Single Molecule Spectroscopy and Imaging

Jörg Enderlein
Zygmunt K. Gryczynski
Rainer Erdmann
Editors

19–21 January 2008
San Jose, California, USA

Sponsored and Published by
SPIE

Volume 6862


SPIE is an international society advancing an interdisciplinary approach to the science and application of light.
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:


ISSN 1605-7422
ISBN 9780819470379

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 © 2008, 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 1605-7422/08/$18.00.

Printed in the United States of America.

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

**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

<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>vii</td>
<td>Conference Committee</td>
<td></td>
</tr>
<tr>
<td>ix</td>
<td>Introduction</td>
<td></td>
</tr>
</tbody>
</table>

### FCS I

**6862 02** Latest applications for 2-focus fluorescence correlation spectroscopy (Invited Paper) [6862-01]
T. Dertinger, Univ. of California, Los Angeles (USA); I. von der Hocht, Forschungszentrum Jülich GmbH (Germany); A. Loman, Eberhard Karls Univ. Tübingen (Germany); R. Erdmann, PicoQuant GmbH (Germany); J. Enderlein, Eberhard Karls Univ. Tübingen (Germany)

**6862 04** Single molecules in fluorescence fluctuation spectroscopy: effective volume and photon counting histogram [6862-03]
J. Ackermann, FluIT Biosystems GmbH (Germany); H. Mathis, B. Greiner, BioMOS, Fraunhofer Gesellschaft (Germany)

### FCS II

**6862 08** Fluorescence correlation spectroscopy to study antibody binding and stoichiometry of complexes [6862-08]
K. M. Swift, E. D. Matayoshi, Abbott Labs. (USA)

### NEW DEVELOPMENTS IN METHODS AND SYSTEM I

**6862 09** Recent advances in time-correlated single-photon counting (Invited Paper) [6862-09]

**6862 0A** Single molecule protein folding kinetics in a co-axial microfluidic mixer [6862-10]
K. M. Hamadani, S. Weiss, Univ. of California, Los Angeles (USA)

**6862 0B** Detection and quantification of lipid membrane binding on silica micro-tube resonator sensor [6862-11]
T. Ling, S. Majd, M. Mayer, L. J. Guo, Univ. of Michigan (USA)

**6862 0C** Label-free detection of cytokines using optical microcavities (Young Investigator Award) [6862-12]
A. M. Armani, S. E. Fraser, California Institute of Technology (USA)

**6862 0D** Toward single-molecule detection with very compact DNA sequencer based on single-photon avalanche diode array [6862-13]
I. Rech, S. Marangoni, A. Gulinatti, Politecnico di Milano (Italy); M. Ghioni, S. Cova, Politecnico di Milano (Italy) and MPD Micro-Photon-Devices (Italy)
NEW DEVELOPMENTS IN METHODS AND SYSTEM II

6862 0E  Dynamics of TBP binding to the TATA box (Invited Paper) [6862-14]
P. Schluesche, G. Heiss, Ludwig-Maximilians-Univ. München (Germany); M. Meisterernst, Helmholtz Ctr. Munich German Research Ctr. for Environmental Health (Germany) and Westfalian Wilhelms Univ. (Germany); D. C. Lamb, Ludwig-Maximilians-Univ. München (Germany) and Univ. of Illinois at Urbana-Champaign (USA)

6862 0F  Hybrid photodetector for single-molecule spectroscopy and microscopy (Invited Paper) [6862-15]
X. Michalet, A. Cheng, J. Antelman, Univ. of California, Los Angeles (USA); M. Suyama, Hamamatsu Photonics K.K. (Japan); K. Arisaka, S. Weiss, Univ. of California, Los Angeles (USA)

6862 0G  Integrated microfluidic diagnostic platform for marker and environmental detection [6862-16]
H. P. Mathis, C. Müller, B. Greiner, G. Blaess, Fraunhofer-Institut FIT, BioMOS (Germany)

6862 0H  Single molecule detection for in vitro diagnostics [6862-17]
T. kiner, J. Ackermann, FluIT Biosystems GmbH (Germany); H. P. Mathis, B. Greiner, Fraunhofer Institute for Applied Information Technology (Germany); T. Tonn, D. Tschachojan, N. Kukoc-Zivovinov, S. Geiring, FluIT Biosystems GmbH (Germany)

6862 0I  Noninvasive high-speed optical imaging of biochemical interactions in microfluidic devices [6862-18]
R. Arora, G. I. Petrov, V. V. Shcheslavskiy, V. V. Yakovlev, Univ. of Wisconsin, Milwaukee (USA)

FRET

6862 0M  Monitoring the conformational dynamics of a single potassium transporter by ALEX-FRET [6862-22]
N. Zarrabi, Univ. of Stuttgart (Germany); T. Heitkamp, J.-C. Greie, Univ. of Osnabrück (Germany); M. Börsch, Univ. of Stuttgart (Germany)

NEW DEVELOPMENTS IN METHODS AND TECHNOLOGY

6862 0O  Overcoming the depth discrimination barrier in widefield microscopes: 3D single molecule tracking with high axial accuracy [6862-24]
S. Ram, Univ. of Texas Southwestern Medical Ctr. (USA); J. Chao, P. Prabhat, Univ. of Texas Southwestern Medical Ctr. (USA) and Univ. of Texas at Dallas (USA); E. S. Ward, Univ. of Texas Southwestern Medical Ctr. (USA); R. J.ober, Univ. of Texas Southwestern Medical Ctr. (USA) and Univ. of Texas at Dallas (USA)

6862 0P  Maximum-likelihood position sensing and actively controlled electrokinetic transport for single-molecule trapping [6862-25]
L. Davis, Z. Sikorski, W. Robinson, G. Shen, X. Li, B. Canfield, I. Lescano, B. Bomar, W. Hofmeister, J. Germann, J. King, Y. White, A. Terekhov, Univ. of Tennessee Space Institute (USA)
Novel detection scheme for optical biosensing using whispering gallery modes in clusters of dielectric particles [6862-27]
A. François, S. Krishnamoorthy, M. Himmelhaus, Bio-Nanotechnology Research Project (Japan)

PLASMONS AND METAL INTERACTION

Single molecule photophysics near metallic nanostructures (Invited Paper) [6862-29]
J. Zhang, Y. Fu, K. Ray, M. H. Chowdhury, H. Szymacinski, K. Nowaczyk, J. R. Lakowicz, Univ. of Maryland School of Medicine (USA)

Fluorescence enhancement on silver nanostructures: studies of components of ribosomal translation in vitro [6862-30]
W. Mandecki, UMDNJ, New Jersey Medical School (USA); S. Bharill, J. Borejdo, Univ. of North Texas Health Science Ctr. (USA); D. Cabral, B. S. Cooperman, I. Farrell, Univ. of Pennsylvania (USA); L. Fetter, Alcatel-Lucent Technologies (USA); E. Goldman, UMDNJ, New Jersey Medical School (USA); Z. Gryczynski, Univ. of North Texas Health Science Ctr. (USA); H. Jakubowski, UMDNJ, New Jersey Medical School (USA); H. Liu, Univ. of Pennsylvania (USA); R. Luchowski, E. Matveeva, Univ. of North Texas Health Science Ctr. (USA); D. Pan, H. Qin, Univ. of Pennsylvania (USA); D. Tennant, Alcatel-Lucent Technologies (USA); I. Gryczynski, Univ. of North Texas Health Science Ctr. (USA)

Optical cavity mode excitations in metal-coated microspheres [6862-31]
M. Himmelhaus, Bio-Nanotechnology Research Project, Fujirebio Inc. (Japan)

SMS IN BIOLOGY

Specific molecular aggregation of photosynthetic pigment-protein complex LHCII [6862-33]
W. I. Gruszecki, E. Janik, W. Grudzinski, Maria Curie-Sklodowska Univ. (Poland); P. Kernen, Zyomyx Inc. (USA); M. Gospodarek, Lublin Technical Univ. (Poland); W. Maksymiec, Z. Krupa, Maria Curie-Sklodowska Univ. (Poland)

Imaging of G protein-coupled receptors in solid-supported planar membranes at the single molecule level [6862-34]
I. Märki, M. Leutenegger, M. Geissbuehler, Ecole Polytechnique Fédérale de Lausanne (Switzerland); R. Robelek, Univ. of Regensburg (Germany); E.-K. Sinner, Max-Planck-Institut für Polymerforschung (Germany); T. Lasser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

POSTER SESSION

Fluorescence microscope using total internal reflection [6862-37]
S. J. Bae, S. Y. Lee, U. Knag, Korea Electrotechnology Research Institute (South Korea)

Advances in label-free optical biosensing: direct comparison of whispering gallery mode sensors with surface plasmon resonance [6862-38]
A. François, S. Krishnamoorthy, M. Himmelhaus, Bio-Nanotechnology Research Project (Japan)
Suppressing nonspecific adsorption of proteins on the single-molecular level [6862-39]

N. Marmé, JILA, National Institute of Standards and Technology (USA), Univ. of Colorado (USA), and Univ. of Heidelberg (Germany); H. Lee, JILA, National Institute of Standards and Technology (USA) and Univ. of Colorado (USA); A. Friedrich, German Cancer Research Ctr. (Germany); C.-W. Park, Univ. of Heidelberg (Germany); J. Fiore, D. J. Nesbitt, JILA, National Institute of Standards and Technology (USA) and Univ. of Colorado (USA); J.-P. Knemeyer, JILA, National Institute of Standards and Technology (USA), Univ. of Colorado (USA), and German Cancer Research Ctr. (Germany)

Author Index
Conference Committee

Symposium Chairs

James Fujimoto, Massachusetts Institute of Technology (USA)
R. Rox Anderson, Wellman Center for Photomedicine, Massachusetts General Hospital (USA) and Harvard School of Medicine (USA)

Program Track Chairs

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

Conference Chairs

Jörg Enderlein, Eberhard Karls Universität Tübingen (Germany)
Zygmunt K. Gryczynski, University of North Texas Health Science Center (USA)
Rainer Erdmann, PicoQuant, GmbH (Germany)

Program Committee

Sabato D'Auria, Consiglio Nazionale delle Ricerche (Italy)
Ewa M. Goldys, Macquarie University (Australia)
Johan Hofkens, Katholieke Universiteit Leuven (Belgium)
Borys Kierdaszuk, Uniwersytet Warszawski (Poland)
Gabor Laczkó, University of Szeged (Hungary)
Joseph A. Miragliotta, Johns Hopkins University (USA)
Maria Teresa C. A. Neves-Petersen, Aalborg Universitet (Denmark)
Markus Sauer, Universität Bielefeld (Germany)
Andong Xia, Institute of Chemistry (China)

Session Chairs

Welcome and Introduction
Jörg Enderlein, Eberhard Karls Universität Tübingen (Germany)
Zygmunt K. Gryczynski, University of North Texas Health Science Center (USA)
Rainer Erdmann, PicoQuant, GmbH (Germany)

FCS I
Rainer Erdmann, PicoQuant, GmbH (Germany)

FCS II
Thomas Dertinger, University of California, Los Angeles (USA)
New Developments in Methods and System I
Jörg Enderlein, Eberhard Karls Universität Tübingen (Germany)

New Developments in Methods and System II
Felix Koberling, PicoQuant, GmbH (USA)

FRET
Samantha Fore, University of California, Davis (USA)

New Developments in Methods and Technology
Zygmunt K. Gryczynski, University of North Texas Health Science Center (USA)

Plasmons and Metal Interaction
Christian Eggeling, Max-Planck-Institut für biophysikalische Chemie (Germany)

SMS in Biology
Markus Sauer, Universität Bielefeld (Germany)

PicoQuant Young Investigator Award
Zygmunt K. Gryczynski, University of North Texas Health Science Center (USA)

Poster Session
Rainer Erdmann, PicoQuant, GmbH (Germany)
Introduction

From January 19–24, 2008 SPIE organized a special symposium titled Biomedical Spectroscopy, Microscopy, and Imaging, as part of North America’s largest photonics event, SPIE’s Photonics West. The conference on Single Molecule Spectroscopy and Imaging was surely one of the highlights of this symposium, and about 120 scientists enjoyed more than 40 presentations.

The goal of the conference was to provide a state-of-the-art interdisciplinary forum for spectroscopists, biochemists, and engineers to exchange information on ultra-sensitive optical detection and spectroscopy down to the single-molecule level, and its applications in chemoanalysis, biophysics, 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 super-resolution imaging with a special focus on biological and biomedical applications. The presentations clearly showed 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 bio-molecules.

Special attention was given by the conference chairs Jörg Enderlein (University of Tübingen), Zygmunt Karol Gryczynski (The Center for Commercialization of Fluorescence Technologies,) and Rainer Erdmann (PicoQuant GmbH) to the activities of young investigators. A special Young Investigator Award worth $750 USD was sponsored by PicoQuant to motivate these students to submit and present their work at such a leading event.

Although all student contributions were of outstanding quality, the chairs decided to give the 2008 prize to Andrea Armani from the group of Scott Fraser for the paper: “Label-free detection of cytokines using optical microcavities.” (see also 1) as she demonstrated a fascinating new method for the analysis of single molecules overcoming the current limitation of adding labels.

The award also offers the winner a registration fee waiver for the leading European meeting on Single Molecule Spectroscopy and Ultra Sensitive Analysis in the Life Sciences, to be held 17–19 September 2008 in Berlin.

The next Single Molecule Spectroscopy and Imaging conference will be held again in San Jose as part of Photonics West from 19–24 January 2009. SPIE and the chairs of this conference encourage all scientists in the field to join us again and submit their latest scientific results.
We thank all authors of this year’s conference who submitted their papers on time, and our thanks goes also to the SPIE staff for helping us to make this conference so successful.

Jörg Enderlein,
Zygmunt K. Gryczynski
Rainer Erdmann

1 A. M. Armani*, R. P. Kulkarni, S. E. Fraser, R. C. Flagan, K. J. Vahala
“Label-free, single-molecule detection with optical microcavities”