exchange information on ultrasensitive optical detection and spectroscopy down to the single-molecule level, and its applications in chemoanalysis, biological and biomedical research, medical diagnostics, and microscopy.

The conference covered a wide range of different single-molecule techniques such as fluorescence correlation spectroscopy (FCS), fluorescence lifetime Imaging (FLIM), or Foerster resonance energy transfer (FRET), with a special focus on biological and biomedical applications. The presentations showed clearly that single molecule spectroscopy has become an important tool in fundamental biological and biomedical research, as it allows the study of function and interaction of individual biomolecules.

The sessions about superresolution microscopy were among the highlights. Starting with a general overview given in a keynote lecture by Prof. Stefan Hell, the subsequent talks presented many new and exciting results, demonstrating for example, the possibility of achieving optical far-field resolution down to 1 nm.

The conference chairs, Jörg Enderlein (Georg-August-Univ. Göttingen), Zygmunt K. Gryczynski (Ctr. for Commercialization of Fluorescence Technologies, The Univ. of North Texas Health Science Ctr.), and Rainer Erdmann (PicoQuant GmbH), paid special attention to the presentations of Young Investigators. A specially dedicated Young Investigator Award with a generous cash prize was sponsored by PicoQuant to motivate these students to submit and present their work at such a leading event.

The large amount of excellent talks this year made it impossible to pick a single winner. In the end, PicoQuant honoured the outstanding work of several young scientists by increasing the award amount and splitting it between Sigrun Henken-johann (Univ. Bielefeld, paper 7185-02), Jonas Fölling (MPI Göttingen, paper 7185-20) and Nathan P. Wells (Los Alamos National Lab., paper 7185-33).

The award also offers the winners a registration fee waiver for the leading European Meeting on Single Molecule Spectroscopy and Ultra Sensitive Analysis in the Life Sciences, to be held in September 15–18, 2009, in Berlin.

The next Single Molecule Spectroscopy and Imaging conference will be held in San Francisco as part of Photonics West, 23–28 January 2010. SPIE and the conference chairs encourage all scientists in the field to join us again for presenting their latest scientific results.

We thank all of the authors of this year's session who submitted their papers in time, and our thanks go also to the SPIE staff for helping us tremendously to make this session such a successful event.

Jörg Enderlein Zygmunt K. Gryczynski Rainer Erdmann