

# ***Multiphoton Microscopy in the Biomedical Sciences XVI***

**Ammasi Periasamy**  
**Peter T. C. So**  
**Karsten König**  
*Editors*

**14–16 February 2016**  
**San Francisco, California, United States**

*Sponsored by*  
SPIE

*Cosponsored by*  
Becker & Hickl GmbH (Germany)  
Carl Zeiss (United States)  
Chroma Technology Corp. (United States)  
Coherent Inc. (United States)  
Semrock Inc. (United States)  
ISS, Inc. (United States)  
JenLab GmbH (Germany)  
Leica Microsystems (United States)  
Spectra-Physics®, a Newport Company (United States)

*Published by*  
SPIE

**Volume 9712**

Proceedings of SPIE, 1605-7422, V. 9712

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

Multiphoton Microscopy in the Biomedical Sciences XVI, edited by Ammasi Periasamy, Peter T. C. So, Karsten König,  
Proc. of SPIE Vol. 9712, 971201 · © 2016 SPIE · CCC code: 1605-7422/16/\$18 · doi: 10.1117/12.2239754

The papers 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. Additional papers and presentation recordings may be available online in the SPIE Digital Library at [SPIDigitalLibrary.org](http://SPIDigitalLibrary.org).

The papers 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 these proceedings:

Author(s), "Title of Paper," in *Multiphoton Microscopy in the Biomedical Sciences XVI*, edited by Ammasi Periasamy, Peter T. So, Karsten König, Proceedings of SPIE Vol. 9712 (SPIE, Bellingham, WA, 2016) Six-digit Article CID Number.

ISSN: 1605-7422  
ISSN: 2410-9045 (electronic)  
ISBN: 9781628419467

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](http://SPIE.org)

Copyright © 2016, 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](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 1605-7422/16/\$18.00.

Printed in the United States of America.

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

**SPIE. DIGITAL  
LIBRARY**

[SPIDigitalLibrary.org](http://SPIDigitalLibrary.org)

---

**Paper Numbering:** *Proceedings of SPIE* follow an e-First publication model. A unique citation identifier (CID) number is assigned to each article at the time of publication. Utilization of CIDs allows articles to be fully citable as soon as they are published online, and connects the same identifier to all online and print versions of the publication. SPIE uses a six-digit CID article numbering system structured as follows:

- 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.

# Contents

vii	<i>Authors</i>
ix	<i>Conference Committee</i>
xiii	<i>Introduction</i>
xv	<i>JenLab Young Investigator Award</i>

---

## JENLAB YOUNG INVESTIGATOR AWARD PAPERS PRESENTATION

9712 02	<b>Four-wave mixing based light sources for real-world biomedical applications of coherent Raman microscopy (JenLab Young Investigator Award) [9712-61]</b>
9712 03	<b><i>In vivo</i> imaging flow cytometry based on laser scanning two-photon microscopy at kHz cross-sectional frame rate [9712-59]</b>

---

## KEYNOTE SESSION

9712 04	<b>Correlated FLIM and PLIM for cell metabolism (Keynote Paper) [9712-1]</b>
---------	--

---

## BIOMEDICAL APPLICATIONS OF COHERENT RAMAN II

9712 0G	<b>Hyperspectral stimulated Raman scattering and multiphoton imaging for digital pathology of colonic disease [9712-13]</b>
---------	---

---

## COHERENT RAMAN TECHNICAL DEVELOPMENT

9712 0M	<b>M-CARS and EFISHG study of the influence of a static electric field on a non-polar molecule [9712-19]</b>
9712 0N	<b>Synchronized and timing-stabilized pulse generation from a gain-switched laser diode for stimulated Raman scattering microscopy [9712-20]</b>

---

## FLIM/FRET/FCS I

9712 0P	<b>Binding of the immunomodulatory drug Bz-423 to mitochondrial F<sub>0</sub>F<sub>1</sub>-ATP synthase in living cells by FRET acceptor photobleaching (Invited Paper) [9712-22]</b>
---------	---

9712 0Q **Investigation of prostate cancer cells using NADH and Tryptophan as biomarker: multiphoton FLIM-FRET microscopy [9712-23]**

---

**FLIM/FRET/FCS II**

---

9712 0R **Temporal and spatial binning of TCSPC data to improve signal-to-noise ratio and imaging speed (Invited Paper) [9712-24]**

9712 0S **Tunable PIE and synchronized gating detections by FastFLIM for quantitative microscopy measurements of fast dynamics of single molecules (Invited Paper) [9712-25]**

9712 0T **ns-time resolution for multispecies STED-FLIM and artifact free STED-FCS (Invited Paper) [9712-26]**

---

**TECHNOLOGY DEVELOPMENT I**

---

9712 0V **Metabolic microscopy of head and neck cancer organoids [9712-29]**

9712 0X **A phasor approach analysis of multiphoton FLIM measurements of three-dimensional cell culture models [9712-31]**

9712 0Y **Using multiphoton fluorescence lifetime imaging to characterize liver damage and fluorescein disposition in liver *in vivo* [9712-86]**

---

**TECHNOLOGY DEVELOPMENT II**

---

9712 11 **Recent developments in widely tunable and high peak power ultrafast laser sources and their adoption in biological imaging [9712-34]**

---

**SECOND/THIRD HARMONIC GENERATION I**

---

9712 17 **Characterization of human arterial tissue affected by atherosclerosis using multimodal nonlinear optical microscopy [9712-39]**

9712 18 **Forward versus backward polarization-resolved SHG imaging of collagen structure in tissues [9712-40]**

---

**SECOND/THIRD HARMONIC GENERATION II**

---

9712 1D **Multiphoton imaging with a nanosecond supercontinuum source [9712-45]**

---

**TECHNOLOGY DEVELOPMENT III**

---

9712 1F **Large field of view multiphoton microscopy of human skin (Invited Paper) [9712-47]**

- 9712 1G **Design of a portable wide field of view GPU-accelerated multiphoton imaging system for real-time imaging of breast surgical specimens (Invited Paper)** [9712-48]
- 9712 1H **Compact fixed wavelength femtosecond oscillators as an add-on for tunable Ti:sapphire lasers extend the range of applications towards multimodal imaging and optogenetics** [9712-49]

---

#### TECHNOLOGY DEVELOPMENT IV

---

- 9712 1M **Multi-photon microscope driven by novel green laser pump** [9712-54]
- 9712 1P **Quantitative structural markers of colorectal dysplasia in a cross sectional study of ex vivo murine tissue using label-free multiphoton microscopy** [9712-57]

---

#### POSTER SESSION

---

- 9712 1R **Observation of tendon repair in animal model using second-harmonic-generation microscopy (Student Poster Session Competition Award)** [9712-70]
- 9712 1S **Nonlinear optical Stokes ellipsometric (NOSE) microscopy for imaging the nonlinear susceptibility tensors of collagen (Student Poster Session Competition Award)** [9712-77]
- 9712 1T **Multiphoton fluorescence lifetime imaging of metabolic status in mesenchymal stem cell during adipogenic differentiation** [9712-28]
- 9712 1V **Comparison of in vivo and ex vivo imaging of the microvasculature with 2-photon fluorescence microscopy** [9712-60]
- 9712 1W **The nature of multiphoton fluorescence from red blood cells** [9712-63]
- 9712 1Z **In vivo two-photon imaging measuring the blood-brain barrier permeability during early postnatal brain development in rodent** [9712-66]
- 9712 20 **High-resolution stimulated Raman scattering microscopy by focal-field modulation** [9712-67]
- 9712 21 **Mapping of intracellular concentrations of macromolecules by two-photon excited fluorescence lifetime imaging** [9712-69]
- 9712 22 ***In situ* quantitative evaluation of osteoblastic collagen synthesis under cyclic strain by using second-harmonic-generation microscope** [9712-71]
- 9712 2C **Time-gated FLIM microscope for corneal metabolic imaging** [9712-82]
- 9712 2E **FLIM data analysis of NADH and Tryptophan autofluorescence in prostate cancer cells** [9712-84]



## Authors

Numbers in the index correspond to the last two digits of the six-digit citation identifier (CID) article numbering system used in Proceedings of SPIE. The first four digits reflect the volume number. Base 36 numbering is employed for the last two digits and indicates the order of articles within the volume. Numbers start with 00, 01, 02, 03, 04, 05, 06, 07, 08, 09, 0A, 0B...0Z, followed by 10-1Z, 20-2Z, etc.

Ahsen, Osman, 1G  
Andersen, Peter E., 1M  
Balu, Mihaela, 1F  
Baraige, Fabienne, 1D  
Barbieri, Beniamino, 0S  
Baria, Enrico, 17  
Batista, Ana, 2C  
Beier, Hope T., 0R  
Blanquet, Véronique, 1D  
Börsch, Michael, 0P  
Brey Mayer, J., 04  
Brooker, Jeff, 1G  
Brucker, S. Y., 0X  
Bystrova, A. S., 1T  
Cable, Alex, 1G  
Cahill, Lucas, 1G  
Capitaine, E., 0M  
Chandra, Dhyan, 0Q  
Cicchi, Riccardo, 17  
Clausen, Mathias P., 0T  
Connolly, James L., 1G  
Coskun, Ulas, 0S  
Couderc, Vincent, 0M, 1D  
Crawford, Darrell, 0Y  
Cui, Meng, 03  
Dantus, Marcos, 1W  
DeWalt, Emma L., 1S  
Djurhuus, Martin, 1M  
Domingues, José Paulo, 2C  
Dow, Ximeng Y., 1S  
Dowler, Rhys, 0T  
Dudenkova, V. V., 1T  
Eggeling, Christian, 0T  
Erdmann, Rainer, 0T  
Evans, Conor L., 1W  
Fang, Yi-Cheng, 0N  
Faulkner-Jones, Beverly E., 1G  
Ferreon, Allan Chris, 0S  
Fujimoto, James G., 1G  
Galiani, Silvia, 0T  
Giacomelli, Michael G., 1G  
Glick, Gary D., 0P  
Gottschall, Thomas, 02  
Gräber, Peter, 0P  
Greening, Gage J., 1P  
Gusachenko, Ivan, 18  
Hakulinen, T., 1H  
Hase, Eiji, 1R, 22  
Hornegger, Joachim, 1G  
Hou, Jue, 1F  
Huang, Zhiwei, 0G, 20  
Husvogt, Lennart, 1G  
Jauregui, Cesar, 02  
Jensen, Ole Bjarlin, 1M  
Johnson, Kathryn M., 0P  
Kalinina, S., 04  
Kaneyasu, J. F., 0M  
Kano, H., 0M  
Klein, J., 11, 1H  
Koberling, Felix, 0T  
Koenig, Marcelle, 0T  
Koletar, Margaret, 1V  
Kong, Lingjie, 03  
Kraemer, Benedikt, 0T  
Kuzmin, Andrey, 21  
Lai, Keith K., 1P  
Lakner, P. H., 0X  
Latour, Gaël, 18  
Lefort, Claire, 0M, 1D  
Leproux, Philippe, 0M, 1D  
Lévêque, Philippe, 1D  
Liao, Shih-Chu Jeff, 0S  
Limpert, Jens, 02  
Lin, Jian, 0G, 20  
Liu, Lixin, 21  
Louot, C., 0M  
Marti, Dominik, 1M  
Massi, Daniela, 17  
Matsubara, Oki, 22  
Meleshina, A. V., 1T  
Meyer, Tobias, 02  
Mikami, Hideharu, 1F  
Minamikawa, Takeo, 1R, 22  
Möller, Y., 0X  
Monaghan, M. G., 0X  
Morgado, António Miguel, 2C  
Muldoon, Timothy J., 1P  
Murphy, Michael, 1W  
Nesi, Gabriella, 17  
O'Connor, Rodney P., 1D  
Olayoye, M. A., 0X  
O'Melia, Meghan J., 0Q, 2E  
Opipari, Anthony W., Jr., 0P  
Osseiran, Sam, 1W  
Ould-Moussa, N., 0M  
Ozeki, Yasuyuki, 0N  
Pagnoux, D., 0M  
Patting, Matthias, 0T

Pavone, Francesco S., 17  
Peng, Xiao, 21  
Periasamy, Ammasi, 0Q, 2E  
Petersen, Jan, 0P  
Pliss, Artem, 21  
Popp, Jürgen, 02  
Potma, Eric O., 1F  
Prasad, Paras N., 21  
Prieto, Sandra P., 1P  
Qu, Junle, 21  
Quadrado, Maria João, 2C  
Rehman, Shaguffa, 0Q, 2E  
Reisch, Paja, 0T  
Roberts, Michael S., 0Y  
Rodríguez-Contreras, Adrián, 1Z  
Rotellini, Matteo, 17  
Rück, A., 04  
Sato, Katsuya, 1R, 22  
Saytashev, Ilyas, 1W  
Schanne-Klein, Marie-Claire, 18  
Schenke-Layland, K., 0X  
Schmitt, Michael, 02  
Schmitt, Paul D., 1S  
Shah, Amy T., 0V  
Sheykin, Yury, 1G  
Shi, Lingyan, 1Z  
Shirmanova, M. V., 1T  
Silva, Susana F., 2C  
Simpson, Garth J., 1S  
Skala, Melissa C., 0V  
Sled, John G., 1V  
Spence, Dana M., 1W  
Starke, Ilka, 0P  
Stefanovic, Bojana, 1V  
Steinman, Joe, 1V  
Studier, Hauke, 0Y  
Sullivan, Shane Z., 1S  
Sun, Yuansheng, 0S  
Svindrych, Zdenek, 0Q, 2E  
Takahashi, Mitsuhiko, 1R  
Tang, Jianyong, 03  
Tannert, Sebastian, 0T  
Thorling, Camilla A., 0Y  
Teulon, Claire, 18  
Tokunaga, Kyoya, 0N  
Tombelaine, Vincent, 1D  
Tromberg, Bruce J., 1F  
Tünnermann, Andreas, 02  
Vardeh, Hilde, 1G  
Wallrabe, Horst, 0Q, 2E  
Walsh, Alex J., 0R  
Wang, Zi, 0G  
Yasui, Takeshi, 1R, 22  
Yokoyama, Hiroyuki, 0N  
Yoshitake, Tadayuki, 1G  
Zagaynova, E. V., 1T  
Zheng, Wei, 0G, 20

# Conference Committee

## *Symposium Chairs*

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

**R. Rox Anderson**, Wellman Center 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**, University of Southern California (United States) and  
Spectral Molecular Imaging, Inc. (United States)

## *Conference Chairs*

**Ammasi Periasamy**, University of Virginia (United States)

**Peter T. C. So**, Massachusetts Institute of Technology (United States)

**Karsten König**, Universität des Saarlandes (Germany)

## *Conference Program Committee*

**Wolfgang Becker**, Becker & Hickl GmbH (Germany)

**Alberto Diaspro**, Istituto Italiano di Tecnologia (Italy)

**Chen-Yuan Dong**, National Taiwan University (Taiwan)

**Kevin W. Eliceiri**, University of Wisconsin-Madison (United States)

**Scott Fraser**, The University of Southern California (United States)

**Paul M. W. French**, Imperial College London (United Kingdom)

**Hans C. Gerritsen**, Universiteit Utrecht (Netherlands)

**Enrico Gratton**, University of California, Irvine (United States)

**Min Gu**, Swinburne University of Technology (Australia)

**Stefan W. Hell**, Max-Planck-Institut für Biophysikalische Chemie  
(Germany)

**Paul J. Campagnola**, University of Wisconsin-Madison (United States)

**Satoshi Kawata**, Osaka University (Japan)

**Fu-Jen Kao**, National Yang-Ming University (Taiwan)

**Arnd K. Krueger**, Spectra-Physics®, a Newport Company  
(United States)

**Joseph R. Lakowicz**, University of Maryland School of Medicine  
(United States)

**Steve M. McDonald**, Coherent, Inc. (United States)

**Angelika C. Rueck**, Universität Ulm (Germany)

**Junle Qu**, Shenzhen University (China)  
**Steven S. Vogel**, National Institutes of Health (United States)  
**Paul W. Wiseman**, McGill University (Canada)  
**X. Sunney Xie**, Harvard University (United States)  
**Chris Xu**, Cornell University (United States)  
**Bernhard Zimmermann**, Carl Zeiss Jena GmbH (Germany)  
**Warren R. Zipfel**, Cornell University (United States)

*Session Chairs*

- 1 Keynote Session  
**Karsten König**, Universität des Saarlandes (Germany)
- 2 Biomedical Applications of Coherent Raman I  
**Eric Potma**, University of California, Irvine (United States)
- 3 Biomedical Applications of Coherent Raman II  
**Ji-Xin Cheng**, Purdue University (United States)
- 4 Coherent Raman Technical Development  
**Marcus T. Cicerone**, National Institute of Standards and Technology (United States)
- 5 FLIM/FRET/FCS I  
**Yuansheng Sun**, ISS, Inc. (United States)
- 6 JenLab Young Investigator Award Papers Presentation  
**Karsten König**, Universität des Saarlandes (Germany)
- 7 FLIM/FRET/FCS II  
**Michael Börsch**, Friedrich-Schiller-Universität Jena (Germany)
- 8 Technology Development I  
**Peter T. C. So**, Massachusetts Institute of Technology (United States)
- 9 Technology Development II  
**Peter T. C. So**, Massachusetts Institute of Technology (United States)
- 10 Second/Third Harmonic Generation I  
**Conor Evans**, Harvard School of Medicine (United States)
- 11 Second/Third Harmonic Generation II  
**Paul J. Campagnola**, University of Wisconsin-Madison (United States)
- 12 Technology Development III  
**Francesco S. Pavone**, European Laboratory for Non-linear Spectroscopy (Italy)

13 Technology Development IV  
**Karsten König**, Universität des Saarlandes (Germany)

Poster Session

**Holly Aaron**, University of California, Berkeley (United States)

**Kevin W. Eliceiri**, University of Wisconsin-Madison (United States)

**Alex J. Walsh**, Air Force Research Laboratory (United States)



## Introduction

Multiphoton microscopy has been established as the 3D imaging method of choice for studying biomedical specimens from single cells and whole animals to patients with sub-micron resolution. 25 years have passed since the realization of two-photon laser scanning microscopy. The ever-expanding scope of applications and the continuing instrumental innovations require a forum where new ideas can be exchanged and presented. Our conference at the SPIE BIOS 2016 meeting continues to address this need.

The 16th year of this conference began with three keynote lectures from leaders in the field: Angelika C. Rueck, Universität Ulm (Germany), "Correlated phosphorescence and fluorescence lifetime imaging for cell metabolism" [9712-1]; Peter T. C. So, Massachusetts Institute of Technology (United States), "Depth-resolved incoherent and coherent wide-field high-content imaging" [9712-2]; and Xiaoliang S. Xie, Harvard University (United States), "Biomedical applications of SRS microscopy" [9712-3].

For the 5th year in a row, the conference was extremely pleased to have the JenLab Young Investigator Award in addition to our regular poster awards. This award was donated by Karsten König, President and Founder of JenLab GmbH (Germany). The award selection committee included Arnd Krueger, Spectra Physics®, a Newport Company (United States), Francesco S. Pavone, Università degli Studi di Firenze (Italy), Paul J. Campagnola, University of Wisconsin-Madison (United States), and the two conference chairs, Karsten König and Peter T. C. So. The selection process included the abstract, manuscript, and poster presentation. Two finalists were selected for oral presentation after their poster presentations.

The two finalists were

1. Lingjie Kong, Purdue University (United States), for "In vivo imaging flow cytometry based on laser scanning two-photon microscopy at kHz cross-sectional frame rate" [9712-59], and
2. Thomas Gottschall, Friedrich-Schiller-Universität Jena (Germany), for "Four-wave mixing based light sources for real-world biomedical applications of coherent Raman microscopy" [9712-61].

**Thomas Gottschall**, Friedrich-Schiller-Universität Jena (Germany) was selected as the **winner of the JenLab Young Investigator Award 2016**.

For the 16th year in a row, the conference organized poster awards for students and postdoctoral fellows. The poster awards were donated by our conference sponsors, including: Becker & Hickl GmbH, Chroma Technology Corp., Coherent Inc., ISS, Inc., Spectra Physics®, a Newport Company, Leica Microsystems, Semrock Inc., and Carl Zeiss.

The 3 poster award winners were:

1. Hequn Wang, Massachusetts General Hospital (United States), for "Noninvasive visualization of pheomelanin using coherent Raman scattering microscopy" [9712-62],
2. Eiji Hase, The University of Tokushima (Japan), for "Observation of tendon repair in animal model using second-harmonic-generation microscopy" [9712-70], and
3. Ximeng You, Purdue University (United States), for "Nonlinear optical Stokes ellipsometric (NOSE) microscopy for imaging the nonlinear susceptibility tensors of collagen" [9712-77].

Some of the most valuable contributions in this volume are articles written by highly experienced practitioners of multiphoton microscopy. They have enumerated the most important considerations in designing multiphoton microscopes and imaging experiments. Further, updates on the state-of-the-art commercial multiphoton microscope systems were presented. This volume also includes proceedings describing some recent advances in major multi-photon microscope components and applications, including laser light sources, ultra-fast optics, filters, FRET, FLIM, FCS, Raman, CARS, SRS and CRS microscopy and spectroscopy, single molecule, endoscopy, energy metabolism measurements including NADH, FAD, tryptophan in cells and tissues, and various scientific and clinical applications.

On a personal note, the conference chairs are grateful for the participation of all authors and session chairs, and acknowledge the innovation-driven manufacturers and sponsors of this conference (Becker & Hickl GmbH, Chroma Technology Corp., Coherent Inc., ISS, Inc., JenLab GmbH, Spectra-Physics®, a Newport Company, Leica Microsystems, Semrock Inc., and Carl Zeiss) for their enthusiastic support in organizing this conference successfully for the last 16 years. We look forward to other exciting conferences in the future and welcome your continued participation and support.

**Ammasi Periasamy  
Peter T. C. So  
Karsten König**

# **JenLab Young Investigator Award**

*presented to*

**Thomas Gottschall**

Friedrich-Schiller-Universität Jena (Germany)

*for*

**Four-wave mixing based light sources for real-world biomedical applications of coherent Raman microscopy [9712-61]**

*Session Chair*

**Peter T. C. So**, Massachusetts Institute of Technology (United States)

*Award Presenter*

**Karsten König**, President of JenLab GmbH (Germany)

