

PROGRESS IN BIOMEDICAL OPTICS AND IMAGING  
Vol. 10, No. 43

# **Advanced Microscopy Techniques**

**Paul J. Campagnola**

**Ernst H. K. Stelzer**

**Gert von Bally**

*Editors*

**14–16 June 2009**

**Munich, Germany**

*Sponsored and Published by*

Optical Society of America (United States)

SPIE

*Cooperating Organisation*

German Biophotonics Research Program (Germany)

*Cosponsored by*

Photonics4Life—European Network of Excellence for Biophotonics

Air Force Office of Scientific Research (United States)

**Volume 7367**

Proceedings of SPIE-OSA Biomedical Optics, 1605-7422, v. 7367

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 *Advanced Microscopy Techniques*, edited by Paul J. Campagnola, Ernst H. K. Stelzer, Gert von Bally, Proceedings of SPIE-OSA Biomedical Optics Vol. 7367 (SPIE, Bellingham, WA, 2009) Article CID Number.

ISSN 1605-7422  
ISBN 9780819476432

Copublished 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  
and  
Optical Society of America  
2010 Massachusetts Ave., N.W., Washington, D.C., 20036 USA  
Telephone 1 202/223-8130 (Eastern Time) · Fax 1 202/223-1096  
<http://www.osa.org>

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

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](http://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.



[SPIEDigitalLibrary.org](http://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

ix	Conference Committee
xi	Introduction

---

## CONFOCAL/3D MICROSCOPY

---

- 7367 04 **Investigation of retinal microstructure by adaptive optics scanning laser ophthalmoscope with 1-micrometer wavelength probe** [7367-15]  
Y. Yasuno, K. Kurokawa, S. Makita, Univ. of Tsukuba (Japan) and Computational Optics and Ophthalmology Group (Japan); M. Miura, Tokyo Medical Univ. (Japan) and Computational Optics and Ophthalmology Group (Japan); K. Kawana, F. Okamoto, T. Oshika, Univ. of Tsukuba (Japan) and Computational Optics and Ophthalmology Group (Japan)
- 7367 07 **Position-referenced microscopy: regions of interest localization and subpixel image comparison by means of pseudo-random patterns embedded in cell culture boxes**  
[7367-48]  
J. A. Galeano Zea, P. Sandoz, L. Robert, Institut FEMTO-ST, CNRS, Univ. de Franche-Comté (France); E. Gaiffe, J.-L. Prétet, C. Mougin, Univ. de Franche-Comté (France)

---

## PHOTOPHYSICS

---

- 7367 0C **Controlling fluorescent proteins by manipulating the local density of photonic states (Invited Paper)** [7367-61]  
C. Blum, Y. Cesa, J. M. van den Broek, A. P. Mosk, Univ. Twente (Netherlands); W. L. Vos, Univ. Twente (Netherlands) and FOM Institute for Atomic and Molecular Physics (Netherlands); V. Subramaniam, Univ. Twente (Netherlands)
- 7367 0D **Barium titanate nanoparticles used as second harmonic radiation imaging probes for cell imaging** [7367-25]  
C.-L. Hsieh, Ecole Polytechnique Fédérale de Lausanne (Switzerland) and California Institute of Technology (United States); R. Grange, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Y. Pu, D. Psaltis, Ecole Polytechnique Fédérale de Lausanne (Switzerland) and California Institute of Technology (United States)
- 7367 0E **Dose limited fluorescence microscopy of living cells** [7367-57]  
H. Schneckenburger, Hochschule Aalen (Germany) and Univ. Ulm (Germany); M. Wagner, P. Weber, S. Schickinger, T. Bruns, Hochschule Aalen (Germany); W. S. L. Strauss, Univ. Ulm (Germany)

---

## OPTICAL SECTIONING

---

- 7367 0G **The zebrafish digital embryo: *in toto* reconstruction of zebrafish early embryonic development with digital scanned laser light sheet fluorescence microscopy** [7367-21]  
P. J. Keller, A. D. Schmidt, European Molecular Biology Lab. (Germany); J. Wittbrodt, European Molecular Biology Lab. (Germany), Univ. of Heidelberg (Germany), and Karlsruhe Institute of Technology (Germany); E. H. K. Stelzer, European Molecular Biology Lab. (Germany)
- 7367 0H **Optically sectioned imaging by oblique plane microscopy** [7367-53]  
C. Dunsby, Imperial College London (United Kingdom)

---

## NLO I: APPLICATIONS

---

- 7367 0M **Time- and spectral-resolved multiphoton imaging of fresh bladder biopsies** [7367-11]  
R. Cicchi, LENS—European Lab. for Non-linear Spectroscopy (Italy) and Univ. of Florence (Italy); A. Crisci, G. Nesi, Univ. of Florence Medical School (Italy); A. Cosci, LENS—European Lab. for Non-linear Spectroscopy (Italy) and Univ. of Florence (Italy); S. Giancane, M. Carini, Univ. of Florence Medical School (Italy); F. S. Pavone, LENS—European Lab. for Non-linear Spectroscopy (Italy) and Univ. of Florence (Italy)
- 7367 0O **Myosin helical pitch angle as a quantitative imaging biomarker for characterization of cardiac programming in fetal growth restriction measured by polarization second harmonic microscopy** [7367-06]  
I. Amat-Roldan, Hospital Clinic-IDIBAPS and CIBER-ER (Spain) and ICFO—Instituto de Ciencias Fotónicas (Spain); S. Psilodimitrakopoulos, ICFO—Instituto de Ciencias Fotónicas (Spain); E. Eixarch, I. Torre, B. Wotjas, F. Crispi, F. Figueras, Hospital Clinic-IDIBAPS and CIBER-ER (Spain); D. Artigas, P. Loza-Alvarez, ICFO—Instituto de Ciencias Fotónicas (Spain); E. Gratacos, Hospital Clinic-IDIBAPS and CIBER-ER (Spain)
- 7367 0R **Harmonic holographic microscopy with circularly polarized excitation** [7367-31]  
C.-L. Hsieh, Ecole Polytechnique Fédérale de Lausanne (Switzerland) and California Institute of Technology (United States); R. Grange, Ecole Polytechnique Fédérale de Lausanne (Switzerland); Y. Pu, D. Psaltis, Ecole Polytechnique Fédérale de Lausanne (Switzerland) and California Institute of Technology (United States)

---

## NLO II: METHODS

---

- 7367 0S **Contrast enhancement in second harmonic imaging: discriminating between muscle and collagen** [7367-12]  
S. Psilodimitrakopoulos, ICFO—Instituto de Ciencias Fotónicas (Spain); D. Artigas, ICFO—Instituto de Ciencias Fotónicas (Spain) and Univ. Politècnica de Catalunya (Spain); G. Soria, Institut d'Investigacions Biomèdiques de Barcelona, CSIC (Spain); I. Amat-Roldan, ICFO—Instituto de Ciencias Fotónicas (Spain) and Hospital Clinic-IDIBAPS and CIBER-ER (Spain); I. Torre, E. Gratacos, Hospital Clinic-IDIBAPS and CIBER-ER (Spain); A. M. Planas, Institut d'Investigacions Biomèdiques de Barcelona, CSIC (Spain); P. Loza-Alvarez, ICFO—Instituto de Ciencias Fotónicas (Spain)

- 7367 0U **Quasi white light multiphoton imaging** [7367-59]  
C. de Mauro, D. Alfieri, Light4Tech Firenze S.r.l. (Italy); M. Arrigoni, D. Armstrong, Coherent, Inc. (United States); F. S. Pavone, Univ. degli Studi di Firenze (Italy)
- 7367 0V **A comparison between coherent and spontaneous Raman scattering for biological imaging** [7367-22]  
B. R. Bachler, M. Cui, S. R. Nichols, J. P. Ogilvie, Univ. of Michigan (United States)

---

#### LOCALIZATION AND HIGH PRECISION

---

- 7367 0X **High-resolution fluorescence microscopy using three-dimensional structured illumination** [7367-02]  
P. F. Gardeazábal Rodríguez, P. Blandin, I. Maksimovic, E. Sepulveda, E. Muro, B. Dubertret, V. Lorette, Lab. Photons et Matière, CNRS, Ecole Supérieure de Physique et Chimie Industrielles (France)
- 7367 0Y **Super-resolved position and orientation of fluorescent dipoles** [7367-13]  
F. Aguet, S. Geissbühler, I. Märki, T. Lasser, M. Unser, Ecole Polytechnique Fédérale de Lausanne (Switzerland)
- 7367 10 **Live cell imaging with surface plasmon-mediated fluorescence microscopy** [7367-18]  
K. Balaa, Institut Langevin, CNRS, Univ. Paris Diderot (France); V. Devauges, Lab. de PhotoPhysique Moléculaire, CNRS, Univ. Paris-Sud (France); Y. Goulam, Institut Langevin, CNRS, Univ. Paris Diderot (France); V. Studer, Ecole Supérieure de Physique et de Chimie Industrielles (France); S. Lévéque-Fort, Lab. de PhotoPhysique Moléculaire, CNRS, Univ. Paris-Sud (France); E. Fort, Institut Langevin, CNRS, Univ. Paris Diderot (France)
- 7367 11 **FRET detection for neurobiological applications using a total internal reflection fluorescence lifetime imaging microscope** [7367-47]  
V. Devauges, P. Blandin, Lab. de Photophysique Moléculaire, CNRS, Univ. Paris Sud (France), Lab. Charles Fabry de l'Institut d'Optique, CNRS, Univ. Paris Sud (France), and Univ. Paris Sud (France); J. C. Cossec, CRICM UPMC/Inserm, CNRS (France); S. Lécart, Univ. Paris Sud (France); C. Marquer, M. C. Potier, CRICM UPMC/Inserm, CNRS (France); F. Druon, Lab. Charles Fabry de l'Institut d'Optique, CNRS, Univ. Paris Sud (France) and Univ. Paris Sud (France); P. Georges, Lab. Charles Fabry de l'Institut d'Optique, CNRS, Univ. Paris Sud, (France) and Univ. Paris Sud (France); S. Lévéque-Fort, Lab. de PhotoPhysique Moléculaire, CNRS, Univ. Paris Sud (France) and Univ. Paris Sud (France)

---

#### HOLOGRAPHIC METHODS

---

- 7367 14 **3D tracking and multi-wavelength techniques for digital holographic microscopy based cell analysis (Invited Paper)** [7367-62]  
B. Kemper, P. Langehanenberg, S. Kosmeier, S. Przibilla, A. Vollmer, S. Ketelhut, G. von Bally, Ctr. for Biomedical Optics and Photonics, Univ. of Muenster (Germany)
- 7367 16 **Digital holographic microscopy at fundamental and second harmonic wavelengths** [7367-19]  
E. Shaffer, C. Depeursinge, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

- 7367 18 **Application of color digital holographic microscopy for analysis of stained tissue sections** [7367-01]  
X. Mo, Ctr. for Biomedical Optics and Photonics, Univ. of Muenster (Germany) and Beijing Institute of Technology (China); B. Kemper, P. Langehanenberg, A. Vollmer, Ctr. for Biomedical Optics and Photonics, Univ. of Muenster (Germany); J. Xie, Beijing Institute of Technology (China); G. von Bally, Ctr. for Biomedical Optics and Photonics, Univ. of Muenster (Germany)

---

#### POSTER SESSION

---

- 7367 19 **Reflective confocal laser scanning microscopy and nonlinear microscopy of cross-linked rabbit cornea** [7367-03]  
A. Krüger, Laser Zentrum Hannover e.V. (Germany); M. Hovakimyan, Universitaetsaugenklinik Rostock (Germany); D. F. Ramirez, Laser Zentrum Hannover e.V. (Germany); O. Stachs, R. F. Guthoff, Universitaetsaugenklinik Rostock (Germany); A. Heisterkamp, Laser Zentrum Hannover e.V. (Germany)
- 7367 1A **Optical tweezers force measurements to study parasites chemotaxis** [7367-05]  
A. A. de Thomaz, L. Y. Pozzo, Univ. Estadual de Campinas (Brazil); A. Fontes, Univ. Federal de Pernambuco (Brazil); D. B. Almeida, Univ. Estadual de Campinas (Brazil); C. V. Stahl, J. R. Santos-Mallet, S. A. O. Gomes, Fundacao Oswaldo Cruz (Brazil); D. Feder, Univ. Federal Fluminense (Brazil); D. C. Ayres, S. Giorgio, C. L. Cesar, Univ. Estadual de Campinas (Brazil)
- 7367 1D **Controlling the emission of organic dyes for high sensitivity and super-resolution microscopy** [7367-20]  
T. Cordes, I. H. Stein, C. Forthmann, C. Steinhauer, M. Walz, W. Sumnerer, B. Person, J. Vogelsang, P. Tinnefeld, Ludwig-Maximilians-Univ. (Germany)
- 7367 1E **Applying image restoration to fluorescence lifetime imaging microscopy (FLIM)** [7367-23]  
C.-W. Chang, M.-A. Mycek, Univ. of Michigan (United States)
- 7367 1F **Development and assessment of image reconstruction algorithms using a low-cost bench-microscope based on a linear CMOS image sensor** [7367-24]  
M. P. Macedo, Univ. of Coimbra (Portugal) and Instituto Superior de Engenharia de Coimbra (Portugal); C. M. B. A. Correia, Univ. de Coimbra (Portugal)
- 7367 1H **Temporal imaging chamber (TIC) for en face imaging of epidermal absorption in vitro** [7367-28]  
C. Simonsson, M. Smedh, C. Jonsson, M. B. Ericson, Göteborg Univ. (Sweden)
- 7367 1J **Study of 3D cell morphology and effect on light scattering distribution** [7367-35]  
A. E. Ekenyong, J. Ding, L. V. Yang, N. R. Leffler, J. Q. Lu, East Carolina Univ. (United States); R. S. Brock, Virginia Commonwealth Univ. (United States); X.-H. Hu, East Carolina Univ. (United States)
- 7367 1K **Three dimensional numerical simulation of complex optical systems using the coherent transfer function** [7367-39]  
R.-A. Lorbeer, A. Heisterkamp, Laser Zentrum Hannover e.V. (Germany)

- 7367 1O **Confocal microscopy for automatic texture analysis of elastic fibers in histologic preparations** [7367-45]  
R. L. Adam, G. Vieira, D. P. Ferro, A. A. de Thomaz, C. L. Cesar, K. Metze, Univ. Estadual de Campinas (Brazil) and Instituto Nacional de Fotônica Aplicada à Biologia Celular (Brazil)
- 7367 1P **MEMS-based confocal laser scanning microscope for in vivo imaging** [7367-46]  
J. Helfmann, R. Schütz, I. Gersonne, G. Illing, Laser- und Medizin-Technologie GmbH, Berlin (Germany)
- 7367 1R **Point spread function measured in human skin using two-photon fluorescence microscopy** [7367-52]  
S. Guldbbrand, C. Simonsson, M. Smedh, M. B. Ericson, Göteborg Univ. (Sweden)
- 7367 1S **Tomographic screening of 3-dimensional cell cultures** [7367-56]  
T. Bruns, V. Richter, M. Wagner, Hochschule Aalen (Germany); W. S. L. Strauss, Univ. Ulm (Germany); H. Schneckenburger, Hochschule Aalen (Germany) and Univ. Ulm (Germany)

*Author Index*



# Conference Committee

## General Chairs

**Mary-Ann Mycek**, University of Michigan (United States)  
**Wolfgang Drexler**, Cardiff University (United Kingdom)

## Program Chairs

**Christoph K. Hitzenberger**, Medizinische Universität Wien (Austria)  
**Brian W. Pogue**, Dartmouth College (United States)

## Conference Chairs

**Paul J. Campagnola**, University of Connecticut Health Center (United States)  
**Ernst H. K. Stelzer**, European Molecular Biology Laboratory (Germany)  
**Gert von Bally**, Centrum für Biomedizinische Optik und Photonik, Westfälische Wilhelms-Universität Münster (Germany)

## Program Committee

**Kishan Dholakia**, University of St. Andrews (United Kingdom)  
**Kevin Eliceiri**, University of Wisconsin-Madison (United States)  
**Paul French**, Imperial College London (United Kingdom)  
**Jesper Glückstad**, Danmarks Tekniske Universitet Fotonik (Denmark)  
**Charles P. Lin**, Massachusetts General Hospital (United States)  
**Jerome Mertz**, Boston University (United States)  
**Vinod Subramaniam**, Universiteit Twente (Netherlands)  
**Rainer Uhl**, Ludwig-Maximilians-Universität München (Germany)

## Session Chairs

Confocal/3D Microscopy  
**Paul J. Campagnola**, University of Connecticut Health Center (United States)

Photophysics I  
**Rainer Uhl**, Ludwig-Maximilians-Universität München (Germany)

Photophysics II  
**Ernst H. K. Stelzer**, European Molecular Biology Laboratory (Germany)

Optical Sectioning

**Gert von Bally**, Centrum für Biomedizinische Optik und Photonik,  
Westfälische Wilhelms-Universität Münster (Germany)

NLO I: Applications

**Jesper Glückstad**, Danmarks Tekniske Universitet Fotonik (Denmark)

NLO II: Methods

**Charles P. Lin**, Wellman Laboratories of Photomedicine, Massachusetts  
General Hospital (United States)

Localization and High Precision

**Jerome Mertz**, Boston University (United States)

Holographic Methods

**Kishan Dholakia**, University of St. Andrews (United Kingdom)

# **Introduction**

This volume is a collection of technical papers presented at the 2009 European Conferences on Biomedical Optics held in Munich, Germany from June 14–18. These collected papers were given in the technical conference of Advanced Microscopy Techniques (AMT).

The technical conference was divided into 8 topical sessions and one poster session. The papers in these sessions covered a wide range of topics: confocal/3D microscopy, photophysics, holographic methods, non-linear optical microscopy, optical sectioning methods, and localization and high precision microscope methods. These topics have a common thread in that the technology was developed to meet long-standing problems in biology where, for example, methods were developed for faster imaging with better viability, label-free imaging, and for higher resolution to obtain better structural level data. As you will find in the roster, members of the program committee represent the emerging microscopy methods discussed in these sessions.

The technical conference included three excellent invited presentations:

"Controlling fluorescent proteins by manipulating the local density of photonic states" by Christian Blum, et al., Biophysical Engineering, Univ. of Twente, Netherlands. (73670C)

"3D tracking and multi-wavelength techniques for digital holographic microscopy based cell analysis" by Bjoern Kemper, et al., Center for Biomedical Optics and Photonics (CeBOP), Univ. of Muenster, Germany (736714)

"Light sheet based fluorescence microscopes (LSFM, SPIM, DSLM) reduce phototoxic effects by several orders of magnitude" by Philipp Keller, Cell Biology and Biophysics Unit and Developmental Biology Unit, EMBL Heidelberg, Germany (presentation only)

We would like to thank authors and presenters of this year's conference for their contributions. The outstanding conference program was put together by the fine effort of the program committee and we are thankful to the members of the program committee for their contributions.

We are sure that readers will find this proceedings volume useful and informative.

**Paul Campagnola  
Ernst Stelzer  
Gert von Bally**

